Gundersen, K.; Jensen, H. L. *Acta Agric. Scand.* **1955**, *6*, 100–114.
Harder, W.; Kuenen, J. G.; Matin, A. *J. Appl. Bacteriol.* **1977**, *43*, 1–24.
Helm, V.; Reber, H. *Eur. J. Appl. Microbiol. Biotechnol.* **1979**, *7*, 191–199.
Herbert, D.; Phipps, P. J.; Strange, R. E. In "Methods in Microbiology"; Norris, J. R.; Ribbons, D. W., Eds.; Academic Press: New York, 1971; Vol. 5B, pp 249–252.
Kaufman, D. D. *Weeds* **1966**, *14*, 90–91.
Kearney, P. C.; Oliver, J. E.; Kontson, A.; Fiddler, W.; Pensabene, J. W. *J. Agric. Food Chem.* **1980**, *28*, 633–635.
Laanio, T. L.; Kearney, P. C.; Kaufman, D. D. *Pestic. Biochem. Physiol.* **1973**, *3*, 271–277.
Leipzig, N. A.; Hockenbury, M. R. *Proc. Ind. Waste Conf.* **1979**, *34*, 195–205.
Lusby, R. W.; Oliver, J. E.; Kearney, P. C. *J. Agric. Food Chem.* **1980**, *28*, 641–644.
Malaney, G. W. *J.—Water Pollut. Control Fed.* **1960**, *32*, 1300–1311.
McCormick, N. G.; Feeherry, F. E.; Levinson, H. S. *Appl. Environ. Microbiol.* **1976**, *31*, 949–958.
Reber, H.; Helm, V.; Karanth, N. G. K. *Eur. J. Appl. Microbiol. Biotechnol.* **1979**, *7*, 181–189.
Spain, J. C. Ph.D. Thesis, University of Texas, Austin, TX, University Microfilms International No. 7920216, Ann Arbor, MI, 1979.
Stephenson, P.; Benedek, A.; Malaiyandi, M.; Lancaster, E. A. *Ozone: Sci. Eng.* **1979**, *1*, 263–279.
Suess, A.; Fuchsbichler, G.; Eben, Ch. *Z. Pflanzenernaehr. Bodenkd.* **1978**, *141*, 57–66.
Tewfik, M. S.; Evans, W. C. *Biochem. J.* **1966**, *99*, 31P–32P.
Villanueva, J. R. *J. Biol. Chem.* **1964**, *239*, 773–776.
Young, J. C.; Affleck, S. B. *Proc. Ind. Waste Conf.* **1974**, *29*, 154–164.
Zeyer, J.; Kearney, P. C. *Pestic. Biochem. Physiol.* **1982**, *17*, 215–223.

Received for review April 2, 1982. Accepted October 20, 1982.

Concentration, Insulin Potentiation, and Absorption of Chromium in Beer

Richard A. Anderson* and Noella A. Bryden

The chromium content of 27 domestic and imported brands of beer was determined to evaluate the importance of beer as a dietary source of chromium. Chromium concentration of selected beers ranged from 0.48 to 56 ng/mL; concentration of 8 brands of beer was below 2 ng/mL, 11 ranged from 2 to 10, and 8 brands of beer contained more than 10 ng of Cr/mL. Chromium content of the ingredients was not sufficient to account for the high levels of Cr found in some beers; therefore, a significant amount of Cr in beer was due to unidentified sources. Chromium in beer, brewing water, and corn grits displayed insignificant in vitro insulin potentiating activity, malt displayed low levels of insulin activity, and hops, which was the highest in total Cr, inhibited in vitro insulin activity. Chromium in beer was absorbed by humans similar to the Cr found endogenously in foods. These data demonstrate that the Cr concentration of beer varied widely and that some beer contained significant quantities of chromium that was absorbed by humans.

Marginal chromium deficiency of man and animals leads to impaired glucose and lipid metabolism that can be prevented or alleviated by proper chromium nutrition (Schwarz and Mertz, 1959; Jeejeebhoy et al., 1977). Even individuals who eat a well-balanced diet may not be ingesting the suggested minimum recommended safe and adequate amount of chromium (Kumpulainen et al., 1979). Brewers' yeast is one of the richest sources of chromium but relatively few people normally eat brewers' yeast. Many people, however, frequently drink beer, the end product of the brewers' yeast fermentation process. The average per capita consumption of beer in the United States is approximately 9 oz/day compared to 8.6 oz for milk (Katz, 1981).

Among the constituents of beer, several metals are nutritionally important, including sodium, potassium, calcium, magnesium, and trace quantities of copper, iron, and zinc (Binns et al., 1978). Chromium should also be added to that list. In the present study, significant quantities of Cr were present in several brands of beer; on the basis of the average Cr content of the 27 brands of beer analyzed in this study, an average 12-oz (355 mL) beer would supply 7% of the minimum recommended safe and adequate intake for Cr. Relative absorption, sources of Cr, and forms of Cr in beer and ingredients were also determined.

U.S. Department of Agriculture, Science and Education Administration, NER, Beltsville Human Nutrition Research Center, Vitamin and Mineral Nutrition Laboratory, Beltsville, Maryland 20705.

MATERIALS AND METHODS

Beer samples were purchased from local liquor stores or donated by individuals working at the Beltsville Human Nutrition Research Center. Ingredients used in brewing beer were provided by Carling National Brewery, Baltimore, MD, and Stroh Brewery, Detroit, MI. Insulin potentiation of samples was determined as described (Anderson et al., 1978a). Beer samples (10 brands) were assayed directly; beer ingredients were extracted with 0.1 N ammonium hydroxide (Anderson et al., 1978b) and analyzed in duplicate on 2 separate days for insulin potentiating activity.

Chromium Analysis of Urine and Beer. Chromium was determined with flameless atomic absorption spectroscopy by method of additions on nonashed urine samples (25 μL) with a Perkin-Elmer 5000 and a HGA-500 furnace with pyrolytic coated tubes. Furnace conditions for direct analysis of Cr in urine were the following: first drying, 100 °C; ramp, 15 s; hold, 20 s; internal argon drying flow, 300 mL/min; second drying, 130 °C; ramp, 15 s; hold, 60 s; internal argon flow, 300 mL/min; atomize, 2700 °C; ramp, 0 s; hold, 4 s; internal argon flow, 50 mL/min; clean out, 2700 °C; ramp, 1 s; hold, 4 s; internal argon flow, 300 mL/min. Urine samples were collected in plastic-lined

Table I. Chromium Concentration of Selected Domestic and Imported Beer Samples

beer no.	can (C) or bottle (B)	country	Cr, ng/mL, mean ± SD	range, ng/mL
1 (2)[a]	C	USA	0.48	0.44-0.52
2 (19)	C	USA	0.50 ± 0.10	0.34-0.67
2 (2)	B	USA	0.88	
3 (1)	B	USA	0.94	
4 (2)	B	USA	1.2	
5 (24)	B	USA	1.12 ± 0.06	1.1-1.2
5 (1)	C	USA	4.8	
6 (1)	C	USA	1.9	
7 (1)	B	USA	2.4	
8 (2)	C	USA	2.5	2.5-2.6
9 (2)	C	USA	2.6	2.4-2.7
10 (2)	B	USA	2.9	2.6-3.2
11 (1)	C	USA	4.8	
12 (2)	C	USA	8.8	8.6-9.0
13 (4)	C	USA	7.4 ± 5.6	1.1-12
13 (7)	B	USA	38.0 ± 17	11-52
14 (2)	B	USA	9.2	9.0-9.4
14 (2)	C	USA	17.5	17-18
15 (4)	B	USA	10.0 ± 0.9	9.2-11
16 (1)	C	USA	16.0	
17 (1)	C	USA	18.0	
18 (3)	C	USA	21 ± 2.3	20-24
19 (30)	B	USA	28.5 ± 2.0	25-32
19 (8)	C	USA	56 ± 2.3	54-60
20 (5)	B	Australia	0.54 ± 0.04	0.52-0.60
21 (1)	B	Ireland	0.80	
22 (1)	B	Mexico	1.9	
23 (1)	B	China	2.5	
24 (4)	B	Germany	3.2 ± 0.06	3.2-3.3
25 (5)	B	Germany	4.8 ± 0.3	4.4-5.0
26 (1)	B	Czechoslovakia	7.0	
27 (1)	B	Canada	30.0	

[a] The number in parentheses denotes the number of samples tested.

24-h urine containers (Scientific Products, McGaw Park, IL). Two pooled urine samples, whose Cr concentration had been verified by two independent techniques, were assayed at least 2 times daily as internal checks on the analytical reliability of the chromium determinations (Veillon et al., 1982). We found a daily variation of less than 10%. Beer ingredients, except water, were analyzed following acid digestion (Wolf, 1981). National Bureau of Standards standard reference materials (SRM), bovine liver (SRM 1577) and brewers' yeast (SRM 1569), were analyzed as checks on the relative accuracy of the method. Under our conditions, a mean ± standard deviation for the chromium content of bovine liver (SRM 1577) of 0.085 ± 0.01 μg/g for 11 determinations was obtained (certified value, 0.088 ± 0.012 μg/g), and for brewers' yeast (SRM 1569) we obtained a value of 2.08 ± 0.25 μg/g (certified value, 2.12 ± 0.05 μg/g).

Conditions for Cr analysis of beer were similar to those for urine except that samples were diluted at least 4-fold with water prior to analysis and first ramp was 10 s, hold 20 s, hold after second dry 10 s, ash 1100 °C, and hold 30 s. One beer sample was run at least twice a day to determine any daily variation which was less than 10%.

RESULTS AND DISCUSSION

Chromium content of several U.S. and imported brands of beer was determined. Chromium concentration of beer samples ranged from 0.48 to 56 ng/mL (Table I). Therefore the total Cr content of a 12-oz. beer (355 mL) varied from 0.17 to 20 μg. Of the 27 beer samples analyzed, the chromium concentration of 8 brands of beer was below 2 ng/mL, 11 ranged from 2 to 10 ng/mL, and 8 brands of beer contained more than 10 ng of Cr/mL. The chromium content of some brands of beer varied. For example, the chromium content of sample 13 ranged from 1.1 to 12 ng/mL for beer distributed in cans and 11 to 52 ng/mL for the same beer distributed in bottles. Since beer distributed in bottles should be similar to that in cans ("Practical Brewing", 1976), the Cr concentration of this beer varied more than 40-fold. The chromium content of sample 13 appeared to be higher in bottles but that of sample 19 was higher in cans; Cr concentration of other beer samples was the same in both bottles and cans. This appears to illustrate the variability of the Cr content of the same brand of beer rather than the variability due to the container. No association was observed between Cr content of beer and country of origin. We also did not find that any particular brewery produced beer of consistently higher Cr content. Beer samples no. 7, 13, 16, and 19 were all brewed in the same brewery; sample no. 7 was one of the lowest Cr-containing beers while no. 19 was the highest of the samples analyzed. Beer samples from the same lot appeared to be quite uniform with respect to Cr concentration. For example, 24 samples of beer no. 19 (bottle) were all within 15% of the mean Cr concentration for this beer. However, some beer samples from different lots of this brand of beer differed by more than 100% and samples from different lots of beer no. 13 varied by several hundred percent. Some brands of beer appeared to be very consistent. For example, Cr concentrations of beer from at least three different lots of beer no. 5 were nearly identical.

Sources of Chromium. To determine the possible sources of chromium in beer, we analyzed samples of ingredients from two breweries. Beer samples from one of the breweries that donated ingredients to be tested were among the lowest in total Cr and those from the second brewery were among the highest. The relatively high Cr content of some beer samples and the large variation in Cr content of different brands of beers brewed at the same location suggested that brewing water was not the major

Table II. Chromium Content and Insulin Potentiation of Beer Ingredients

	brewery 1		brewery 2	
ingredient	Cr, ng/g	% insulin potentiation[a]	Cr, ng/g	% insulin potentiation[a]
brewing water	0.50	0	0.36	0
corn grits	no sample	0	<5	0
malt	48	60	32	70
hops	1163	−70	797	−60

[a] Samples were extracted with a 20-fold excess of 0.1 N ammonium hydroxide; 100 μL was assayed directly (see Materials and Methods). Percent insulin potentiation is the increase in activity above insulin alone. Negative values indicate that samples inhibited insulin action.

Table III. Urinary Excretion of Male Subjects Who Drank Beer with High and Low Levels of Cr

	μg of Cr excreted/24 h				
subject no.	basal[a] (no beer)	+high Cr beer	ratio[b]	+low Cr beer	ratio[c]
1	0.14	0.50	3.6	0.14	1.0
2	0.18	0.42	2.3	0.28	1.6
3	0.25	0.54	2.1	0.37	1.4

[a] Mean Cr excretion for two different 24-h periods in which subjects drank no beer. [b] Ratio between urinary Cr excreted on the day when subjects drank 2130 mL of beer containing 28.5 ± 2 ng of Cr/mL (total of 61 μg of Cr) and basal excretion. [c] Ratio between urinary Cr excreted on the day when subjects drank 2130 mL of beer containing 0.50 ± 0.1 ng of Cr/mL (total of 1.1 μg of Cr) compared to the basal excretion.

source of variation. Indeed, the Cr content of brewing water samples from the two breweries was 0.5 ng of Cr/mL or less (Table II). Corn grits were relatively low in Cr (<5 ng/g) and malt contained 32 or 48 ng of Cr/g (Table II). The chromium concentration of the hops was the highest of the ingredients tested, 1163 ng/g from one brewery and 797 ng/g from the other. However, the chromium content of the individual ingredients do not appear to account for the total chromium found in some beer samples. In brewing, corn grits, malt, and hops are diluted approximately 1:20, 1:10, and 1:1000, respectively ("Practical Brewing", 1976). Therefore, based on our values for the Cr concentration of ingredients for beer, the upper limit of the contribution of each component per liter of beer would be the following: water, 0.5 μg; corn grits, 0.3 μg; malt, 4.8 μg; hops, 1.2 μg. The total would be approximately 6.8 μg/L of beer (assuming all the chromium would be incorporated into the beer). Additional chromium above 6.8 μg/L appears to originate from unknown sources. Release of significant quantities of Cr from stainless steel vessels during processing of foods has been reported recently (Offenbacher and Pi-Sunyer, 1982). The chromium content of yeast is not given since brewers' yeast would be removed from the beer prior to consumption.

Biological Activity of Samples. Specific organic forms of chromium potentiate insulin activity while inorganic complexes and most organic complexes have little or no effect on in vitro insulin activity (Anderson, 1981). These organic complexes are also postulated to be absorbed much more efficiently than simple Cr compounds (Mertz et al., 1974). We found no discernible insulin potentiating activity in 5, 25, and 100 μL beer samples. Corn grits yielded little or no insulin potentiating activity; however, ammonium hydroxide extracts of the malt displayed significant insulin potentiating activity. Beers using rice instead of corn in the brewing process also did not display insulin potentiating activity nor did the Cr concentration of these samples differ from some of those in which corn was used. Hops, which contained the highest concentration of Cr of the ingredients tested, inhibited the action of insulin 60–70% (Table II). We have observed inhibition of insulin activity with high levels of other chromium compounds (Anderson et al., 1978a). For comparative purposes, similar ammonium hydroxide extracts from brewers' yeast potentiate insulin 300–600% (Anderson et al., 1978b).

Absorption of Cr from Beer. Essentially all absorbed Cr is excreted in the urine (Anderson, 1981); therefore, urinary Cr excretion can be used as a relative measure of Cr absorption. The relative absorption of the chromium in beer by three male subjects (26, 27, and 35 y), who drank 2130 mL (72 oz) of a high-Cr beer (no. 19, bottle), was determined. Subjects were also asked to drink an equivalent amount of a low-Cr beer (no. 2, can) on a separate day to ascertain that any changes in 24-h urinary excretion of Cr were not due to nonspecific effects of beer. Basal levels of daily urinary Cr excretion were determined prior to each day beer was included in the diet. The high-chromium beer contained 28.5 ± 2 ng of Cr/mL (Cr content of every beer consumed was measured). Beer consumed contained 61 μg of Cr which would approximately double the normal estimated daily intake of Cr (Kumpulainen et al., 1979; Schroeder et al., 1962; Levine et al., 1968). If the form of Cr found endogenously in beer is absorbed similar to that found in other dietary sources, an approximate doubling of the urinary Cr excretion would be expected. Urinary Cr excretion increased for all three subjects. Increases ranged from 2.1- to 3.6-fold (Table III). Low-Cr beer consumed by the subjects contained only about 1 μg of Cr and did not markedly increase Cr excretion. When subjects drank the low-Cr beer, total urinary Cr excretion did not differ significantly from their basal levels. However, when subjects drank the high-Cr beer, urinary Cr excretion was significantly greater than their basal levels ($P < 0.05$). Although we did not measure the Cr intake of foods eaten, subjects were asked to eat similar foods on the control and days when beer was consumed. In a previous study (R. Anderson et al., unpublished observations) when there were no restrictions on dietary intake, daily variation in Cr excretion was usually less than 25% and often within 10%. Further, basal Cr excretion in this study was an average of 2 separate days at least 1 week apart.

In summary, the Cr content, among and within brands of beer, varied widely but was high enough in several brands to contribute significantly to the dietary intake of Cr. Chromium content of all beer samples we analyzed averaged 9.6 μg/L. On the basis of that value, an average 12-oz beer would supply 3.4 μg of Cr or about 7% of the recommended minimum safe and adequate intake for Cr. Furthermore, the percent of Cr that human subjects absorbed from beer apparently was similar to that absorbed from other dietary sources.

Registry No. Chromium, 7440-47-3; insulin, 9004-10-8.

LITERATURE CITED

Anderson, R. A. *Sci. Total Environ.* **1981**, *17*, 13–29.
Anderson, R. A.; Brantner, J. H.; Polansky, M. M. *J. Agric. Food Chem.* **1978a**, *26*, 1219–1221.
Anderson, R. A.; Polansky, M. M.; Roginski, E. E.; Mertz, M. *J. Agric. Food Chem.* **1978b**, *26*, 858–861.
Binns, F.; Ensor, R. J.; Macpherson, A. L. *J. Sci. Food Agric.* **1978**, *29*, 71–74.

Jeejeebhoy, K. N.; Chu, R. C.; Marliss, E. B.; Greenberg, J. R.; Bruce-Robertson, A. *Am. J. Clin. Nutr.* **1977**, *30*, 531–538.
Katz, P. C. *Brew. Dig.* **1981**, *56*, 26–29.
Kumpulainen, J. T.; Wolf, W. R.; Veillon, C.; Mertz, W. *J. Agric. Food Chem.* **1979**, *27*, 490–494.
Levine, R. A.; Streeten, D. H. P.; Doisy, R. J. *Metab., Clin. Exp.* **1968**, *17*, 114–124.
Mertz, W.; Toepfer, E. W.; Roginski, E. E.; Polansky, M. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1974**, *33*, 2275–2280.
Offenbacher, E. G.; Pi-Sunyer, F. X. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1982**, *41*, 391.
"Practical Brewing", 2nd ed.; Master Brewers of the Americas: Madison, WI, 1976.
Schroeder, H. A.; Balassa, J. J.; Tipton, I. H. *J. Chronic Dis.* **1962**, *15*, 941–964.
Schwarz, K.; Mertz, W. *Arch. Biochem. Biophys.* **1959**, *85*, 292–295.
Viellon, C. V.; Patterson, K. Y.; Bryden, N. A. *Anal. Chim. Acta* **1982**, *136*, 233–241.
Wolf, W. *Beltsville Symp. Agric. Res.* **1981**, *4*, 175–196.

Received for review April 23, 1982. Accepted November 1, 1982.

A New Method for the Identification of the Origin of Ethanols in Grain and Fruit Spirits: High-Field Quantitative Deuterium Nuclear Magnetic Resonance at the Natural Abundance Level

Gerard J. Martin,* Maryvonne L. Martin, Francoise Mabon, and Marie J. Michon

It is shown that very different internal natural distributions of the deuterium isotope exist in ethanol samples from various origins. Quantitative ^2H NMR at the natural abundance level provides a new and efficient tool for investigating these distributions, and original selective parameters are introduced. Thus, the R parameter varies from about 2.2 for gins and rums which are obtained from corn and sugar cane, respectively, to 2.7 for ethanols extracted from sugar beet, and bourbon whiskies are unambiguously differentiated from malt scotch whiskies. The method is also capable of providing the overall ^2H content obtainable by mass spectroscopy. Moreover, the selectivity of the NMR method enables information to be obtained on the biosynthetic mechanisms which intervene in the formation of the ethanol and on the influence of the climatological factors.

The identification of ethanol in spirits and beverages is always a challenge for the chemist concerned with analytical problems or with chemical and biochemical mechanisms. Until now, radioactivity methods based on the use of ^{14}C (Simon et al., 1975) or ^3H tracers (Simon and Medina, 1968) or mass spectrometry determinations of the ^{13}C and ^2H contents of ethanol (Bricout et al., 1975; Bricout, 1978) have been used to solve, at least in part, this question. However, a major drawback of these methods is that only the overall isotopic content of the molecule is directly available. In fact, although chemical transformation of ethanol prior to mass analysis (Rauschenbach et al., 1979) may give an indication of the isotope content of the methyl group, the interpretation of the results usually implies some assumptions concerning the biomechanisms of deuterium fractionation during the fermentation (Ponticorvo, 1968).

In the last few years the development in the technology of high-field nuclear magnetic resonance has made possible the recording of ^2H NMR spectra of samples at the natural abundance level and, recently, we have shown that substantial differences exist in the intramolecular deuterium distribution of various ethyl and vinyl (Martin and Martin, 1981a,b) derivatives. In the light of these results we develop here a new, nondestructive, and rapid method, for identifying the origin of ethanols in various spirits and beverages. This method which has been used to measure the quantity of sucrose added in wines (Martin and Martin, 1981c) may also be the source of valuable information on deuterium fractionation and redistribution in biochemical pathways, and we shall discuss the results of NMR in reference to the commonly accepted biomechanisms of ethanol fermentation.

Laboratoire de Chimie Organique Physique, C.N.R.S.-E.R.A. 315 Faculté des Sciences, 44072—Nantes Cedex, France.

EXPERIMENTAL SECTION

Principle of the Method. The proton decoupled ^2H spectrum of ~95% ethanol consists of four lines which have the same chemical shifts as those of the signals observed in the corresponding proton spectrum (Figure 1). Due to the very small natural abundance of deuterium in organic molecules ($100–160 \times 10^{-6}$) the ^2H NMR spectrum of pure ethanol can be explained in terms of the relative contributions of the different monodeuterated molecules: CH_2DCH_2OH (I); CH_3CHDOH (II); CH_3CH_2OD (III). In order to compare the quantity of the deuterated species II to that of I, which can be normalized to the statistical value of 3, we define the parameter

$$R_s(i) = 3S_i/S_I \qquad (i = II, III) \qquad (1a)$$

where S denotes the integrated area of signal i. In practice the signal heights H_i are measured with a better precision than the area and it may be convenient to define the internal ratios

$$R_h(i) = 3H_i/H_I \qquad (1b)$$

These parameters represent the true value of the relative amount of resonating nuclei and are therefore equal to R_s, only on the condition that the line widths are identical. Otherwise the R_h ratios must be considered as empirical parameters. In fact, if the ratio of the line widths of signals I and II, $\Delta\nu_{1/2}(II)/\Delta\nu_{1/2}(I)$, does not change significantly in a series of different samples, the $R_h(II)$ parameter offers a precise way of comparing the deuterium distribution in molecules I and II of different ethanol samples. Due to the large fluctuations in the line width of the OD signal

0021-8561/83/1431-0311$01.50/0