cholate level in the enterohepatic circuit requisite to normal lipid absorption. The observed pattern of absorptive activity along the intestine would seem to be ideally suited to the dual function of keeping the cholates in the lumen to promote lipid absorption for as long as possible and yet allowing their eventual recovery to the animal.

The existence of such a gradient in absorptive activity indirectly supports the idea that some specific system exists in the mucosal epithelium to absorb cholate. The magnitude of the cholate gradient becomes more striking when one considers that the mucosal surface area per unit length of small intestine decreases with distance from the pylorus(3).

It is interesting to note that this gradient for bile salt *in vivo* is oriented in the opposite direction to the absorptive activity gradients for glucose along the small intestine of the rat(4), and for palmitic acid along the hamster small intestine(5) observed in experiments with surviving small intestine *in vitro*.

Kremen, *et al.*(6) have shown in dogs that a much greater defect in fat absorption occurs when the distal half of the small intestine is resected than when the proximal half (below the duodenum) is removed. The possibility should be considered that the marked fat absorption defect observed by these authors after distal resection resulted from elimination of a highly active area of bile salt absorption with a consequent decrease in amount of cholate in the enterohepatic circuit, and did not necessarily reflect a greater inherent ability of the distal small intestine to absorb fat.

Summary. Using an *in vivo,* acute loop technic it has been shown in the rat that absorptive activity for conjugated cholate increases nearly 4-fold as the small intestine is descended from duodenum to terminal ileum.

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Pyridoxine Deficiency in Hyperthyroidism.* (26164)

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Hyperthyroidism is associated with an increased requirement not only of calories, but also of essential micronutrients. When they are not provided in the daily dietary, or fail to be utilized, weight loss and nutritional deficiencies may ensue.

Among the main micronutrients whose quantitative requirements are elevated in experimental hyperthyroidism are certain of the B complex vitamins. Thus, Cowgill and Palmieri concluded from experimentally produced thyrotoxicosis that there is some relation between amount of B requirement for maintainance of weight and metabolism of the organism(1). In view of the fact that B-6 is involved in a great many enzymatic reactions, it seemed advantageous to investigate pyridoxine metabolism in hyperthyroidism in man.

Material and methods. The presence of B-6 complex is required for metabolic breakdown of tryptophan. In induced B-6 deficiency, derangement of tryptophan metabolism is manifested by increased excretion of xanthurenic acid after a tryptophan load(2). Administration of B-6 corrects this aberration

* We are indebted to Dr. H. Menduke, Jefferson Med. College, for statistical evaluation of data.

TABLE I. Xanthurenic Acid Excretion in Patients with Hyperthyroidism.

Case No.	Sex	Age	Before tryptophan Vol	Creat.	Xa.	After tryptophan Vol	Creat.	Xa.	After tryptophan + B6 Vol	Creat.	Xa.
1	♀	42	1250	1.1	3	900	1.1	11	1200	1.1	11
2	♀	39	1210	.6	10	1110	.6	33	1310	.7	12
3	♀	25	350	.4	2	770	.7	31	500	.4	8
4	♀	22	1220	.7	6	1140	.6	105	1490	.7	16
5	♀	50	1270	1.0	7	970	.7	24	1080	.8	4
6	♀	48	2340	.8	1	2190	.8	195	900	.7	14
7	♀	23	2250	.8	22	3250	1.0	150	680	.6	7
8	♀	66	740	.54	6	890	.87	62	1225	.7	15
9	♀	48	540	.4	2	670	.5	67	500	.4	11
10	♀	50	2370	.6	6	1920	.4	45	1300	.5	6
11	♂	55	2330	1.1	3	2130	1.3	19	1920	.9	7
12	♀	40	1075	.9	20	1260	.9	80	1670	1.1	20
13	♀	20	1400	.9	12	1260	.6	141	2400	1.0	39
14	♀	50	430	.5	2	500	.5	44	800	.8	15

Vol = Volume in ml. Creat. = Creatinine in g/24 hr. Xa. = Xanthurenic acid in mg/24 hr.

and xanthurenic acid excretion is greatly decreased or becomes normal(3). 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. Fourteen patients (13 females and 1 male) were studied from the medical services of the Hahnemann Med. College and Hospital and the Philadelphia General Hosp.† There was no selection other than contingent on established clinical criteria of thyrotoxicosis, such as weight loss, tachycardia, easy fatigability, thyroid enlargement and laboratory findings consistent with the disease, *i.e.*, increased PBI and I^{131} uptake, and elevated metabolic rate, and rapid I^{131} conversion (Table II).

Controls. Fourteen patients (9 females, 5 males) of similar age, free from hyperthyroidism, served as controls. Both groups of patients were instructed to consume a liberal regular house diet (average intake of 3000 calories); to our knowledge they were not taking any vitamin supplements, but were given 10 mg of Thiamine and 5 mg of Riboflavin daily during the study, since coenzymes containing these vitamins are known to be involved in tryptophan metabolism. Xanthurenic acid excretion was determined by a modified method of Rosen(4) on a 24 hr urine specimen. A control xanthurenic acid 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 hr urine collection obtained. On the morning of the third day, 50 mg of pyridoxine-HCl (U.S.P.) was administered intramuscularly 30 minutes before a second dose of 10 g of tryptophan was given, followed by the third and final 24 hr urine collection. Urine specimens were collected under toluence, and after the reaction was adjusted to pH 5.5 with 5 normal hydrochloric acid, aliquots were stored in amber glass bottles at 4°C. Accuracy of collections was checked by carrying out urinary creatinine determinations by the method of Folin(5). When a variation in creatinine of more than 25% was found, the patient was dropped from the study. Complete collections were deemed essential, since studies had revealed that the largest amounts of xanthurenic acid excretion appeared in the first 8 hours after oral ingestion of tryptophan. None of the patients taking tryptophan experienced any severe side reactions. No change in blood urea nitrogen 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.*

† We are indebted to Drs. Charles Shuman and Stephen Dubell, Temple Univ. Hospital, Philadelphia, Pa., for permission to include 2 hyperthyroid patients studied for pyridoxine deficiency. This work has been supported in part by a grant from HEW, Public Health Service.

TABLE II. Parameters of Thyroid Function.

Case No.	I^{131}, %	PBI	Conversion ratio, %	Basal metabolic rate
1	61	14	81	+46
2*	90	12	27	+30
3	58	12	52	+64
4	50	11	—	+47
5	95	21	89	+35
6	92	20	77	+60
7	84	15	—	+59
8	47	7	56	—
9	59	17	61	—
10	65	20	91	+59
11	84	—	91	+72
12	54	9	—	—
13	—	22	—	+41
14	47	8	61	—

Normal laboratory values: I^{131} uptake, 10 to 40%; protein-bound iodine (PBI), 3.5-7.5 γ %; I^{131} conversion ratio, 13 to 42%; basal metabolic rate, minus 10 to plus 10.

* Low conversion ratio in case 2 is due to cessation of propylthiouracid therapy 2 wk prior to I^{131} studies.

(6) in 6 patients, since the enzyme is known to be involved in pyridoxine metabolism. The significance of transaminase determinations as an index of pyridoxine deficiency will be described elsewhere jointly with transaminase results in cardiac failure(7). None of the present subjects showed evidence of heart failure, as determined by the usual clinical criteria.

Results. Tables I and III present results of xanthurenic acid excretion, creatinine, and volume of 24 hr urine collections on all subjects. Fourteen hyperthyroid 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. 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.

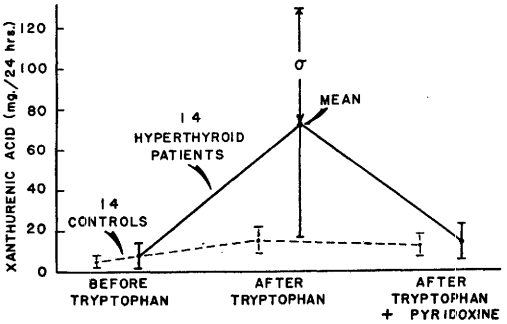

FIG. 1. Response of hyperthyroid and of control patients to tryptophan load and to tryptophan load after pyridoxine.

The hyperthyroid groups and control groups were quite comparable with respect to both mean and standard deviation in pre-tryptophan level of xanthurenic acid excre-

TABLE III. Non-thyroid Patients.

Case No.	Sex	Age	Control Vol	Control Creat.	Control Xa.	AT-1 Vol	AT-1 Creat.	AT-1 Xa.	AT-B6 Vol	AT-B6 Creat.	AT-B6 Xa.	Diagnosis
1	♀	32	3000	1.0	8	1750	.9	13	1350	.8	13	Rheumatic heart
2	♀	47	1100	1.1	1	1095	1.1	9	855	1.0	7	Melancholia
3	♂	19	900	.64	3	1740	1.71	10	1300	1.18	8	Fractured femur
4	♀	60	3100	1.5	5	3260	1.4	20	2600	1.4	15	Diabetes mellitus
5	♂	22	1400	1.59	4	570	1.14	8	1540	1.51	9	Anxiety neurosis
6	♂	34	910	1.2	1	1750	1.5	22	1340	1.0	11	Duodenal ulcer
7	♀	42	1200	.89	9	2350	1.09	27	2940	1.09	20	Hypochronic anemia, unknown etiology
8	♀	37	3085	1.8	9	1965	1.9	19	3010	1.8	20	Diabetes mellitus
9	♀	62	1985	1.5	8	2089	1.6	18	2100	1.6	18	Arteriosclerotic heart disease
10	♀	30	700	.86	9	1560	.93	14	1310	1.24	12	Hyperventilation syndrome
11	♂	25	1185	1.8	3	1745	1.4	6	680	1.1	1	Pulmonary osteoarthropathy
12	♀	26	830	1.0	1	810	1.1	18	560	1.1	16	Psychoneurosis
13	♀	60	2970	2.3	2	2210	1.3	11	3200	1.9	12	Diabetes mellitus
14	♂	44	1090	1.6	8	2550	1.5	20	1800	1.5	12	Chronic osteomyelitis

Vol = Volume in ml. Creat. = Creatinine in g/24 hr. Xa. = Xanthurenic acid in mg/24 hr.

tion with means of 7.3 and 5.1 mg/24 hr respectively. Following tryptophan load, each showed a highly significant mean increase over pre-tryptophan level (probability of "t" less than 0.001 in each case). The mean increase for hyperthyroid 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 (71.0 *vs.* only 15.4 mg/24 hr). In addition, the variance for the hyperthyroid group was considerable and significantly greater than that of the control group (standard deviation of 56.0 and 6.1 mg/24 hr respectively). This greater variance in the hyperthyroid group was to be expected because these patients differed greatly in severity of metabolic derangement, while the control group was more homogenous in that none suffered from hyperthyroidism. The highest level attained by a control patient was 27 mg/24 hr, while four-fifths of the hyperthyroid 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 17 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 hyperthyroid 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 (13.2 mg/24 hr for the hyperthyroid group and 12.5 mg/25 hr for the control group). They also showed comparable variances after pyridoxine (standard deviation of 8.7 and 5.2 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 (Fig. 1).

Discussion. The statistically significant high levels of xanthurenic acid in patients with hyperthyroidism and the improvement in derangement of tryptophan metabolism with a resultant return to decidedly lower levels of xanthurenic acid upon administration of pyridoxine, tend to indicate that the nutritional status of hyperthyroid patients with respect to pyridoxine is subnormal. The only reference in the literature in regard to tryptophan metabolism in hyperthyroidism is that by Wachstein and Lobel(8). In their series of 44 patients with various diseases, studied with the tryptophan load test, 3 patients with hyperthyroidism had 24 hr urine xanthurenic acid excretion of 84 mg, 33 mg and 31 mg respectively. After administration of a total of 50 mg pyridoxine to 2 patients and 100 mg pyridoxine to a third patient, xanthurenic acid excretion after tryptophan load was zero. The patient who originally excreted 84 mg xanthurenic acid had a third tryptophan load test, but no pyridoxine, 3 weeks later the results of the xanthurenic acid excretion were considerably higher than in the first test (128 mg). It is to be regretted that no information is given as to the patient's exact clinical status and thyroid function parameters at a time when xanthurenic acid excretion was the highest (128 mg). The question arises as to the mechanism responsible for the deranged tryptophan metabolism in hyperthyroidism. Calculated values of daily intake of B-6 in the subjects under our study were 2.0 to 2.5 mg.‡ Daily requirement for normal human adults is estimated to be from 1 to 2 mg (Nat. Research Council, 1958). It is apparent that an oral intake of B-6 considered adequate for a normal person is totally inadequate for a patient stressed with hyperthyroidism. An analagous situation with regard to thiamine exists in the disease(9). Another factor which may contribute to abnormal tryptophan metabolism may be failure of B-6 to be incorporated in the enzyme system required for normal tryptophan metabolism. However, when pyridoxine was ad-

‡ Values for B-6 were calculated from Tables of Richard W. Vilter: Wohl and Goodhard, *Modern Nutrition in Health and Disease*, 1960, p. 349, Lea and Febiger.

ministered intramuscularly to our patients, bio-chemical response to tryptophan was normal, tending to indicate that availability of B-6 was probably the limiting factor for normal tryptophan metabolism in our patients with hyperthyroidism. The patients in this study showed no overt clinical manifestations of pyridoxine deficiency. However, biochemical changes, particularly an abnormal tryptophan load test, become demonstrable before symptoms manifest themselves in experimentally induced human pyridoxine deficiency(10,14).

One of the patients with hyperthyroidism showed obvious signs of muscle weakness while under study; daily treatment with pyridoxine without other antithyroid therapy resulted in considerable gain in muscle strength. Based on Spies' work on the influence of pyridoxine intravenously on muscle weakness in pellagrins(12), Rosenberg *et al.* (11) administered B-6 to a group of patients who suffered extreme muscle weakness and exhaustion, in whom no other symptoms of B Complex deficiency were evident. Among this group were 3 patients with hyperthyroidism who received pyridoxine intravenously, with no anti-thyroid therapy. As a result of this procedure, they noted increased ability to perform work.

It is reasonable to infer that some symptoms, *e.g.*, muscle weakness in hyperthyroidism, may not be due directly to excessive activity of the thyroid hormone, but rather reflect a conditioned manifestation of pyridoxine deficiency, not unlike thiamine(9,13) and nicotinic acid deficiency(15). More extensive studies on the chemical changes at the cellular level of biopsied muscle from hyperthyroid patients may advance our knowledge of the causal relationship between pyridoxine deficiency and muscle weakness in hyperthyroidism.

Summary. The occurrence of pyridoxine deficiency was determined in 14 patients with hyperthyroidism and 14 control euthyroid patients by the tryptophan load test. Urinary xanthurenic acid excretion following the tryptophan load test was significantly greater in hyperthyroid patients than in controls. Mean excretion of xanthurenic acid in hyperthyroid cases was 71.9 mg/24 hr with standard deviation of 56.0. Mean xanthurenic acid excretion of control cases was 15.4 with standard deviation of 6.1. After pyridoxine administration, mean xanthurenic acid excretion was 13.2 with standard deviation of 8.7 in the hyperthyroid cases, while in the control group, xanthurenic acid excretion was 12.5 with standard deviation of 5.2. Since pyridoxine is required for orderly catabolism of tryptophan and pyridoxine corrected this metabolic aberration, it is not unreasonable to state that in hyperthyroidism, the availability of pyridoxine is limited. Until the actual significance of these findings becomes more clearly defined, it would seem reasonable to suggest that use of pyridoxine in addition to anti-thyroid medication may prove of distinct benefit in treatment of the hyperthyroid patients.

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