*Summary.* A procedure for preparation of follicle-stimulating hormone from human pituitaries has been described. Approximately a 15-fold purification could be achieved by a procedure involving fractionation with $(NH_4)_2SO_4$ and a cation exchange resin.

*Addendum:* FSH fraction SII has been tested for gonad-stimulating activities in human subjects by Dr D. M. Bergenstal of National Cancer Institute, Bethesda, Md. A female subject (age 32), one day after cessation of menstruation, was injected subcutaneously with 2.5 mg FSH concentrate every 6 hours for 6 days and followed by castration. Ovaries were highly stimulated with the appearance of multiple follicular cysts. Details of these clinical studies will be reported by Dr. Bergenstal elsewhere.

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Received June 19, 1958. P.S.E.B.M., 1958, v98.

## Association of Pyridoxine Deficiency and Convulsions in Alcoholics.*† (24203)

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Alcoholic withdrawal syndrome includes convulsions. It is generally believed that these seizures relate to idiopathic or post-traumatic epilepsy or alcohol withdrawal(1,2). As infants fed a formula deficient in Vit. $B_6$ (3) or adults with chemically induced Vit. $B_6$ lack (*i.e.*, administration of isoniazide(4) or desoxy-pyridoxine(5,6)) have occasional *grand mal* seizures, we wondered if seizures in alcoholics might not be associated with Vit. $B_6$ deficiency, particularly as their diets are grossly inadequate. The term "rum fit" has been used to describe these seizures occurring in the immediate 36- to 72-hour period after cessation of an alcoholic spree. Henceforth this nomenclature will be used to describe these patients. This study purports to determine if rum fitters may be Vit. $B_6$ deficient.

*Materials and methods.* Five rum fitters, 2 patients with alcoholism and associated epilepsy§, 6 patients in various phases of alcoholism but without fits, and 1 normal non-alcoholic individual were studied. The group consisted of 13 males and 1 female. All were seen immediately on admission to the hospital. They were given a Vit. $B_6$-free diet consisting of "Dexin"‖ in water mixture and daily vitamin supplement excluding Vit. $B_6$.¶ An initial urine was collected. Following this, the patients were fed 10 g dl-tryptophane mixed with Dexin in water, either orally or by gas-

* The authors wish to thank the house officers of the Harvard Medical Services at the Boston City Hospital for their cooperation in this study.

† This work was supported by grant from Nutrition Fn., N. Y., the Office of Surgeon General, Dept. of Army, and Merck Sharp & Dohme, Rahway, N. J., to Harvard University.

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§ These patients also had convulsions when not drinking and when their diets were good.

‖ A partial starch hydrolysate, Burroughs-Wellcome Co., Tuckahoe, N. Y.

¶ The daily vitamin supplement consisted of: thiamine 50 mg (i.m.) and 10 mg riboflavin, 100 mg niacin, and 100 mg ascorbic acid (p.o.).

TABLE I. Xanthurenic Acid Excretion before and after Pyridoxine.

| Diagnosis | Patient | Xanthurenic acid (mg/24 hr) after 10 g dl-tryptophane: No $B_6$ | After 100 mg pyridoxine HCl |
|---|---|---|---|
| Phases of alcoholism | | | |
| Acute and chronic ethanolism | 1* | 0 | 0 |
| Acute hallucinosis-tremulousness | 2* | 24.0 | 0 |
| Acute peripheral neuropathy | 3 | 0 | 0 |
| Wernicke-Korsakoff syndrome | 4* | 0 | 0 |
| | 5 | 55.0 | 0 |
| | 6* | 51.0 | 0 |
| Alcoholism with associated epilepsy | 7† | 0 | 0 |
| | 8 | 44.0 | 0 |
| Rum fitters | 9* | 110.0 | 0 |
| | 10† | 146.0 | 16.5 |
| | 11† | 158.0 | 20.0 |
| | 12† | 38.0 | 0 |
| | 13 | 0 | 0 |
| Normal non-alcoholic healthy | 14 | 0 | 0 |

* These patients in addition had the classical stigmata of cirrhosis.

† These patients exhibited cirrhosis + delirium tremens.

tric tube, and started on a complete 24-hour urine collection. Then 100 mg pyridoxine hydrochloride was injected intramuscularly and the study repeated for a second 24 hours. The volumes of these two 24-hour urine collections were measured and an aliquot saved, either refrigerated (20°C) or frozen −4°C) until used. Electroencephalograms (EEG) were obtained in 5 of the 7 patients with *grand mal* seizures. Patients were allowed physiologic saline, hypotonic glucose, dilantin, thorzine, phenobarbital, or antibiotics as needed. No patient had known active tuberculosis. None was given isoniazide or iproniazide(4). Xanthurenic acid (XA) excretion for each of the 2 tryptophane load tests was measured(7). This method depends upon the reaction of XA with ferric ammonium sulfate in a "tris" buffer. The green color produced is proportional to the XA and is measured in the Coleman Junior spectrophotometer at 610 $\mu$. The pre-tryptophane urine sample from each patient was used as an internal colorimetric control.

*Results.* The Table records urinary XA of each patient and shows that the highest excretions occurred in rum fitters; lower values appear in the other 2 categories: alcoholism with associated epilepsy, and phases of alcoholism. After 100 mg pyridoxine intramuscularly, there was a marked decrease in XA excretions. The data are averaged and represented graphically. By comparison of average values for the 3 groups, the graph shows 4 times the XA excretion in rum fitters in the first tryptophane load test. The other 2 groups are similar.

XA excretion after a tryptophane load is accepted as the standard method for demonstrating Vit. $B_6$ deficiency in man(8). Rum fitters show the highest correlation with this evidence of Vit. $B_6$ lack. XA, where demonstrated, was in every case diminished by Vit. $B_6$. The small urine XA in non-rum fitters may well represent lesser degrees of Vit. $B_6$ shortage in sub-convulsive levels, as the dietary intake of these patients was also poor.

All values were calculated by the same standard curve at the same time. The oldest urine was 5 months old when its recorded value was obtained. XA varied somewhat with the standard curve, each set of reagents prepared, and to decrease in absolute value with storage. For example, levels of 600 mg and 225 mg XA in patients 10 and 11, respectively, were obtained when fresh urine was available. This would account for values being somewhat lower than those previously

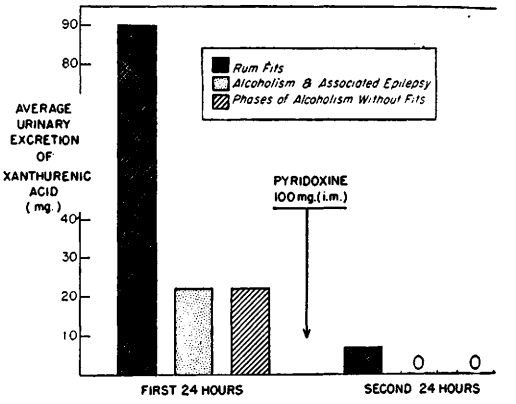


FIG. 1. Urinary excretion of xanthurenic acid after 10 g dl-tryptophane given at beginning of each 24-hr period. The first period is before and the second after administration of 100 $\mu$g pyridoxine.

reported in experimental Vit. $B_6$ deprivation in man(9).

The failure to demonstrate increased XA in urine after tryptophane loading does not exclude lack of pyridoxine group of vitamins. Another metabolite of tryptophane metabolism, such as 3-hydroxykynurenine, acetyl kynurenine or kynurenine, may have been the accumulated products in these cases(10). It is possible, as suggested by Roberts and Baxter(11), that the ultimate compound deficient in Vit. $B_6$ shortage is gamma amino butyric acid (GABA) which is formed by decarboxylation of glutamic acid, a reaction activated by pyridoxal-5-phosphate as coenzyme.

Three of the 5 rum fitters subsequently recovered through a stormy course with associated delirium tremens. One rum fitter presented as *status epilepticus*. The others had 1-2 isolated seizures. EEG's were normal in patients with rum fits. One of the 2 patients with alcoholism and associated epilepsy had an abnormal EEG (No. 7). All rum fitters showed multiple signs suggestive of deficiency of members of the Vit. B complex group of vitamins. the most common was depapillation and reddening of tongue, diffusely reddened oral mucous membranes, and injected conjunctivae. None showed evidence of scurvy.

Convulsions in alcoholics have been related to alcohol withdrawal and to idiopathic or post-traumatic epilepsy. It has been possible to separate a group of these patients who exhibited Vit. $B_6$ deficiency and who had no evidence of previous epilepsy, or of abnormal EEG's.

*Summary.* 1. Increased xanthurenic acid excretion after 10 g dl-tryptophane was demonstrated in 3 of 5 patients with rum fits. This metabolic defect was corrected by Vit. $B_6$ in a second tryptophane load test. 2. Patients with alcoholism and associated epilepsy, acute and chronic alcoholism, cirrhosis, acute hallucinosis-tremulousness, acute peripheral neuropathy, Wernicke-Korsakoff syndrome, and a non-alcoholic, healthy individual did not demonstrate significant Vit. $B_6$ deficiency by this test. 3. It is postulated that Vit. $B_6$ deficiency is etiologically related to rum fits.

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Received June 20, 1958. P.S.E.B.M., 1958, v98.

## Experimental Lathyrism in the Rat. Nature of Defect in Epiphysial Cartilage. (24204)

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Profound alterations have been described in the cartilages, bones and aortas of animals fed the seeds of *lathyrus odoratus* or treated with certain chemical compounds such as beta-aminopropinonitrile (BAPN), aminoacetonitrile (AAN)(1), and most recently semicarbazide(2). No agreement has been reached concerning the basic metabolic defect