Vitamin B_6 Components in Some Meats, Fish, Dairy Products, and Commercial Infant Formulas

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Pyridoxine, pyridoxal, pyridoxamine, and total unchromatographed vitamin B_6 values for meats, fish, dairy products, and commercial infant formulas were determined microbiologically using *S. carlsbergensis*. The hydrolyzed food extracts were chromatographically separated using a Dowex 50 resin column prior to microbiological assay. The vitamin B_6 values as assayed ranged for meats from 3 to 8 μg. B_6/gram, fish from 0.5 to 4 μg. B_6/gram, dairy products from 0.01 to 4 μg. B_6/gram, and commercial infant formulas from 0.4 to 4 μg. B_6/gram.

Vitamin B_6 is known to be widely distributed in plant and animal tissues, yet reliable quantitative data on the amounts contained in some foods are not extensive or may be entirely lacking. Even less information concerning the distribution of pyridoxine, pyridoxal, and pyridoxamine in natural materials is available.

Rabinowitz and Snell (1948) reported values for the individual forms of vitamin B_6 for a very limited number of natural products. Polansky *et al.* (1964) reported values for the three B_6 components in grains and cereal products, in fruits and nuts (Polansky and Murphy, 1966), and in fresh and dried vegetables (Polansky, 1969). There are several papers giving the total vitamin B_6 content of a number of foods (Burger *et al.*, 1956; Hardinge and Crooks, 1961; and Lieck, 1958).

No one assay procedure has been satisfactory for all three B_6 forms, although the growth response of a yeast *Saccharomyces carlsbergensis* has been used widely (Woodring, 1960) to measure total vitamin B_6 following the procedure of Atkin *et al.* (1943) or some modification of it. Procedures for separating and determining the B_6 components in hydrolyzed food extracts are important and essential developments for the vitamin B_6 assay. Methods have been described for using Dowex 50 ion exchange columns to separate the vitamin B_6 components of hydrolyzed food extracts into individual fractions and assaying the separated components with *S. carlsbergensis* (MacArthur and Lehmann, 1959; Polansky *et al.*, 1964; Toepfer and Lehmann, 1961). Data obtained by these procedures compare well with those obtained by rat bioassay for total vitamin B_6 in a few selected food samples (Toepfer *et al.*, 1963).

Vitamin B_6 occurs in most natural materials in both the free and bound form; and therefore, is unavailable to microorganisms unless liberated by acid hydrolysis. The most effective single extraction procedure was to autoclave the samples with 0.055*N* HCl or H_2SO_4 for 1 to 5 hours (Atkin *et al.*, 1943; Rabinowitz and Snell, 1947; Rubin *et al.*, 1947); this was reported to be much more effective for animal products than extraction with higher acid concentrations.

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This paper presents values for pyridoxine, pyridoxal, and pyridoxamine determined in chromatographed fractions of extracts from forty meat, fish, dairy product, and commercial infant formula samples. Total vitamin B_6 values in unchromatographed extracts, determined microbiologically and calculated as pyridoxine, are also reported. Supporting proximate composition data are included to make it possible to relate these vitamin B_6 data with data from other sources on similar samples.

EXPERIMENTAL

Samples. Food samples were purchased on the Washington, D. C., area market except for fresh eggs which came from the Poultry Research Branch, Agricultural Research Service, U. S. Department of Agriculture. In most cases, purchases were made at several stores and/or several brands of an item were obtained. Edible portions of all items were prepared and separable fat was removed from the lean for all meat samples. The laboratory sample used for analysis was prepared by combining each item on an equal weight basis. Eight subsamples were sealed in sanitary enameled tin cans and stored at −40° F until analyzed. Vitamin B_6 and proximate composition analyses were made on separate subsamples.

Procedures. For each analysis, a freshly opened subsample of food was used. For meats, fish, and cheese, the subsample, while still frozen, was put through a home electric food chopper with a plate having openings of $^3/_{16}$ inch. From 3 to 15 grams of the finely ground, unthawed food, 3 to 40 grams of the thawed liquid sample, or 1 gram of the finely divided dry sample were extracted. All samples were extracted in 200 ml. 0.055*N* HCl by autoclaving at 15 pounds steam for 5 hours. The extract was cooled to room temperature, adjusted to pH 4.5, diluted to 250-ml. volume, and filtered through Whatman No. 40 filter paper.

The procedures for the chromatographic separation and the microbiological assay were previously reported (Toepfer and Lehmann, 1961). A slight change has been made in the preparation of the Dowex AG 50W-X8 resin used in the chromatographic separation and this change has also been previously reported (Polansky *et al.*, 1964).

Total solids, nitrogen, and ash were determined by procedures of the Association of Official Agricultural Chemists (Association of Official Analytical Chemists) (1965), modified as previously described (Polansky *et al.*, 1964). Fat was also determined by official AOAC methods: meats and fish by continuous extraction of dried samples with petroleum ether in a Soxhlet apparatus for 16 hours, fresh and dried eggs by acid-hydrolysis, fluid milk products and dry milk by the appropriate Roese-Gottlieb procedure involving hydrolysis with ammonium hydroxide, and cheese by the Roese-Gottlieb method of digestion with ammonium hydroxide followed by acid hydrolysis and ether extraction.

RESULTS AND DISCUSSION

The superiority of low concentrations of acid over high concentrations for extraction of vitamin B_6 as reported by Rabinowitz and Snell (1947) and Rubin *et al.* (1947) was also observed by this laboratory for animal products. These articles reported that autoclaving at 20 pounds steam pressure gave the best extraction, but preliminary studies in this laboratory showed that 15 pounds steam pressure gave as good or better extraction of B_6 in animal products.

Extraction of meat products with 0.44*N* HCl [the acid concentration used for routine extraction of plant products in this laboratory (Polansky *et al.*, 1964)] instead of 0.055*N* HCl gave approximately the same pyridoxine and pyridoxal values, but only about half of the pyridoxamine. Considerably more day to day variation was observed in the pyridoxal and pyridoxamine values for meat products than from any other food products analyzed. Rubin *et al.* (1947) reported that the optimum pH for extraction of B_6 was 1.7 to 1.8. He also said, "Maximal values are obtained with *S. carlsbergensis* only under strictly controlled and limited experimental conditions. Variations in assays with this organism occur largely as a result of variations in extraction of vitamin B_6 from biological materials, owning to the sharply defined conditions of pH, extraction ratio, time, and pressure required for maximal liberation." It is possible that our pyridoxal and pyridoxamine irregularity in meats is due to some extraction problem of meat that is different from other food products.

Egg whites are very low in vitamin B_6. This made it necessary to use a large amount of sample for extraction, thus requiring additional acid to obtain the optimum pH of the extraction medium. The hypoallergenic commercial infant formulas were hydrolyzed with both 0.055*N* HCl and 0.44*N* HCl, since it was a milk type of food, but not an animal product. The results were comparable so the 0.055*N* HCl extraction values were used for this paper.

The values for the vitamin B_6 components of meats, fish, dairy products, and commercial infant formulas are given in Table I. Vitamin B_6 values are averages from at least three independently run assays. Proximate composition data for these samples, included in Table I, are averages of triplicate determinations in at least two separate assay periods.

The total unchromatographed vitamin B_6 values are also given in Table I. The chromatographed total vitamin B_6 values for the animal products were statistically higher than the unchromatographed values as shown by an analysis of variance. This was not surprising as a relatively high per cent of the vitamin B_6 in these products was pyridoxamine. Pyridoxamine gave less growth response to *S. carlsbergensis* than the other two forms of B_6; therefore, when the components are chromatographed and analyzed separately, then totaled, the value should be higher than when all three forms are analyzed together and calculated against a pyridoxine curve. The total of the three components gave a true B_6 value as compared to the value obtained without chromatographic separation. A previous report (Toepfer *et al.*, 1963) showed agreement between values obtained by chromatography and by rat bioassay.

Pyridoxal and pyridoxamine were the predominant forms of vitamin B_6 in hydrolyzed extracts of animal products with only small amounts of pyridoxine being present. Of the animal products analyzed, only four had more than 10% of their B_6 as pyridoxine: pork loin, 14%; cottage cheese, 24%; evaporated milk, 25%; and egg whites, 50%. Four out of the five commercial infant formulas contained predominantly pyridoxine because they had added pyridoxine hydrochloride and/or were made up of non-animal products. There is an indication that any product that is fully cooked or canned contains at least 70% pyridoxamine, while the original product contained less pyridoxamine and more pyridoxal. During the heating process, transamination must occur and pyridoxal is converted to pyridoxamine. This was also found in other studies made in this laboratory.

The meat products analyzed ranged from 3 to 8 μg. of vitamin B_6 per gram. It is not possible to make direct comparisons among cuts as the samples were not obtained so as to make these comparisons. Beef liver contains the most B_6, with chicken breast muscle coming next and then chicken livers. Both the liver samples contained 70% of their B_6 as pyridoxamine. The chicken breast and leg and pork loin contain a higher per cent of their B_6 as pyridoxal than as pyridoxamine. The beef and leg of lamb contain a slightly higher per cent as pyridoxamine than as pyridoxal.

The fish samples ranged from 0.5 μg. of B_6/gram for fresh oysters to 4 μg. B_6/gram for canned tuna. The liquid part of the oyster sample contained about 1/3 as much B_6 as the muscle, while the liquid in the canned salmon and tuna samples contained a little more B_6 than the muscle. The oysters and the shrimp contained a slightly higher portion of pyridoxamine than pyridoxal while flounder fillets contained 70% pyridoxal. The canned fish was high in pyridoxamine as discussed earlier.

The dairy products and eggs as analyzed ranged from 0.01 μg. vitamin B_6/gram for egg whites to 4 μg. B_6/gram for whole dried egg. Cottage cheese had only 0.17 μg. B_6/gram and blue cheese with 1.29 μg. B_6/gram was the highest of cheese samples. All cheese except cottage contained their B_6 predominantly as pyridoxamine. Egg white was extremely low in B_6; egg yolk contained nearly 3 μg./gram, but by the time the white was mixed with it to make a whole egg there was only about 1 μg. B_6/gram. Whole egg had 44% of its vitamin B_6 as pyridoxal and 54% as pyridoxamine, while 90% of B_6 in whole dried egg was pyridoxal. Fluid milk samples ranged from 0.3 μg. B_6/gram to 0.5 μg. B_6/gram with nonfat dry milk about 10 times higher. Milk contained predominantly pyridoxal, while buttermilk and evaporated milk contained mostly pyridoxamine.

There was considerable variation in the amount of B_6 present in the commercial infant formulas even when considered on a reliquefied or diluted basis. The vitamin B_6 content of these products as assayed ranged from 0.4 to 4 μg. B_6/gram. The modified nonfat liquid cows milk formula, even with added pyridoxine hydrochloride, had only about half as much B_6 as whole milk. The only infant formula that was an animal product and had no added B_6 contained

Table I. Values for Vitamin B_6 Components and Proximate Composition of Some Meats, Fish, Dairy Products, and Commercial Infant Formulas

Sample Description	Proximate Composition Values, %				Vitamin B_6 Values, µg./gram				
						Chromatographed			
	Total Solids	Fat	Kjeldahl Nitrogen	Ash	Unchromatographed	Sum	Pyridoxine	Pyridoxal	Pyridoxamine
Meat									
Beef, raw									
Chuck	29.8	7.7	3.45	0.95	2.97	3.22	0.30	1.46	1.46
Liver	31.8	3.0	3.32	1.45	7.22	8.21	0.67	1.80	5.74
Round	28.7	4.1	3.68	1.09	5.10	5.97	0.43	2.42	3.12
Round steak	28.3	3.7	3.36	1.12	4.24	5.41	0.44	2.27	2.70
Chicken, raw									
Breast	26.0	1.4	3.68	0.89	6.97	6.83	0.44	5.08	1.31
Breasts with skin	29.6	6.7	3.36	0.81	5.66	5.57	0.37	4.11	1.09
Liver	24.3	2.0	2.73	1.14	4.65	6.70	0.32	1.55	4.83
Legs and thighs	25.6	4.9	3.10	0.95	2.73	3.25	0.27	1.80	1.18
Legs and thighs with skin	28.4	8.3	2.97	0.89	2.43	2.89	0.24	1.61	1.04
Skin	47.4	29.6	2.36	0.57	0.79	0.94	0.08	0.55	0.31
Lamb									
Leg, raw	25.6	3.9	3.16	1.06	2.66	3.34	0.15	1.33	1.86
Pork									
Ham fully cooked	27.7	2.8	3.28	4.38	3.14	3.19	0.33	0.52	2.34
Loin, raw	36.6	14.4	3.33	0.96	3.87	4.18	0.58	2.68	0.92
Fish									
Flounder, fillets, fresh	16.4	0.2	2.24	2.30	1.43	1.37	0.10	0.97	0.30
Oysters, fresh, frying size:									
Raw meat only	14.8	1.9	0.89	0.58	0.49	0.50	0.03	0.20	0.27
Liquid	4.4	0.1	0.33	0.39	0.17	0.18	0.01	0.08	0.09
Shrimp, frozen, shelled, deveined	22.6	0.1	3.28	1.32	1.13	1.22	0.07	0.56	0.59
Salmon, Sockeye, red, canned									
Meat only	34.4	6.5	4.14	2.33	2.40	2.43	0.05	0.22	2.16
Liquid	16.5	5.1	1.44	2.56	2.58	2.75	0.05	0.14	2.56
Bones					2.36	2.69	0.06	0.24	2.39
Skin	38.7	19.9	2.71	2.33	1.96	2.29	0.05	0.17	2.07
Tuna, canned, water packed									
Meat	31.5	0.5	4.71	1.40	3.48	3.78	0.05	0.25	3.48
Liquid	10.1	0.1	1.30	1.86	4.68	4.39	0.06	0.22	4.11
Dairy Products and Eggs									
Cheese									
Blue	58.9	28.7	3.46	5.58	0.97	1.29	0.12	0.08	1.09
Cottage	21.4	4.0	2.08	1.27	0.16	0.17	0.04	0.07	0.06
Natural Cheddar	68.0	34.2	4.00	4.12	0.43	0.52	0.02	0.04	0.46
Natural Swiss	64.5	27.6	4.39	3.72	0.34	0.54	0.03	0.10	0.41
Processed pasteurized American	62.0	29.9	3.37	5.46	0.45	0.67	0.03	0.06	0.58
Processed pasteurized Swiss	59.8	25.2	3.93	5.74	0.35	0.43	0.03	0.09	0.31
Eggs									
Whites, fresh	11.6	0.02	1.60	0.74	0.02	0.01	0.005	0.004	0.001
Yolks, fresh	50.8	30.4	2.62	1.77	2.01	2.81	0.05	1.23	1.53
Whole, fresh	25.7	11.0	1.96	1.11	0.73	1.01	0.02	0.44	0.55
Whole, dried	94.2	40.8	7.34	3.44	3.65	4.00	0.22	3.61	0.17
Milk									
Buttermilk, pasteurized	9.1	0.5	0.49	0.85	0.28	0.33	0.01	0.11	0.21
Evaporated	26.2	6.2	1.02	1.57	0.44	0.52	0.13	0.06	0.33
Homogenized, pasteurized	12.0	3.4	0.48	0.74	0.35	0.34	0.01	0.26	0.07
Nonfat dry	96.7	0.3	5.45	8.16	3.73	3.82	0.12	2.80	0.90
Yogurt, pasteurized	15.7	2.0	0.75	1.13	0.38	0.43	0.03	0.27	0.13
Commercial Infant Formulas									
Milk, cows, modified, nonfat liquid[a,b]	24.1	6.1	0.57	0.87	0.37	0.37	0.19	0.04	0.14
Milk, cows, modified, powdered[a]	98.6	21.2	2.19	4.27	1.16	1.42	0.03	0.90	0.49
Milk, cows, skimmed, powdered[b,c]	98.1	22.3	1.92	4.21	3.97	3.95	2.75	0.88	0.32
Milk, soy product, liquid[b,d]	25.1	5.3	0.95	1.26	2.20	2.03	1.80	0.12	0.11
Milk, soy product, powdered[d]	97.4	30.2	3.94	6.80	2.96	2.89	1.74	0.59	0.56

[a] Similac.
[b] Pyridoxine hydrochloride added.
[c] Bremil.
[d] Mull-soy.

predominantly pyridoxal, similar to whole milk, while the other contained mostly prydoxine as mentioned previously.

Total vitamin B_6 values reported in the literature showed that the animal products fell in the same general range as reported here except for egg whites; Hardinge and Crooks (1961) reported a considerably higher value for egg white while the value of Lieck and Sondergoard (1958) was in good agreement with the one reported here.

In general, meats could be considered as a good source of vitamin B_6, fish as a fair to good source, with dairy products being a poor to fair source.

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