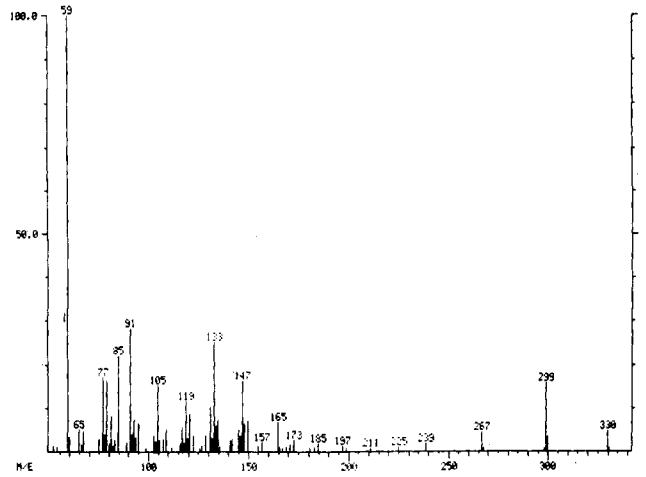

Figure 4. Solid-probe mass spectrum of 16-methoxycafestol.

C1-C2, shows analogous peaks at m/z 131 and 146. At the high end of the spectrum, cafestol, which forms a disilyl derivative (MW 460), has a peak at m/z 357 corresponding to a loss of a 103 radical [$-CH_2O - Si(CH_3)_3$]* arising from the silyl ether on the primary alcohol at C17. A corresponding M – 103 peak at m/z 299 occurs in the spectrum of the new diterpene. This is evidence for the fact that the new diterpene can only form a monosilyl derivative (MW 402) and that silylation occurs at C17. The m/z 59 ion, which is the base peak in the spectrum of the new diterpene's silyl derivative, is also the base peak in the spectra of both its free alcohol (Figure 4) and its esterified derivative (not shown). The peak at m/z 299 is also prominent in all three spectra. These observations can be explained by assuming that in the diterpenes the CH_2OH group at C16 is readily lost whether or not derivatized. With the proposed structure for the new diterpene (MW 330), a loss of M – 31 would result in an ion at m/z 299. In cafestol this loss results in a peak at m/z 285. Once this occurs in the new diterpene, further fragmentation could give rise to the m/z 59 ion $[C_3H_7O]$* containing carbons C15, C16, and the methoxy group.

The addition of 16-methoxycafestol would bring to four the number of diterpenes identified in coffee. A third diterpene, cafestol-2-one, was found by Richter and Spiteller (1979) and isolated as the 11-*O*-β-D-glucopyranoside. It is interesting to speculate that the 16-methoxy derivative of kahweol might also exist in Canephoras. Although given the small amount of kahweol, it would undoubtedly be present at very low levels.

Registry No. Kahweol linoleate, 108214-29-5; kahweol palmitate, 81760-45-4; cafestol palmitate, 81760-46-5; kahweol oleate, 108214-30-8; kahweol stearate, 108214-31-9; kahweol eicosanoate, 108214-32-0; 16-methoxycafestol, 108214-28-4.

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Mineral Metabolism and Bone Strength of Rats Fed Coffee and Decaffeinated Coffee

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Two studies were conducted to determine effects of ingestion of nutritionally complete diets with 6.6% coffee or 6.6% decaffeinated coffee on growth, mineral metabolism, hematological status, and bone strength of weanling rats (study 1) and anemic young (50-day-old) rats (study 2). Rats fed coffee had elevated concentrations of iron in their livers, kidneys, and tibias (study 1) and absorbed iron more efficiently (study 2) than control rats. In both studies, rats fed coffee had elevated liver copper levels and elevated tibia concentrations of zinc, calcium, magnesium, and phosphorus. Rats fed decaffeinated coffee also had elevated zinc tibia levels. These differences in bone mineral levels were not associated with differences in bone strength or elasticity.

In 1982 the average American coffee drinker consumed approximately 3.4 cups of coffee/day (Diamond, 1983). Recently, consumption figures have dropped slightly, but coffee continues to be a popular beverage (International Coffee Organization, 1985).

Morck et al. (1983) found that human subjects incorporated less ^{59}Fe into their red blood cells when coffee was added to a standardized, radiolabeled meal. They believed this effect represented a change in iron absorption. However, coffee and one of its components, caffeine, have been found to influence a number of physiological functions (i.e., thermogenesis, gastrointestinal motility, GI secretions, kidney function) that could ultimately affect utilization of dietary iron for heme synthesis (Von Borstel, 1983; Acheson et al., 1980; Feldman et al., 1981; Cohen and Booth 1975; Sunano and Miyazaki, 1973; Wald et al., 1976; Palm et al., 1984; Yeh et al., 1986; Massey and Berg, 1985). The practical importance of several of these factors can

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not be assessed in a "1-meal" study such as that performed by Morck et al. because animals can adapt to caffeine ingestion (Dews, 1982; Naismith et al., 1969).

Other investigators have found that administration of coffee or caffeine increased urinary losses of calcium (Heaney and Recker, 1982; Massey and Wise, 1984; Massey and Berg, 1985; Yeh et al., 1986). The practical importance of these losses on bone metabolism have not been assessed.

Although many investigators have assumed that most of the physiological effects of coffee were due to caffeine, there are several other components in coffee (i.e., polyphenols, amino acids, various products formed during roasting) that could affect mineral utilization (Sivetz and DesRosier, 1979). For example, Lykken et al. (1986) observed that Maillard reaction products formed during browning depressed that absorption of zinc. Moreover, several investigators have observed tha ingestion not only of regular coffee but also of decaffeinated coffee stimulated gastric secretions (Feldman et al., 1984; Cohen and Booth, 1975).

Therefore, the purposes of these studies were (1) to assess effects of chronic consumption of coffee on absorption and metabolism of iron, copper, calcium, magnesium, zinc, and phosphorus; (2) to compare effects of coffee and decaffeinated coffee on mineral utilization; and (3) to investigate effects of coffee and decaffeinated coffee consumption on physical characteristics of bone.

PROCEDURES

In two studies, rats were fed nutritionally complete diets that contained amounts of coffee or decaffeinated coffee equivalent to consumption of 15 cups of coffee daily by adult humans. This estimate is based on the fact that Nestle Co., Inc., suggests on the label of Taster's Choice freeze-dried instant coffee that consumers "add one rounded teaspoon coffee per cup". We found that a "rounded teaspoon" as estimated by three different individuals 15 times averaged 2.66 g. An average male consumes 600 g dry-weight food/day. Thus, we added 6.6% (w/w) instant coffee (15 cups × 2.66 g/cup/600 g) to the test diets.

In study 1, rats were randomly assigned to one of three dietary treatments: a basal diet (treatment 1Basal) (n = 6); the basal diet supplemented with 6.6% (w/w) instant coffee (Taster's Choice freeze-dried instant, Nestle Co., Inc., White Plains, NY) (treatment 1Coffee) (n = 7); the basal diet supplemented with 6.6% instant decaffeinated coffee (Taster's Choice freeze-dried instant decaffeinated) (treatment 1Decaff) (n = 6). Diets were provided ad libitum throughout the 22-day study.

For 25 days prior to the start of study 2, rats were fed an iron-deficient diet (9.4 μg of Fe/g diet). At the end of this depletion phase the average hematocrit of the rats was 26%. The rats were then fed for 22 days five dietary treatments (n = 6/treatment): a nutritionally complete basal diet (treatment 2Basal) fed ad libitum; the basal diet supplemented with 6.6% instant coffee (treatment 2Coffee); the basal diet pair-fed to the level consumed by rats treatment 2Coffee (treatment 2Basal-PFC); the basal diet supplemented with 6.6% instant decaffeinated coffee (treatment 2Decaff); the basal diet pair-fed to the level consumed by rats fed treatment 2Decaff (treatment 2Basal-PFD). Deionized water was supplied ad libitum throughout both studies.

Animals and Diets. Weanling male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were housed individuallly in stainless-steel, wire cages in facilities that met the standards of the American Association for Accreditation of Laboratory Animal Care.

Table I. Analyzed Mineral Composition of Diets

treatment	Ca[a]	P[a]	Mg[b]	Zn[b]	Fe[b]	Cu[b]	Mn[b]
			Study 1				
1Basal	5.44	4.01	508	14	48	6.5	40
1Coffee	5.42	4.60	745	18	50	6.8	43
1Decaff	5.43	4.48	774	15	50	6.6	44
			Study 2				
2Basal, 2Basal-PFC, 2Basal-PFD	4.99	3.96	503	33	32	6.9	43
2Coffee	5.03	4.34	776	32	34	7.2	42
2Decaff	5.02	4.31	785	32	32	6.5	43
iron-deficient (prestudy)	5.04	3.97	535	36	9.4	5.9	50

[a] In milligrams/gram. [b] In micrograms/gram.

Basal purified diets were formulated according to general guidelines prepared by the American Institute of Nutrition (1977) and contained 20% lactalbumin (Teklad Test Diets, Madison, WI), 5% cellulose (Teklad Test Diets), 5% corn oil (Mazola, Best Foods, Englewood Cliffs, NJ), 1% AIN-76 vitamin mixture (Teklad Test Diets), 3.5% modified AIN-76 mineral mixture (Teklad Test Diets), 0.2% choline, 50% sucrose, and 15.3% cornstarch. Coffees were ground to a fine powder and substituted for equal quantities of cornstarch in the test diets.

The analyzed calcium, phosphorus, magnesium, zinc, iron, copper, and manganese contents of the diets are reported in Table I. The instant coffees did not add significant amounts of copper, zinc, iron, or calcium but did contribute significant amounts of phosphorus and magnesium to the test diet. Ten replicates of the instant coffee were found to contain 0.30 ± 0.03 μg of Cu/g, 3.88 ± 0.12 μg of Zn/g, 32.2 ± 1.2, μg of Fe/g, 1882 ± 15 μg of Ca/g, 3.62 ± 0.07 mg of Mg/g, and 3.54 mg of P/g, and 10 replicates of the decaffeinated coffee were found to contain 0.32 ± 0.02 μg of Cu/g, 2.09 ± 0.04 μg of Zn/g, 34.7 ± 1.3 μg of Fe/g, 2074 ± 15 μg of Ca/g, 3.53 ± 0.11 mg of Mg/g, and 4.41 mg of P/g. These data are consistent with the data in Table I. The test diets tended to contain more magnesium and phosphorus than the control diets.

Sample Collection and Analyses. Rats were weighed three times per week during both studies. In studies 1 and 2, feces were collected on days 16–18 and 15–18 of the test periods, respectively. During study 2, blood was collected from the orbital sinuses of anesthesized rats on days 0, 8, 15, and 22 of the test periods, and hematocrits were determined.

All rats were killed by exsanguination after an overnight fast. Livers, tibiae, ulnae, and kidneys were cleansed of adhering matter and frozen in acid-soaked plastic containers.

Samples of diets, liver homogenates, dried fecal composites, tibae, and kidneys were ashed at 450 °C in a muffle furnace and analyzed for copper, iron, zinc, calcium, and magnesium content by atomic absorption spectroscopy and for phosphorus content (study 2 only) by a colorimetric procedure as described previously (Greger and Snedeker, 1980; Fiske and Subbarow, 1925). Lanthanum (0.1%) was added to all samples and standards during magnesium and calcium determinations. Plasma samples were diluted and analyzed for copper by atomic absorption spectroscopy (Greger and Snedeker, 1980). Bovine liver (1577a) or milk (1549) standards obtained from the National Bureau of Standards were processed with each batch of samples. Liver samples were determined to contain 122 ± 2 μg of Zn/g (mean ± SEM) (n = 95), 153 μg of Cu/g (n = 105), 108 ± 4 μg of Ca/g (n = 69), 586 ± 5 μg of Mg/g (n = 67), and 11.4 ± 0.3 mg of P/g (n = 18) when the certified NBS values were 123 μg of Zn/g, 158 μg of Cu/g, 120 μg of Ca/g,

Table II. Growth and Food Intake of Rats Fed Diets Containing Coffee and Decaffeinated Coffee

treatment	init body wt, g	final body wt,[a] g	food intake,[a] g/day	efficiency of food utilizn,[a] %
		Study 1		
1Basal	53 ± 2[b]	174 ± 3[b]	14.4 ± 0.2[b]	42 ± 0[b]
1Coffee	52 ± 2	130 ± 4[a]	10.9 ± 0.3[a]	36 ± 1[a]
1Decaff	56 ± 3	179 ± 2[b]	14.8 ± 0.3[b]	42 ± 1[b]
		Study 2		
2Basal	153 ± 7	270 ± 12	16.4 ± 0.7[b]	36 ± 1
2Coffee	152 ± 6	237 ± 7	13.6 ± 0.3a	31 ± 0
2Basal-PFC	152 ± 5	242 ± 10	13.6 ± 0.3[a]	33 ± 2
2Decaff	151 ± 5	252 ± 8	15.3 ± 0.6[b]	33 ± 2
2Basal-PFD	151 ± 6	256 ± 11	15.3 ± 0.6[b]	34 ± 1

[a] Means for a study in a column without a common superscript letter are significantly ($p < 0.05$) different. [b] Mean ± SEM.

600 μg of Mg/g, and 11.1 mg of P/g, respectively. Milk samples were determined to contain 48.0 ± 0.6 μg of Zn/g ($n = 4$), 12.5 ± 0.3 mg of Ca/g ($n = 11$), and 1.17 ± 0.02 mg of Mg/g ($n = 9$) when the certified values were 46.1 μg of Zn/g, 13.0 mg of Ca/g, and 1.20 mg of Mg/g.

Ceruloplasmin activity of plasma samples was determined by utilizing a colorimetric procedure (Schosinsky et al., 1974). Neutral detergent residue of the coffees and diets was determined by the method of Goering and Van Soest (1970). The physical characteristics (i.e., elastic load, maximum load) of ulnae were measured with an Instron materials tester, Model 1130 (Instron Corp., Canton, MA), by standardized procedures, and the limit of elasticity and ultimate stress of the bones were calculated by standardized formulas (Beary, 1969; Crenshaw et al., 1981; Wolinsky et al., 1972).

Effects of the treatments were evaluated statistically by analysis of variance (Ryan et al., 1976). Least significant difference tests were applied when appropriate (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

In study 1, rats fed coffee (treatment 1Coffee) weighed less, consumed less food, and utilized food less efficiently than rats fed decaffeinated coffee (treatment 1Decaff) or the basal diet (treatment 1Basal) (Table II). In study 2 there were no significant differences among groups of rats in final body weights or food utilization. However, those rats fed coffee consumed less food, especially during the first week of the study, than rats fed the control diet ad libitum or decaffeinated coffee. The reduced sensitivity in regard to weight gain of rats in study 2 of coffee may reflect that they were 25 days older, were anemic, and were fed somewhat higher levels of zinc than rats in study 1. In both studies the effect of coffee on food intake and activity level of rats appeared to decrease somewhat during the study. This ability of animals to adapt with time to caffein ingestion has been observed by other investigators (Dews, 1982; Naismith et al., 1969).

Trace Elemental Metabolism. Morck et al. (1983) observed that addition of coffee to a meal reduced incorporation of ^{59}Fe into red blood cells of human subjects. Dietary treatments had little effect on hematocrit of rats in either study. At the end of study 1, the hematocrit of rats fed diets 1Basal, 1Coffee, and 1Decaff were 40%, 43%, and 43%, respectively. At the end of the iron depletion period prior to study 2, the average hematocrits of rats in each group were similar (Table III). Although the final hematocrits of rats in study 2 were not affected by the dietary treatments, the hematocrits of rats fed the basal diet ad libitum rose faster during the first 8 days of repletion than those of rats fed decaffeinated coffee or pair-fed the basal diet.

Table III. Changes in Hematocrits of Anemic Rats Fed Iron-Repletion Diets Containing Coffee and Decaffeinated Coffee in Study 2

treatment	hematocrit, % day 0[a]	hematocrit, % day 22	Δ(hematocrit), %: days 0–8[b]	Δ(RBC mass): days 0–7[b]
2Basal	24 ± 2[c]	49 ± 1	19 ± 2[b]	3.40 ± 0.30[c]
2Coffee	26 ± 1	53 ± 1	15 ± 2[a,b]	2.14 ± 0.16[a,b]
2Basal-PFC	27 ± 1	50 ± 1	12 ± 1[a]	1.67 ± 0.41[a]
2Decaff	27 ± 0	52 ± 1	12 ± 1[a]	2.52 ± 0.25[b]
2Basal-PFD	27 ± 1	52 ± 1	15 ± 1[a,b]	2.65 ± 0.04[b]

[a] At end of iron depletion period before test period. [b] Means in a column without a common superscript letter are significantly ($p < 0.05$) different. [c] Mean ± SEM. [d] Change in red blood cell mass = [(weight on day 7)(6.7 mL of blood/100-g body weight) (hematocrit on day 7)] − [(weight on day 0)(6.7 mL of blood/100-g body weight)(hematocrit on day 0)].

Table IV. Iron Metabolism of Rats Fed Diets Containing Coffee and Decaffeinated Coffee

treatment	liver Fe[a]	tibia Fe[a]	kidney Fe[a]	app abs of Fe,[a,b] %
		Study 1		
1Basal	57.7 ± 2.7[c,a]	46.1 ± 2.8[a,b]	35.2 ± 1.2[a]	54 ± 2
1Coffee	77.6 ± 5.5[b]	48.7 ± 2.5[b]	42.4 ± 1.4[b]	57 ± 2
1Decaff	55.9 ± 2.6[a]	39.4 ± 0.9[a]	34.6 ± 1.5[a]	53 ± 2
		Study 2		
2Basal	70.0 ± 3.5[b]	37.0 ± 0.8	32.9 ± 2.7	60 ± 2[a]
2Coffee	72.8 ± 1.9[b]	37.4 ± 2.4	34.9 ± 1.3	69 ± 2[b]
2Basal-PFC	75.7 ± 5.0[b]	36.5 ± 0.9	31.5 ± 1.9	53 ± 4[a]
2Decaff	52.1 ± 4.1[a]	38.8 ± 3.5	31.3 ± 2.9	60 ± 3[a]
2Basal-PFD	74.5 ± 3.2[b]	39.4 ± 1.4	34.9 ± 1.1	58 ± 3[a]

[a] Means for a study in a column without a common superscript letter are significantly ($p < 0.05$) different. In micrograms/gram wet weight. [b] Apparent absorption = [(intake − fecal losses)/intake] × 100. [c] Mean ± SEM.

During the first 7 days of iron repletion, rats fed the basal diet ad libitum accumulated more red blood cell mass than the other rats. The iron contents of the diets were similar. Thus, lower iron intake and ultimately lower food intake were major factors influencing the initially slower rise in hematocrits and smaller accumulation of red blood cell mass of rats fed coffee and their pair-fed controls, but perhaps not of rats fed decaffeinated coffee and their pair-fed control. During the first 7 days of the repletion phase of the study, iron intake of rats fed diets 2Basal, 2Coffee, 2Basal-PFC, 2Decaff, and 2Basal-PFD were 0.52 (mean), 0.37, 0.34, 0.49, and 0.48 mg of Fe/day, respectively.

Although the dietary treatments had few hematological effects, the treatments did affect tissue iron levels in rats. In study 1, rats fed coffee had significantly higher concentrations of iron in livers and kidneys than rats fed diets 1Basal and 1Decaff (Table IV). Cohen and Booth (1975) observed ingestion of coffee increased gastric acid secretion; this could result in more iron being in the ferrous state and hence could promote absorption. Similarly Muñoz et al. (1986) observed 3-day-old pups of rats given coffee throughout gestation and lactation had elevated concentrations of iron in their livers. However, these changes probably reflect the smaller size of rats fed coffee in study 1 because the dietary treatments did not affect the total amount of iron in the livers or kidneys of rats. For example, the total amount of iron in the livers of rats fed diets 1Basal, 1Coffee, and 1Decaff were 468, 452, and 492 μg of Fe/liver, respectively. Moreover, in study 2 there were no difference in the concentrations of iron in the livers

Table V. Copper and Zinc Metabolism of Rats Fed Diets Containing Coffee and Decaffeinated Coffee

treatment	liver Cu,[a] μg/g wet wt	kidney Cu, μg/g wet wt	app ads of Cu,[b] %	plasma Cu, μg/100 mL	plasma ceruloplasmin,[c] U/L	tibia Zn,[a] μg/g wet wt	app abs of Zn,[b] %
				Study 1			
1Basal	5.8 ± 0.3[d a]	4.4 ± 0.3	28 ± 4	76 ± 2		156 ± 3[a]	67 ± 4
1Coffee	9.0 ± 0.6[b]	5.2 ± 0.2	27 ± 5	79 ± 4		213 ± 5[c]	56 ± 6
1Decaff	7.3 ± 0.5[a]	5.5 ± 0.5	23 ± 4	81 ± 2		177 ± 5[b]	68 ± 3
				Study 2			
2Basal	7.2 ± 0.2[a]	5.7 ± 0.4	27 ± 4	92 ± 4	53 ± 7	173 ± 4[a]	40 ± 2
2Coffee	9.3 ± 0.7[b]	6.7 ± 0.2	19 ± 5	97 ± 2	63 ± 6	197 ± 3[b]	34 ± 5
2Basal-PFC	6.8 ± 0.3[a]	5.5 ± 0.2	24 ± 2	89 ± 5	45 ± 6	180 ± 4[a]	35 ± 4
2Decaff	9.2 ± 0.6[b]	6.3 ± 0.3	16 ± 4	81 ± 2	46 ± 6	191 ± 2[b]	28 ± 4
2Basal-PFD	6.9 ± 0.2[a]	6.1 ± 0.4	28 ± 2	90 ± 3	51 ± 7	183 ± 4[a]	37 ± 2

[a] Means from a study in a column without a common superscript letter are significantly ($p < 0.05$) different. [b] Percent absorption = [(intake − fecal losses)/intake] × 100. [c] Micromoles of *O*-diansidine dihydrochloride oxidized/minute per liter of plasma. [d] Mean ± SEM.

Table VI. Calcium, Magnesium, and Phosphorus Utilization of Rats Fed Diets Containing Coffee and Decaffeinated Coffee

	tibia, mg/g wet wt			kidney Mg,[a]	app abs, %		
treatment	Ca[a]	Mg[a]	P[a]	μg/g wet wt	Ca[b]	Mg[b]	P[b]
			Study 1				
1Basal	147 ± 4[c a]	2.78 ± 0.08[a]		218 ± 5	76 ± 1	76 ± 3	
1Coffee	164 ± 4[b]	3.21 ± 0.09[b]		222 ± 4	77 ± 2	77 ± 2	
1Decaff	156 ± 3[a,b]	2.0 ± 1.18[a]		223 ± 5	72 ± 2	70 ± 3	
			Study 2				
2Basal	145 ± 2[a]	2.58 ± 0.04[a]	71.7 ± 0.7[a]	184 ± 1[a]	58 ± 1	77 ± 1	68 ± 1
2Coffee	152 ± 1[b]	2.85 ± 0.03[d]	74.6 ± 0.4[b]	197 ± 2[b]	59 ± 3	79 ± 2	72 ± 2
2Basal-PFC	149 ± 2[a,b]	2.66 ± 0.03[a,b]	72.2 ± 0.4[a]	185 ± 4[a]	53 ± 3	77 ± 2	68 ± 1
2Decaff	150 ± 2[b]	2.71 ± 0.02[b,c]	73.1 ± 0.8[a,b]	190 ± 3[a,b]	53 ± 6	77 ± 3	69 ± 2
2Basal-PFD	151 ± 1[b]	2.73 ± 0.02[c]	73.2 ± 0.3[a,b]	190 ± 2[a,b]	58 ± 2	78 ± 1	70 ± 1

[a] Means from a study in a column without a common superscript letter are significantly ($p < 0.05$) different. [b] Percent apparent absorption = [(intake − fecal losses)/intake] × 100. [c] Mean ± SEM.

and kidneys of rats fed coffee and those rats pair-fed the control diet (treatments 2Coffee and 2Basal-PFC) although the rats fed coffee had higher liver iron levels than rats fed the control diet ad libitum.

In order to increase the sensitivity of the rats in terms of iron absorption to the dietary treatments in study 2, rats were made anemic by dietary means prior to study 2 and were fed a lower level of iron and a higher level of zinc in study 2 than in study 1. This may also account for the slightly higher percent absorption of iron observed in study 2 than in study 1.

In study 2 rats fed decaffeinated coffee had significantly lower levels of iron in their livers than animals fed the four other diets. Furthermore, rats fed decaffeinated coffee and the control diets (2Basal, 2Basal-PFC, 2Basal-PFD) absorbed on a percentage basis significantly less iron than rats fed coffee. The feces of rats fed the decaffeinated coffee were blacker and softer than those of rats fed coffee. Negative effects of decaffeinated coffee on apparent absorption of iron, the initial increase in hematocrit after iron depletion, and liver iron levels may reflect the prolonged heat treatment of decaffeinated coffee. This processing evaporates solvents used to decaffeinate the beans and may intensity flavors (Sivetz and DesRosier, 1979; Pintauro, 1975). Extra roasting might increase the production of Maillard reaction products, which have been demonstrated to depress zinc absorption (Lykken et al., 1986). These Maillard reaction products are identified as part of dietary fiber by neutral detergent fiber procedures (Hartley, 1978). However the control, coffee-containing, and decaffeinated coffee-containing diets in this study contained 5.1, 5.0, and 5.2% neutral detergent residue, respectively; the instant coffee and instant decaffeinated coffee contained undetectable and 0.02% neutral detergent residue. Thus, neither coffee nor decaffeinated coffee added significant amounts of presumably nondigestible materials to the diets that could have adversely affected mineral absorption.

The diets containing decaffeinated coffee (1Decaff, 2Decaff) contained more magnesium and phosphorus than the basal diets but so did the diets containing coffee (diets 1Coffee and 2Coffee). Thus, differences in iron utilization by rats fed decaffeinated coffee cannot be attributed to differences in the magnesium and/or phosphorus content of diets containing dicaffeinated coffee. Perhaps the potent secretogogue in decaffeinated coffee suggested by Cohen and Booth (1975) altered iron utilization.

In both studies 1 and 2, the dietary treatments affected the copper levels in livers of rats. In study 1, rats fed coffee had significantly higher concentrations of copper in their livers than rats fed decaffeinated coffee or the control diet ad libitum (Table V). But, the rats fed decaffeinated coffee had significantly more total copper in their livers than rats fed diets 1Basal and 1Coffee (i.e., 64 vs. 47 and 52 μg, respectively). In study 2, rats fed coffee and decaffeinated coffee had significantly higher concentrations of copper in their livers than rats fed the control diets. There was no evidence that coffee or decaffeinated coffee affected apparent absorption of copper. Although plasma copper and ceruloplasmin and kidney copper levels tended to be greater in rats fed coffee, no differences were significant.

Little has been published on the effects of caffeine on copper metabolism, but DiSilvestro and Harris (1983) noted that injection of polyphenols (i.e., catechin) stimulated lysl oxidase (a copper-dependent enzyme) activity in chicks fed adequate but not copper-deficient diets. Coffee solids are estimated to contain about 5% polyphenols (Sivetz and DesRosier, 1979), and ingestion of these polyphenols may have affected copper metabolism in rats fed diets containing coffee or decaffeinated coffee.

In light of our data, we hypothesize that the effect of coffee on the incorporation of ^{59}Fe into red blood cells

Table VII. Physical Properties of Bones of Rats Fed Diets Containing Coffee and Decaffeinated Coffee in Study 2

treatment	elastic load, kg	max load,[a] kg	limit of elasticity, kg/mm^2	ultimate stress, kg/mm^2
2Basal	1.59 ± 0.08[b]	2.22 ± 0.11[b]	21.9 ± 1.6	30.9 ± 2.5
2Coffee	1.30 ± 0.09	1.74 ± 0.10[a]	23.5 ± 1.2	31.4 ± 1.8
2Basal-PFC	1.51 ± 0.06	1.92 ± 0.07[a]	25.0 ± 3.8	31.8 ± 4.8
2Decaff	1.46 ± 0.06	1.89 ± 0.18[a]	21.6 ± 2.7	27.9 ± 3.5
2Basal-PFD	1.39 ± 0.08	1.87 ± 0.08[a]	19.4 ± 1.4	26.1 ± 1.5

[a] Means in a column without a common superscript letter are significantly ($p < 0.05$) different. [b] Means ± SEM.

observed by Morck et al. (1983) was not due to an inhibition of iron absorption by coffee. Rather we hypothesize that ingestion of coffee disturbed iron metabolism during hematopoesis or during iron transport and mobilization perhaps by interfering with copper utilization. Similarly Muñoz et al. (1986) recently suggested that maternal coffee intake impaired mobilization of minerals from the liver reserves of pups and thus interfered with hematopoesis.

In both studies, rats fed coffee or decaffeinated coffee had significantly elevated levels of zinc in their tibias (Table V). The effect of coffee was greater than that of decaffeinated coffee in study 1 only. Palm et al. (1984) observed that rats given coffee as their sole fluid had elevated serum levels of alkaline phosphatase, a zinc-containing enzyme. They did not monitor tissue levels of zinc.

The dietary treatments had no effect on liver and kidney levels (not reported in Table V) or apparent absorption of zinc. Similarly Yeh et al. (1986) observed the injections of caffeine did not affect the zinc balances of animals.

Bone and Macro Mineral Metabolism. Several investigators have hypothesized that ingestion of caffeine-containing beverages may lead to excessive loss of calcium, phosphorus, and magnesium in the urine and ultimately lead to poorer nutritional status in those elements (Heaney and Recker, 1982; Massey and Wise, 1984; Massey and Berg, 1985; Yeh et al., 1986). However, Massey and Wise (1984) and Massey and Berg (1985) only assessed urinary calcium levels for 3 h after a dose of decaffeinated coffee with added caffeine.

Our data did not suggest that coffee had a detrimental effect on nutritional status in regard to calcium. In study 1 rats fed coffee had significantly elevated concentrations of calcium and magnesium in their bones (Table VI), but the total amounts of calcium and magnesium in the bones of rats fed coffee were less than the amounts in the bones of rats fed diets 1Basal or 1Decaff (37 vs. 46 and 47 mg of Ca/tibia and 0.72 vs. 0.87 and 0.88 mg of Mg/tibia, respectively). In study 2, rats fed coffee and decaffeinated coffee did not have significantly greater concentrations of calcium in their tibias than pair-fed controls, only greater levels than those of ad libitum fed controls.

Liver levels of calcium, magnesium, and phosphorus and kidney levels of calcium and phosphorus were not affected by the dietary treatments and are not reported in Table VI. The greater concentration of magnesium in the kidneys of rats fed coffee rather than control diets 2Basal and 2Basal-PFC may reflect the elevated level of magnesium in the coffee-containing diet. However, the decaffeinated coffee-containing diet also contained excess magnesium, and the levels of magnesium in the kidneys of rats fed this diet were not elevated.

The ulnae of rats fed coffee and decaffeinated coffee and their pair-fed controls sustained significantly smaller maximum loads than those of rats fed the control diet ad libitum (Table VII). These results probably reflect the fact that all of the test rats and pair-fed control rats tended to have smaller ulnae than rats fed the control diet ad libitum. There were no significant differences among treatment groups in regard to the ultimate strength and elasticity of bones as judged by standardized measurements and formulae that corrected for bone size (Beary, 1969; Crenshaw et al., 1981; Wolinsky et al., 1972). Thus, the elevated concentrations of calcium, magnesium, and phosphorus in the bones of rats fed coffee appeared to have no practical effects on bone strength. Further studies are needed to assess the effects of longer exposure to coffee on bone strength especially in older animals.

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Registry No. Fe, 7439-89-6; Cu, 7440-50-8; Zn, 7440-66-6; Ca, 7440-70-2; Mg, 7439-95-4; P, 7723-14-0; Mn, 7439-96-5; caffeine, 58-08-2.

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A Comparison of Three Bioassay Techniques for the Detection of Chloramphenicol Residues in Animal Tissues

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In two experiments a total of six young steers weighing 135–196 kg were injected intramuscularly with 11 mg of chloramphenicol (CAP) in propylene glycol/kg body weight twice daily for 3 days. Another steer was injected with double that dose, and two steers were left untreated. Eighteen hours after the last CAP injection all steers were euthanized with a barbiturate overdose. Samples of kidney, liver, and muscle were collected from each steer. No microbial inhibition was obtained with any of the tissues with the swab test on premises (STOP). Only kidney from the steer given 22 mg of CAP/kg was positive by the microbial inhibition test. All muscle samples were positive for CAP by thin-layer chromatography/bioautography (TLCB). The CAP detection limit was 0.6 ppm for TLCB and 5 ppb for a gas chromatographic CAP method. Levels of CAP in muscle and liver decreased when stored at −20 °C.

Until recently, the antibiotic chloramphenicol (CAP) was used in Canada in food-producing animals because of its broad-spectrum antibacterial properties against pathogenic bacteria (Blood et al., 1983; Huber, 1982; Knight, 1981). CAP has been implicated in the occurrence of aplastic anemia in humans (Schmidt, 1983; Settepani, 1984), and as a consequence the use of CAP in food-producing animals has been prohibited in Canada, while the Food and Drug Administration of the United States has never approved the use of CAP in food-producing animals (Knight, 1981; Settepani, 1984). However, the illegal use of CAP in food-producing animals continues to be a concern.

Monitoring for antibiotic residues is done in Canadian abattoirs using the swab test on premises (STOP) (Johnston et al., 1981) and by the identification of suspect animals. The STOP procedure detects the presence of microbial inhibitors but does not identify them. A more specific screening procedure for antibiotics using thin-layer chromatography/bioautography (TLCB) is currently being tested in this laboratory as a confirmatory procedure (Neidert et al., 1987). This will replace, as a confirmatory laboratory test, a microbial inhibition test (MIT) previously used for this purpose. TLCB has been used for the detection and semiquantitation of CAP (Bogaerts et al., 1984). Gas–liquid chromatography (Nelson et al., 1983; USDA–FSIS, 1983) has also been used for CAP analysis. The current investigation was undertaken to compare the results obtained with the three bioassays (STOP, MIT, TLCB) for the detection of CAP residues in tissues from treated animals. For further comparison samples in the second half of the investigation were also analyzed by a quantitative gas chromatographic method for the determination of CAP residues in tissues (USDA–FSIS, 1983).

The effect of sample storage time prior to analysis on CAP levels in tissue was also investigated. Samples were stored at −20 °C and reanalyzed after 2, 5, and 12 or 13 weeks.

MATERIALS AND METHODS

1. Experimental Design and Animal Treatment. *Experiment 1.* Five young Holstein steers weighing 135–165 kg were purchased from a local dairy and maintained for 6 days. Three of the steers were then injected with 11 mg of CAP in propylene glycol/kg body weight twice daily for 3 days (Rogar-Mycine 500, Rogar/STB, London, Ontario), and the other two steers were untreated controls. On days 1 and 3 CAP was injected into the right gluteal muscle, and on day 2 it was injected into the right semitendinosis muscle. Eighteen hours after the last CAP injections, all five steers were euthanized with a barbiturate overdose. Samples (200 g) of kidney and liver, and muscle contralateral to the injection sites, were collected from each animal. Tissues for initial TLCB were immediately homogenized in methanol in the necropsy room. Tissues for STOP and MIT assays were stored 3 h with ice packs before the tests were conducted. The remaining tissues were immediately frozen with dry ice and then stored at −20 °C for 2, 5, and 13 weeks for TLCB analysis.

Experiment 2. Four young Holstein steers were purchased from a local dairy and maintained for 18 days. Three of the steers were then injected with 11 mg of CAP in propylene glycol/kg body weight, and the fourth steer was injected with 22 mg of CAP/kg body weight. At the time of the first injection the steers weighed 138–196 kg. The steers were injected twice daily for 3 days. Other procedures were similar to those described for the first experiment except that the longest time storage study was 12 weeks rather than 13 weeks. Tissues were also analyzed by a quantitative gas chromatographic procedure for CAP analysis. Serum was also collected and analyzed.

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