on liver fat deposition was markedly different—i.e., a marked reduction due to methionine and a small increase due to vitamin B_{12} (ratios 3 and 4).

Vitamin B_{12} acted as an efficient supplement to other amino acid combinations, but its physiological mechanism is not evident from this study. The best illustration of the supplementary value of vitamin B_{12} can be noted in rations 21 and 22. The addition in ration 21 of 0.4% DL-methionine to ration 9 produced a growth of 138.6 grams and a protein efficiency ratio of 2.20, which is less than that secured on ration 9, but the fortification of this ration with vitamin B_{12} in ration 22 not only counteracted the slight amino acid imbalance but was responsible for the production of maximum gain in body weight, 192.8 grams, and an optimum protein efficiency ratio of 2.57. As ration 21 was fortified with methionine, it would appear that vitamin B_{12}, in addition to functioning in synthesis of this sulfur-containing amino acid, increases the utilization of other amino acid combinations as supplements to the proteins in enriched milled wheat flour by a mechanism which will probably be clarified by future research.

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NUTRIENTS IN COFFEE

Nutritional Evaluation of Coffee Including Niacin Bioassay

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The presence of approximately 10 mg. of niacin in 100 grams of ordinary retail coffees, as measured microbiologically, was confirmed by rat assay. Niacin level is dependent on degree of roasting. Experimental dark roasts contained up to 43 mg. of niacin per 100 grams of coffee and equally high levels were found in some specialty coffees obtained on the open market. The niacin is readily extracted in the preparation of beverage. Appreciable, but rather low, levels of seven B vitamins, other than niacin, were present in coffee beverage. Moderate amounts of extractable calcium and iron and low levels of sodium and fluorine were found in roasted coffee.

IT WAS REPORTED IN 1944 THAT ordinary retail roasted coffees tested in the United States contained about 10 mg. of niacin per 100 grams of coffee as measured by microbiological assay (*23*). The niacin was found to be easily extractable with water and samples of restaurant coffee obtained in mid-western United States contained approximately 1 mg. of niacin per 175-ml. cup of coffee. Attempts to carry out bioassays with the chick and the dog met with some difficulties, but the results suggested that the niacin in coffee is biologically active (*24*).

In these studies, comparative microbiological and rat assays were performed on coffee beverage. A survey was made of the niacin content of ordinary and specialty retail coffees marketed in the United States. The relatively low niacin content of green coffees as compared to roasted coffee had suggested that most niacin in roasted coffee is formed during the roasting process, possibly from trigonelline (*N*-methylbetaine of nicotinic acid), which is present at rather high levels in the coffee bean but has no biological niacin activity (*24*). The niacin content of various experimental roasts is reported and data are given on four minerals and eight B vitamins other than niacin.

Materials and Methods

Coffee Samples. All of the determinations of minerals and vitamins, other than niacin, were performed on single samples of: green coffee, a popular roasted retail coffee made from the same batch of green coffee (and with a typical niacin content), and beverage made from this roasted coffee. Various other roasted coffee samples were obtained for niacin assay only.

Experimental Roasts. One-pound batches of coffee were prepared in a Burns gas-fired laboratory roaster. The lightest roast required 8.5 minutes and was judged visually to be lighter than ordinary commercial roasts. The darkest roast required 23 minutes and was judged to be overroasted. The next to the darkest roast required 16 minutes and was judged to have a color comparable to the most heavily roasted commercial coffee.

Analytical Procedures. Microbiological assays were employed for niacin (*14*), choline (*8*), pantothenic acid (*20*),

Table I. Growth Data on Niacin Bioassay

Supplement per 100 Grams Ration	Average Weekly Gain in Grams (4 Weeks)	
Niacin, Mg.	Bioassay A, 6 rats per group, corn grits basal	Bioassay B, 10 rats per group, zein basal
0	4.1	−4.3
0.1	5.6	−2.8
0.2	6.4	3.5
0.4	8.4	7.4
0.8	15.5	19.8
Coffee Concentrate, Ml		
1.2	6.6	0.3
3.0	9.5	11.9

folic acid (*2*), citrovorum factor (*19*), vitamin B_6 (*4*), vitamin B_{12} (*15*), and riboflavin (*16*). The thiochrome method was applied in the case of thiamine (*3*). Procedures that are fairly well standardized were used for determining sodium (*6*), fluorine (*7*), total iron (*18*), and calcium (*10*).

Preparation of Beverage. The beverage was prepared in a 2-gallon aluminum drip coffee maker with 1 pound of a popular retail roasted coffee per 2 gallons of water. The beverage was concentrated by vacuum distillation to one-fortieth of its original volume for incorporation into the bioassay diets. For purposes of comparison, microbiological assays were performed on the concentrates. Tap water was used in the preparation of beverage, except that distilled water was used in making the beverage for mineral determinations.

Bioassay. In the first bioassay, the 40% corn grits ration of Krehl, Teply, and Elvehjem (*11*) was used. In the second, the basal ration consisted of 7% casein, 14% zein, 74% sucrose, 0.55% L-lysine plus essential minerals, and vitamins other than niacin (*22*). Weanling male rats of the Sprague Dawley strain were placed on experiment in individual cages and feed and water were supplied *ad libitum*. Standard nicotinamide and coffee beverage concentrate were mixed intimately with the basal rations. At comparable growth rates, feed efficiency was the same on either supplement. The data (Table I) were submitted to a statistician for evaluation.

Table II. Niacin in Coffee Concentrates

Preparation	Microbiological, Mg./Ml.	Bioassay, Mg./Ml.	
A	0.165	Level A	0.158
		Level B	0.137
		Av.	0.148
B	0.160	Level A	0.120
		Level B	0.170
		Av.	0.140

Results and Discussion

Bioassay. The comparison in Table II of microbiological assay values with those obtained by rat assay shows the latter to be approximately 15% lower. In view of normal experimental variation in both assay procedures, this can be considered good agreement.

Extraction of Niacin from Roasted Coffee. Approximately 80% of the niacin in the roasted coffee was recovered in the drip-coffee beverage prepared for the rat assay. The same degree of extraction was obtained by Cravioto, Guzman, and Suarez (*7*). When ground roasted coffee is placed in boiling water to prepare a decoction, the extraction of niacin rapidly approaches completion (*9, 23, 24*).

Survey of Niacin in Retail Coffee. Additional studies were carried out by using the convenient microbiological procedure. Six popular brands of coffee were obtained on the open market in Madison, Wis., and were found to contain from 7.4 to 11.0 mg. of niacin per 100 grams of coffee with an average content of 9.33 mg. of niacin per 100 grams of coffee. This is in close agreement with values obtained on samples picked up in the same market in 1944 (*24*). Barton-Wright (*5*) reported a value of 13.2 mg. of niacin per 100 grams on one sample of roasted coffee. Several samples of ordinary roast coffees, originating from other parts of the country, had niacin contents of 8.5 to 15.6 mg. per 100 grams. Two samples of chicory-coffee blend had 5.5 and 10.0 mg. of niacin per 100 grams, respectively. A coffee from Colombia had 18.0 mg. of niacin and one from Puerto Rico had 20.0 mg. of niacin per 100 grams. Two brands of demitasse coffee from the United States contained 45.0 and 46.5 mg. of niacin per 100 grams, respectively.

Roasting and Niacin Content. The niacin content of a number of experimental roasts is recorded in Table III. Similar results have been reported by Hughes and Smith (*9*), who found a maximum value of 26.3 mg. of niacin per 100 grams of coffee with a chemical assay method, and, by Cravioto and coworkers whose highest value on microbiological assay was 32.0 mg. of niacin per 100 grams of coffee. These maxima are a little lower than the value of 43.6 mg.

Table III. Roasting and Niacin Content

	Niacin, Mg. per 100 Grams
Green beans	2.2
Just before popping	4.0
Just popped	8.3
New England roast	13.0
French roast	24.9
Italian roast	41.6
Heavy roast	43.6

Table IV. B Vitamins in Coffee

	Mg. per 100 Grams	
	Green	Roasted
Thiamine	0.21	0
Riboflavin	0.23	0.30
Pantothenic acid	1.0	0.23
Choline	59	84
Folic acid	0.020	0.022
Citrovorum factor	0.012	0.003
Vitamin B_6	0.143	0.011
Vitamin B_{12}	0.00011	0.00006

Table V. Minerals in Coffee[a]

	Mg. per 100 Grams		
	Green	Roasted	Beverage
Sodium	4.0	1.4	0.33
Calcium	104	105	4.6
Iron	3.7	4.7	0.21
Fluorine	0.45	0.24	0.018

[a] Beverage prepared with distilled water.

of niacin per 100 grams of coffee reported herein. Hughes and Smith excluded samples "which were considered to be either under-roasted or over-roasted," while Cravioto and associates employed an "almost black" sample as their heaviest roast. In the present experiments the highest level of niacin in the dark roast retail coffees tested was found to be close to the highest value for experimental roasts.

Possible Origin of Niacin in Roasted Coffee. The presence of 1% trigonelline in green coffee suggested that the increase of niacin during roasting might be due to conversion of this compound to niacin (*24*). Slotta and Neisser (*21*) found that the trigonelline content of coffee decreased on roasting. Hughes and Smith reported up to 90% destruction of trigonelline and formation of substantial amounts of niacin during roasting of coffee or heating of trigonelline in a sealed tube. Moores and Greninger (*12*) employed a different assay method and found that no more than 15% of the trigonelline was destroyed during roasting of coffee. However, as only about 1% of the trigonelline in green coffee would have to be converted to niacin to provide 10 mg. per 100 grams of roasted coffee, it appears likely that most of the niacin comes from this source.

Coffee and Niacin Requirement. The National Research Council's recommended daily dietary niacin allowance for the normal adult male is 15 mg. (*13*) and the mininum daily requirement proposed by the Food and Drug Administration is 10 mg. If coffee beverage contains about 1 mg. of niacin per cup, average consumption of 3.5 cups per day (*17*) could supply about one fourth of the allowance or one third of the minimum daily requirement. Current incidence of frank niacin deficiency is negligible in the United States and niacin requirements can easily be met with common foods that are readily available. The niacin supplement from coffee might be important to certain individuals.

The absence of pellagra in some areas of the world, where the diet appears to be low in niacin and good quality protein, has not been satisfactorily explained. There are other areas where pellagra (not necessarily simple niacin deficiency) is not uncommon.

Other Vitamins. Values for eight B vitamins, other than niacin, are given in Table IV. Except for thiamine, the vitamins survive the roasting process well, considering the high temperatures employed. Assays on beverage prepared from the roasted coffee indicate that the vitamins are easily extracted. The vitamins are present in measurable amounts, although at rather low levels in relation to dietary requirements.

Minerals. Moderate levels of extractable calcium and iron, and low levels of sodium and fluorine were present in roasted coffee (Table V).

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FORAGE ANALYSIS

Effect of Cold Storage on the Fluoride Content of Alfalfa

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The tediousness of determinations of fluoride in vegetation samples, coupled with a frequent need to gather a large number of samples in a short time, usually requires storage of samples prior to analysis. Tests using alfalfa samples were conducted to determine the effect of storage at 0° F. for 4 to 6 weeks on fluoride content. Stored samples show more variation than unstored samples, but no consistent change in fluoride content. The variation can be reduced by adding lime to stored samples. Samples stored in a tight container do not gain or lose moisture consistently.

The spread of industries into agricultural areas in recent years has produced an increased concern over the contamination of vegetation by fluorides. Although the toxic effects of fluorides to vegetation create some concern, a more important consideration is the effect of fluoride contamination upon animals eating the vegetation as forage. This forage problem makes a rather extensive vegetation sampling and chemical analysis program a necessity in evaluating the degree of any air pollution by fluorides. Because the analytical procedures involved in the fluoride determinations of biological materials are time-consuming, samples must often be stored for varying periods before they can be analyzed. Another complicating factor arises when large numbers of samples arrive at the laboratory in a short period of time. Alfalfa samples as used throughout this paper refer to green alfalfa and not to hay made from alfalfa.

These studies were conducted to determine whether any errors were being introduced into the fluoride determinations by the storage of samples. The objectives of these tests were to ascertain if alfalfa samples could be placed in cold storage without first weighing, liming, and taking a moisture aliquot; any change in percentage of moisture in the samples after deep freeze storage, and the effect of cold storage on both limed and unlimed alfalfa samples as compared to a control group. Alfalfa was studied because of its availability, its sensitivity to the intake of air-borne fluorides, and its general importance as an agricultural product.

Procedure

In preparing alfalfa for deep-freeze storage 20 grams of the material, which had been detruncated in the field at the time of collection into approximately