

TABLE 1
COMPARISON OF NIACIN AND PANTOTHENIC ACID
CONTENT IN CEREALS

Cereal		Niacin	Pantothenic acid
Species	Variety	(γ /g)	(γ /g)
Corn	M20	19.5	6.4
	K24	25.8	6.5
Wheat	Seneca	46.0	8.53
	Trumbull	50.4	10.6
	Butler	48.8	10.1
	Thorne	46.8	10.8
Oats	Clinton	12.3	15.27
	Wayne	15.8	16.7
Soybeans	Hawkeye	26.6	20.4
	Lincoln	22.8	29.4

The results show that there is a wide variation among the four cereals studied with respect to the niacin and pantothenic acid content, the differences being greater with niacin than with pantothenic acid. Among the varieties within the species the niacin content showed a wide variation in the case of corn, oats, and soybeans, but very little in the case of wheat. Soybean was the only cereal that showed any marked variation of pantothenic acid.

References

1. RICHEY, F. D., and DAWSON, R. F. *Plant Physiol.*, **23**, 238 (1948).
2. BURKHOLDER, P. R., MCVEIGH, I., and MOYER, D. *Yale J. Biol. Med.*, **16**, 659 (1944).
3. DITZLER, L., HUNT, C. H., and BETHKE, R. M. *Cereal Chem.*, **25**, 273 (1948).
4. HUNT, C. H., RODRIGUEZ, L. D., and BETHKE, R. M. *Ibid.*, **27**, 150 (1950).
5. Assoc. Vitamin Chemists. *Methods of Vitamin Assay*. New York: Interscience Pub. (1947).
6. SNELL, E. E., and WRIGHT, L. D. *J. Biol. Chem.*, **139**, 675 (1941).

On the Cleavage and Oxidation of Pteroylglutamic Acid by Enzyme Solution

Katashi Makino and Kenjiro Yamamoto¹

Department of Biochemistry,
Kumamoto Medical College, Kumamoto, Japan

In spite of many reports which showed that pteroylglutamic acid (PGA) had been liberated from its conjugated forms by conjugase from different sources, there is almost no information about the enzymic cleavage of PGA itself. In our experiments we proved that PGA is split by liver enzyme solution.

Preparation of the enzyme solution: 100 g fresh cattle or rabbit liver was ground with 100 ml water, allowed to stand at room temperature for 2 hr, and then centrifuged. The supernatant was dialyzed through celloidion membrane in running water. All experiments were carried out at 37° C, and the pH of the solution was kept at 7.5 with phosphate buffer. For the test the methylene blue technique was used.

¹ We are greatly indebted to S. Kuwada, director of the Takeda Research Laboratory, for a gift of PGA.

Expt. 1. The following system was taken: 0.1 ml of the cattle liver enzyme solution, 0.1 ml of *M*/15 phosphate buffer, 0.2 ml of *M*/5,000 solution of methylene blue, and 0.1 ml of *M*/200 solution of substrates.

Tube No.	Substrates	Time of reduction
1	PGA	49 min
2	<i>p</i> -Aminobenzoylglutamic acid	38 "
3	L-glutamic acid	26 "
4	Control	6 hr

Expt. 2. The following system was taken: 0.1 ml of the rabbit liver enzyme solution and other components as in expt. 1.

Tube No.	Substrates	Time of reduction
1	PGA	19 min
2	<i>p</i> -Aminobenzoylglutamic acid	17 "
3	L-glutamic acid	17 min, 50 sec
4	Control	7 hr

Expts. 1 and 2 show that PGA can reduce the methylene blue by the liver enzyme solution in the same manner as *p*-aminobenzoylglutamic acid and L-glutamic acid, and the oxidation of the former is little slower than the latter two. The similar result has been confirmed through several repeated experiments. It thus seems more reasonable to think that PGA is hydrolyzed to the pteric acid and the glutamic acid, the latter of which reduces the methylene blue with the glutamic acid dehydrogenase, than to think that PGA itself is oxidized. The assumption seems likely from the fact that the *p*-aminobenzoylglutamic acid has reduced the methylene blue at almost the same rate as that of PGA under similar conditions. But in our experiment there was no detectable *p*-aminobenzoylglutamic acid or *p*-aminobenzoic acid liberated because even the most sensitive diazo color test upon *p*-aminobenzoic acid gave negative results in the reaction mixture.

It must be added that when the dialysis of the enzyme solution is not sufficient and some donators remain as purines, it often happens that the system added with PGA as substrate is more slowly reduced than the control. As PGA has strong inhibitory action upon the xanthine oxidase in milk (1), it seems likely in this case, too, that PGA has inhibited the oxidation of the purines remaining on account of the unsatisfactory dialysis of the liver extract.

Xanthine oxidase and xanthopterin oxidase exist in milk and liver, but it would seem that no enzyme exists to oxidize directly the pteridine nucleus of the intact PGA or pteric acid. This assumption was confirmed by reduction of methylene blue with the pteric acid by means of the liver enzyme. The proof of glutamic acid (formol titration and ninhydrin test) which may be liberated by the cleavage of PGA with the liver enzyme, is now under investigation.

Reference

1. KALCKAR, H. M., and KLENOW, H. J. *Biol. Chem.*, **172**, 349 (1948).