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Pyruvic Acid in Blood and Cerebrospinal Fluid.*

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It has been shown that cocarboxylase (the diphosphoric ester of vitamin B_1) is necessary for the normal catabolism of pyruvic acid.[1] In vitamin B_1 deficiency (Oriental Beri-beri) pyruvic acid accumulates in the body fluids.[2] Methods previously used in the determination are not satisfactory since a significant decrease in blood pyruvate occurs if the blood be allowed to stand at room temperature for even one minute prior to precipitation. This disappearance does not occur if monoiodoacetate in a concentration of 0.2% is used as a stabilizing medium. Utilizing this finding, we have recently described a method for the stabilization and determination of pyruvic acid in the blood.[3]

The present study was undertaken in order (1) to determine the degree of stability of pyruvic acid in the spinal fluid, (2) to compare the levels of pyruvic acid in the blood and cerebrospinal fluid, (3) to make certain preliminary observations concerning the value of pyruvic acid determinations for the diagnosis of vitamin B_1 deficiency.

Method. The method for blood determinations was also used for the spinal fluid, except that the stabilizing medium (monoiodoacetate) was found to be unnecessary.

Stability of Pyruvic Acid in the Spinal Fluid. In contrast to our findings in the blood, the pyruvic acid content of the spinal fluid remains constant even if the sample be allowed to stand at room temperature for one hour prior to precipitation. Additional evidence of the stability of pyruvic acid in the spinal fluid was obtained in the following way: Five cc samples of cerebrospinal fluid were caught in test tubes containing 25 mg of sodium monoiodoacetate, and other samples of the same spinal fluid were allowed to stand at room temperature for 60 minutes prior to precipitation without the use of the

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1 Banga, I. L., Ochoa, S., and Peters, R. A., *Biochem. J.*, 1939, **33**, 1109.

2 Platt, B. S., and Lu, G. D., *Quart. J. Med.*, 1936, **5**, 355.

3 Bueding, E., and Wortis, H., *J. Biol. Chem.*, 1940, **133**, 585.

TABLE I.
Stability of Pyruvate in the Spinal Fluid.

Spinal fluid sample No.	Precipitated after 3 min mg% pyruvic acid	25 mg $CH_2ICOONa$ added. Precipitated after 3 min mg% pyruvic acid	No $CH_2ICOONa$ added. Precipitated after 60 min mg% pyruvic acid
1	0.90	—	0.88
2	0.93	—	0.93
3	—	0.86	0.86
4	—	1.04	1.05
5	—	0.83	0.83
6	1.12	1.09	1.10
7	0.78	—	0.79
8*	—	2.22	2.18
9	—	2.40	2.31

*8 and 9 were cases of pneumococcus meningitis.

stabilizing medium. Identical values were obtained in both samples (Table I).

Pyruvic Acid in the Blood and Cerebrospinal Fluid. Simultaneous samples of blood and cerebrospinal fluid were obtained on 67 patients with various neuropsychiatric and medical disorders. The subjects were fasting and at rest in bed. All determinations were done in duplicate.

The content of blood pyruvic acid in 60 normal subjects was previously[3] found to vary from 0.77-1.16 mg %. We have, therefore, arbitrarily decided to consider as abnormally high those cases with blood pyruvate levels of 1.30 mg % or above.

Considered in this fashion, 51 of our 67 cases had normal values for pyruvic acid in the blood, and 16 of our cases showed elevated figures. The relationship of spinal fluid pyruvate to that of the blood in these 2 groups is seen in Table II.

Preliminary Observations Concerning the Value of Pyruvic Acid Determinations for the Diagnosis of Vitamin B_1 Deficiency. The 51 cases with normal blood levels formed the following diagnostically labelled groups: (a) chronic alcoholism without clinical evidence of vitamin B_1 deficiency (peripheral neuropathy or beri-beri), 15 cases;

TABLE II.
Relationship of Blood to Spinal Fluid Pyruvate.

Group	No. of cases	Mg% pyruvic acid — Blood	C.S.F.	% C.S.F./ blood
1	51	0.79-1.30 (Avg 1.03)	0.42-1.52 (Avg 0.84)	43-118* (Avg 82)
2	16	1.41-2.41 (Avg 1.82)	1.03-2.40 (Avg 1.77)	67-170* (Avg 97)

* In only 6 cases (2 in Group 1 and 4 in Group 2) did the % C.S.F./Blood fall outside the range 70-120%.

(b) Schizophrenia, 10 cases; (c) behavior problems in children, 6 cases; (d) mental deficiency in children, 5 cases; (e) pneumonia, 5 cases; (f) psychopathic personality, 2 cases; (g) hyperthyroidism, 2 cases; (h) senile psychosis, 2 cases; (i) one case each of arsenical poisoning, reactive depression, paroxysmal convulsive disorder of unknown etiology and herpes zoster.

The 16 cases with high blood levels formed these diagnostically labelled groups: (a) chronic alcoholism with acute peripheral neuropathy, 12 cases; (b) beri-beri, 1 case; (c) pneumococcus meningitis, 2 cases; (d) pneumonia with prolonged temperature elevation, 1 case.

Of the 16 cases with elevated pyruvic acid levels, it is noteworthy that 13 (12 of acute peripheral neuropathy and one of beri-beri) occurred in clinical syndromes which are known to be the result of vitamin B_1 deficiency. As a matter of fact, Platt and Lu have previously described elevations of blood pyruvate in cases of fulminating beri-beri.[2, 4] We have criticized the limitations of their method in previous communications.[3, 5] In the 3 remaining cases with elevated blood pyruvate, the increase in total metabolism, as a result of prolonged fever with resultant depletion of vitamin B_1, may have contributed to the high values obtained. It should, however, be particularly noted that none of these cases showed evidences of acute peripheral neuropathy. It may be that the metabolic disturbance must exist for some time before clinical evidences of vitamin B_1 deficiency are apparent. In addition, further work may indicate that elevations in blood pyruvate are related to conditions other than avitaminosis B_1.

Nonetheless, our results take on added interest if we reëxamine our cases of chronic alcoholism without clinical evidences of vitamin B_1 deficiency. These 15 cases are subdivided as follows: (a) chronic alcoholism with old or treated peripheral neuropathy, 8 cases; (b) chronic alcoholism without evidences of involvement of the central or peripheral nervous systems, 4 cases; (c) chronic alcoholism with nicotinic acid deficiency, 2 cases; (d) chronic alcoholism with brain laceration, 1 case. The pyruvic acid levels were normal in every case. On the other hand, our cases of chronic alcoholism with clinical evidences of vitamin B_1 deficiency showed an elevation of blood pyruvate in every instance.

Summary. 1. The stability of pyruvic acid in the spinal fluid is

4 Lu, G. D., *Biochem. J.*, 1939, **33**, 774.

5 Wortis, H., Bueding, E., and Wilson, W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 279.

described. 2. The relationship of blood to spinal fluid pyruvate is reported. The amount found in the cerebrospinal fluid is usually 70-120% of that found in a blood sample taken simultaneously. 3. Of the 16 cases with elevated blood pyruvate, 13 occurred in cases of known vitamin B_1 deficiency. These latter constituted the only cases in the entire study with definite clinical evidences of vitamin B_1 deficiency. In the other 3, it is suggested that a relative deficiency of vitamin B_1 may have existed. In 51 additional cases, without clinical evidence of vitamin B_1 deficiency, the blood pyruvate was normal in every instance.

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A New Type of Vitamin K-Deficient Diets.

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Heretofore the vitamin K-deficient Diet E of Almquist and Stokstad,[1] Ration K-1 of Ansbacher,[2] Diet 508 of Dam and co-workers,[3] or modifications thereof, have been used by the majority of investigators[1-7] in assays, in which chicks served as test animals. Since putrified fish meal is an excellent source of one of the natural antihemorrhagic vitamins, obstacles are frequently encountered in the employ of fish meal diets even under conditions tending to minimize bacterial action.

Recently we found that the difficulties arising from K-vitamin synthesis are not experienced when diets are used which contain neither fish meal nor yeast, and in which vitamin K had been destroyed by prolonged heat treatment. We are now making vitamin K assays with Ration K-7 outlined in the accompanying table.

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1 Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1936, **12**, 329.

2 Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

3 Dam, H., Glavind, J., and Karrer, P., *Helv. Chim. Acta.*, 1940, **23**, 224.

4 Dann, F. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 663.

5 MacFie, J. M., Bacharach, A. L., and Chance, M. R. A., *Brit. Med. J.*, Dec. 23, 1939, 1220.

6 Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 194.

7 Tidrick, R. T., Joyce, F. T., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 853.