250-ml Erlenmeyer flask with 50 ml of glacial acetic acid. The mixture was brought to a boil, and a few drops of an indicator (0.1% in benzene) was added. The solution was then titrated with a standardized perchloric acid-acetic acid solution (0.04–0.2 *M*), the solution being reheated near the end point to sharpen the indicator change. Where relatively insoluble salts —e.g., sodium chloride—were used, it was necessary to bring them gradually into solution by repeated heating and titrating.

In Table 1 are listed results obtained by titrating both procaine and procaine hydrochloride. The presence of the acidic group in the latter compound appeared to have no significant influence on the diacidic character of the original base.

In Table 2 the data obtained by titration of sodium, potassium, and ammonium chloride are presented. The titration of sodium chloride proved to be difficult because of its poor solubility in the solvent. In all cases, however, the salts behaved as monoacidic bases.

The observation that the titration end point faded on heating and that the stoichiometric end point was achieved best by bringing the solutions under titration to a boil appeared to indicate that the part of the apparent basicity of the chloride ion was due to the volatility of the acid formed; that is,

$$\mathrm{Cl^- + CH_3CO_2H_2^+ \rightleftharpoons \overset{\uparrow}{\underset{\mathrm{HCl\ (gas)}}{\mathrm{HCl}}} + CH_3CO_2H.}$$

The equilibrium in the above reaction appears to favor the right-hand side, permitting partial titration of the ion without loss of hydrogen chloride from the system. Near the end point, however, the reverse reaction becomes appreciable unless the gas is driven off by boiling.

This hypothesis was partly verified by applying the titration procedure to potassium bromide. In this case the indicator change occurred with only a slight addition of perchloric acid solution; however, it was possible to approach the stoichiometric end point by repeated boiling of the solution, the indicator reverting to the nonacid color after each heating up to the end point. Results obtained in this fashion are presented in Table 3.

TABLE 3

ACIDIMETRIC TITRATION OF POTASSIUM BROMIDE IN GLACIAL ACETIC ACID

Sample	Equiv/mole found	Indicator
Potassium bromide	0.969	α-Naphtholbenzein
	.973	
	.973	
	.984	
	0.976	

Since HBr is a much stronger acid than HCl, the former would be expected to be more highly ionized in acetic acid than the latter. This would explain the initial indicator change observed on addition of a small amount of the perchloric acid solution to potassium bromide. Because of the volatility of HBr, however, the indicator can be driven back to the basic form, permitting the titration to be carried through to the stoichiometric end point.

These observations have been further verified by results obtained by N. F. Hall and co-workers (*2*), using potentiometric means to follow the relative acidity of the acetic acid solutions.

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A Comparison of the Niacin and Pantothenic Acid Content of Certain Cereal Grains Grown under the Same Environmental Conditions

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For many years the emphasis in food production has been on higher yields, and the introduction of hybrid varieties of cereals has resulted in increased production per acre. Within recent years feeding experiments have shown that there is considerable variation in some of the quality factors—such as vitamins—among the different hybrids of any one cereal. Breeding programs have been undertaken to develop high-quality cereals. The work of Richey and Dawson (*1*), Burkholder (*2*), and Ditzler *et al.* (*3*) tends to show that the genetic factor may be the major influence in determining the niacin content of corn, and Hunt *et al.* (*4*) showed that the niacin content of corn is less subject to modification by environmental conditions than is pantothenic acid.

This report presents the data obtained with respect to the niacin and pantothenic acid content of several varieties of each of four different cereals grown under the same environmental conditions.

The cereals analyzed were two double-cross hybrids of corn (*Zea mays* L.), M20 and K24; four varieties of wheat (*Triticum aestivum* L.), Seneca, Trumbull, Butler and Thorne; two varieties of oats (*Avena sativa* L.), Clinton and Wayne; two varieties of soybeans (*Glycine soja* L.), Hawkeye and Lincoln. All are new, but well-established varieties and are used extensively in Ohio. They were all grown the same season on Wooster silt loam at the Ohio Agricultural Experiment Station on plots that were in a high state of fertility. Each sample of grain was ground in a Wiley mill through a 60-mesh sieve.

The niacin and pantothenic acid content was determined by the microbiological method as outlined in *Methods of Vitamin Assay* (*5*), based on the original method of Snell and Wright (*6*). The organism employed was *Lactobacillus arabinosus* 17-5. The results of the analysis are summarized in Table 1.

TABLE 1

COMPARISON OF NIACIN AND PANTOTHENIC ACID CONTENT IN CEREALS

Cereal		Niacin (γ/g)	Pantothenic acid (γ/g)
Species	Variety		
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.

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On the Cleavage and Oxidation of Pteroylglutamic Acid by Enzyme Solution

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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 collodion 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	*p*-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	*p*-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 pteroic 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 pteroic acid. This assumption was confirmed by reduction of methylene blue with the pteroic 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.

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