AN ABSTRACT OF THE THESIS OF

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Title EXCRETION OF TAURINE BY NORMAL WOMEN

Abstract approved _____
Lorraine Miller

The daily urinary excretion of taurine was studied in six women, 22 to 36 years old. Five subjects collected 24-hour urine specimens for 10 days, and another for 37 days. The subjects were free from any known metabolic disorder. They consumed their normal diet.

Urinary taurine was determined by the method of Pentz et al. (1957). Taurine was separated from the other amino acids in urine by treatment with Dowex 50W (H^+), and reacted with dinitrofluorobenzene to form the dinitrophenol derivative. The derivative was measured photometrically at 360 mμ. In addition to taurine, urinary creatinine was determined by a micro-modification of the method by Folin (Hawk, Oser and Summerson, 1954).

The average daily urinary excretion of taurine during the 10 days varied widely among the six subjects, ranging from 60.9 to 196.0 mg per 24 hours. The average excretion of taurine was not related to the subject's age, height, weight or excretion of creatinine.

The daily excretion of taurine by each subject varied. Three subjects excreted relatively constant amounts of taurine during the 10-day period; their excretion ranged from 51.0 to 83.3 mg, 66.0 to 105.0 mg and 123.3 to 163.5 mg per 24 hours. The remaining three subjects showed greater variation in taurine execretion; their excretion ranged from 79.5 to 145.5 mg, 84.8 to 187.5 mg and 154.4 to 337.5 mg per 24 hours. This variation in the daily urinary excretion of taurine may have been caused by differences in protein, amino acid and free taurine content of the diet, or stress.

A subject who received ACTH to control an allergic reaction to dinitrofluorobenzene excreted increased amounts of taurine on the days the hormone was administered. This subject received seven injections of an ACTH preparation between the 17th and 37th day of urine collection. Taurine excretion was greater on the days ACTH was received than on the day immediately preceding or following each injection.

The ingestion of oral contraceptives by two of the subjects did not appear to affect the urinary excretion of taurine. Menstruation appeared to affect the taurine excretion by the two subjects who menstruated during the 10-day period.

Results reported by Merrow et al. (1966) indicate that taurine in plasma may be more indicative of vitamin B_6 nutriture than that in urine. In view of this, study on the relationship of taurine in plasma to that excreted in urine by adequately nourished individuals and ones deficient in vitamin B_6 would be of considerable interest. Since the diet consumed by the subjects could affect the urinary excretion of taurine, it is recommended that the subjects be fed a constant diet of known protein, amino acid, taurine and vitamin B_6 content.

EXCRETION OF TAURINE BY NORMAL WOMEN

by

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EXCRETION OF TAURINE BY NORMAL WOMEN

INTRODUCTION

Taurine is a normal constituent of urine (Dent, 1948). Rats deficient in vitamin B_6 excreted little or no taurine in urine (Blaschko, Datta and Harris, 1953; Hope, 1955; Bergeret and Chatagner, 1956). This has been attributed, in part, to the decreased activity of cysteinesulfinic acid decarboxylase (Hope, 1955; Greengard and Gordon, 1963), a pyridoxal phosphate-dependent enzyme involved in the conversion of cysteine to taurine. Cysteinesulfinic acid decarboxylase is very sensitive to vitamin B_6 deficiency. Most of the activity of cysteinesulfinic acid decarboxylase of the liver was lost after a few days in rats fed a diet low in vitamin B_6; after two weeks of vitamin B_6 deficiency, no enzyme activity was observed (Hope, 1955).

Human subjects depleted of vitamin B_6 showed no consistent trend in the urinary excretion of taurine (Swan, Wentworth and Linkswiler, 1964; Merrow et al., 1966). However, the effect of vitamin B_6 deficiency in humans on the urinary excretion of taurine cannot be adequately evaluated because data on taurine excretion by normal individuals are limited. Furthermore, little is known about the daily variation in the urinary excretion of taurine. Most of the values for taurine were obtained from single

24-hour urine specimens.

The purpose of this thesis is to review the factors affecting the urinary excretion of taurine and to report the daily excretion of taurine by six women.

REVIEW OF LITERATURE

Function of Taurine

Taurine (2-aminoethanesulfonic acid) is found in the tissues of man, animals and marine invertebrates (Meister, 1965; Holden, 1962). It is excreted in urine (Boquet and Fromageot, 1965a), and at one time was considered only an excretory product (Schmidt, von Adelund and Watson, 1918; Schmidt and Allen, 1920). Taurine is a component of taurocholic acid (Tarver and Schmidt, 1942; Portman and Mann, 1954), a bile acid found in man and animals, and phosphotaurocyamine (van Thoai and Roche, 1960), a high-energy phosphate found in the muscle of marine invertebrates. Taurine can be deaminated to isethionic acid in the heart muscle of dogs (Read and Welty, 1962, 1963; Welty and Read, 1964), the axoplasm of the squid (Koechlin, 1955, as cited by Roe and Weston, 1965), and the brain of the rat (Peck and Awapara, 1967). Isethionic acid appears to be involved in the transmission of nerve impulses.

Synthesis of Taurine from Sulfur-Containing Amino Acids

Taurine is synthesized from cysteine. Oral administration of cysteine to the rat caused an increase in the urinary excretion of taurine (Awapara, 1950, 1955a, 1956; Portman and Mann, 1956). After cysteine-S^{35} was administered to the rat by injection,

labeled taurine was isolated in the heart, muscle, spleen, intestine, kidney, brain, testes and liver (Awapara, 1957).

Methionine, after it is converted to cysteine, can also be a precursor of taurine (figure 1). In the formation of cysteine from methionine, methionine is first demethylated to homocysteine. Homocysteine combines with serine to form cystathionine (Meister, 1965), a reaction catalyzed by the pyridoxal phosphate-dependent enzyme, transsulfurase (Binkley, 1951; Binkley, Christensen and Jensen, 1952; Matsuo and Greenberg, 1958a, b, 1959). Cystathionine is subsequently cleaved by cystathionase, another pyridoxal phosphate-dependent enzyme (Binkley et al., 1952; Matsuo and Greenberg, 1958a, b, 1959; Chatagner and Trautman, 1962), to homoserine and cysteine (Meister, 1965). Dogs and rats fed methionine-S^{35} excreted labeled cystathionine (Tabachnick and Tarver, 1955) and taurine (Tarver and Schmidt, 1939, 1942) in urine. Rats deficient in vitamin B_6 excreted increased amounts of cystathionine and decreased amounts of taurine in urine (Hope, 1957b; Chatagner, Tabechian and Bergeret, 1954).

Major Pathway of Synthesis from Cysteine. The pathways for the synthesis of taurine from cysteine are presented in figure 1.

Taurine is synthesized mainly from cysteine through cysteinesulfinic acid and hypotaurine (Meister, 1965). In this

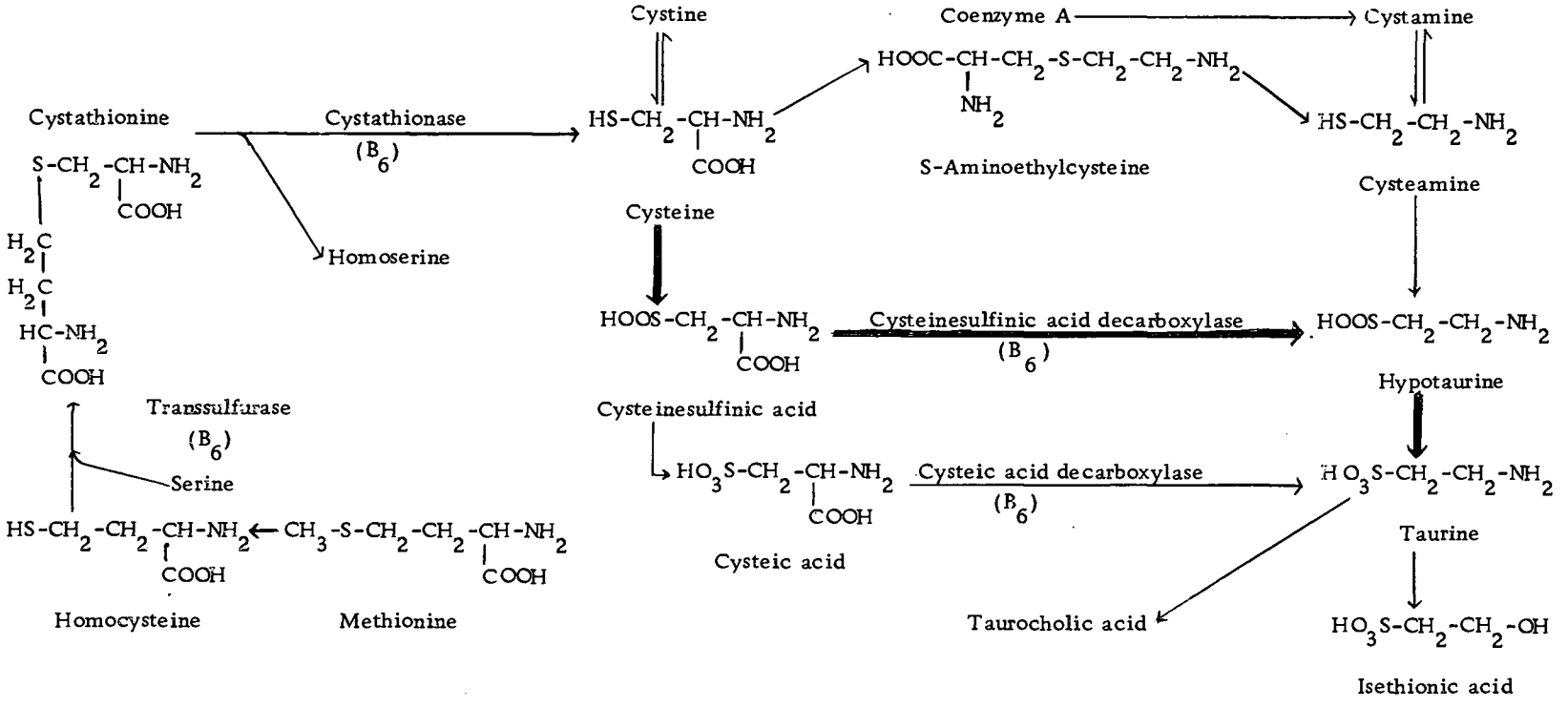

Figure 1. Pathways for the Synthesis of Taurine. These pathways were adapted from Meister, 1965, p. 816, 817. The major pathway for the synthesis of taurine is indicated by the heavier arrors. Reactions requiring pyridoxal phosphate-dependent enzymes are designated with (B_6). Taurine may be converted to isethionic acid and taurocholic acid.

pathway cysteine is first oxidized to cysteinesulfinic acid. This conversion was first shown by Awapara and Doctor (1955) in a rat liver homogenate to which magnesium, adenosine triphosphate, nicotinamide adenine dinucleotide and cytochrome c had been added. Later, Sorbo and Ewety (1965) demonstrated that the necessary cofactors for the oxidation of cysteine to cysteinesulfinic acid by an active factor in rat liver homogenate were oxygen, ferrous iron and reduced pyridine nucleotides.

Cysteinesulfinic acid is decarboxylated by cysteinesulfinic acid decarboxylase, a pyridoxal phosphate-dependent enzyme (Hope, 1955; Greengard and Gordon, 1963; Chatagner, 1964; Bergeret, et al., 1955) to form hypotaurine. The oxidation of cysteine and the decarboxylation of cysteinesulfinic acid appear to occur rapidly. Twenty minutes after cysteine-S^{35} was administered to rats by injection, cysteinesulfinic acid accounted for approximately 1% of the total radioactivity in the liver extract, and hypotaurine 25% (Chapeville and Fromageot, 1955).

Hypotaurine is oxidized to taurine by hypotaurine dehydrogenase, an enzyme requiring nicotinamide adenine dinucleotide (Sumizu, 1962). That hypotaurine is converted to taurine was suggested from results obtained in earlier studies. Awapara and Wingo (1953b) found that cysteine-S^{35} began to accumulate in the liver ten minutes after cysteine-S^{35} was

given to the rat by injection. At the end of 20 minutes hypotaurine-S^{35} was found in small amounts in the liver, and after 30 minutes taurine-S^{35} began to appear. Chapeville and Fromageot (1955) identified hypotaurine-S^{35} as the precursor of taurine-S^{35} in rat liver and kidney after cysteine-S^{35} was administered to the rat by injection. Bergeret et al. (1955), also reported that hypotaurine is the precursor of taurine found in liver and brain homogenates of rats deficient in vitamin B_6 and control rats.

Alternate Pathways. An alternate pathway for the formation of taurine from cysteine is through cysteic acid. When cysteic acid was added to a liver suspension, the concentration of taurine increased while the concentration of cysteic acid decreased (Blaschko et al, 1953). Dogs (Virtue and Doster-Virtue, 1939) and rats (Wellers, 1960) excreted increased amounts of taurine in urine after cysteic acid was fed.

Cysteic acid is decarboxylated by cysteic acid decarboxylase to form taurine. Vitamin B_6 is necessary for the activity of cysteic acid decarboxylase (Blaschko et al., 1953). Hope (1955) suggested that cysteic acid and cysteinesulfinic acid may be decarboxylated by the same enzyme.

Cavallini, Mondovi and De Marco (1955) found S-aminoethylcysteine, a product formed by transsulfuration

between cysteine and aminoethanol (Meister, 1965), could be converted by the rat to cysteamine (2-mercaptoethylamine), cystamine and taurine. Cystamine can also arise as a degradation product of coenzyme A (Meister, 1965). Cavillini, De Marco and Mondovi (1961) later identified the enzyme system converting cystamine to hypotaurine. Rats deficient in vitamin B_6 converted 37.5% of the cysteamine in the diet to taurine (Hope, 1957).

Effect of Vitamin B_6 Deficiency on the Pyridoxal Phosphate-Dependent Enzymes Involved in the Metabolism of Cysteine

Cerecedo, Foy and De Renzo (1948) were the first to suggest that vitamin B_6 is necessary for the metabolism of the sulfur-containing amino acids when they reported that supplements of cysteine and methionine to a diet low in protein caused a lowered resistance to vitamin B_6 deficiency in rats. That high intakes of methionine increased the rate of vitamin B_6 depletion in rats was also reported later by Linkswiler, Baumann and Snell (1951) and Merrow et al. (1966).

Pyridoxal phosphate is required as a coenzyme in the metabolism of other amino acids. In vitamin B_6 deficiency, pyridoxal phosphate-dependent enzymes are inactivated at different rates. According to Tower (1956), the enzyme most susceptible to vitamin B_6 deficiency is liver cysteinesulfinic acid

decarboxylase. Following cysteinesulfinic acid decarboxylase, the order of decreasing susceptibility of other pyridoxal phosphate-dependent enzymes in the liver to vitamin B_6 deficiency are: cysteinesulfinic acid transaminase, glutamic-pyruvic transaminase, glutamic decarboxylase, glutamic-aspartic transaminase, and 5-hydroxytryptophan decarboxylase.

Most of the activity of liver cysteinesulfinic acid decarboxylase was lost in rats fed a vitamin B_6-deficient diet for a few days (Hope, 1955; Greengard and Gordon, 1963), and no enzyme activity was detected after two weeks of deficiency (Hope, 1955). Only in the early stages of deficiency could administration of vitamin B_6 reactivate the enzyme. Greengard and Gordon (1963) suggested that pyridoxine not only acts as a coenzyme, but also as a regulating agent for the production of the protein moiety of cysteinesulfinic acid decarboxylase.

Cysteinesulfinic acid decarboxylase in the brain has a greater affinity for pyridoxal phosphate than cysteinesulfinic acid decarboxylase in the liver. In normal animals the enzyme in the liver appears to be saturated with pyridoxal phosphate, while the enzyme in the brain appears to be unsaturated. In rats deficient in vitamin B_6, the activity of cysteinesulfinic acid decarboxylase in liver decreased while the activity of this enzyme in the brain was not changed (Bergeret et al., 1955). Hope (1957a)

found that rats fed a diet deficient in vitamin B_6 for 11 weeks excreted an average of 1.1 mg of taurine per day in urine while the controls excreted an average of 18.6 mg. The taurine content of the brains of the vitamin B_6-deficient rats, however, was similar to that of the brains of the controls. Hope concluded that the activity of cysteinesulfinic acid decarboxylase in brain and nervous tissue in vitamin B_6-deficient animals was maintained after the activity of the liver enzyme had disappeared. Hope suggested that the nervous system could be the source of small amounts of taurine found in urine during vitamin B_6 deficiency. Another possible source of urinary taurine in vitamin B_6 deficiency suggested by Hope, is the breakdown of cysteamine.

Urinary Excretion of Taurine in Vitamin B_6 Deficiency and Dependency

In rats the urinary excretion of taurine decreases in vitamin B_6 deficiency. Blaschko et al. (1953) found that 12 out of 14 rats fed a diet deficient in vitamin B_6 for 33 or 47 days excreted no taurine, while all of the controls excreted taurine.

The excretion of taurine by an infant with a vitamin B_6 deficiency increased after receiving pyridoxine (Scriver and Hutchins, 1963). Before treatment the urinary excretion of taurine by a 20-month-old infant was 0.12 μM per 1.73 M^2 of surface area.

and 0.35 μM after receiving 15 mg of pyridoxine daily for three months. The taurine concentration of the plasma increased from 0.04 μM per ml to 0.13 μM per ml after pyridoxine therapy. There was no comparable increase in the other amino acids, either in the urine or plasma.

An infant with vitamin B_6 dependency excreted 3.8 mg of taurine per 24 hours while receiving 10 mg of pyridoxine per day. When the supplement of pyridoxine was withdrawn, the urinary excretion of taurine was reduced to 1.7 mg per 24 hours (Scriver, 1960).

Urinary Excretion of Taurine in Vitamin B_6 Deficiency Before and After Loading with Cysteine or Methionine

Since cysteinesulfinic acid decarboxylase appears to be one of the first pyridoxal-phosphate-dependent enzymes to be inactivated by a dietary deficiency of vitamin B_6, McAfee and Williams (1962) suggested that the excretion of taurine after a load dose of cysteine could be used as an indication of vitamin B_6 deficiency. McAfee and Williams found that young rats fed a vitamin B_6-deficient diet for three weeks excreted from 0.01 to 0.05 mg of taurine per 24 hours, while control rats excreted 0.11 mg. When 200 mg of L-cysteine-hydrochloride-monohydrate were administered orally to the animals, the

vitamin B_6-deficient rats excreted from 0.06 to 0.09 mg of taurine, or less than 0.10% of the cysteine supplement as taurine, while the controls excreted 1.7 and 1.8 mg of taurine, or about 1% of the loading dose as taurine. When 100 μg of pyridoxine and 200 mg of L-cysteine-hydrochloride-monohydrate were administered together to both groups of animals, the vitamin B_6 deficient rats excreted from 0.05 mg to 0.09 mg of taurine per 24 hours and the controls excreted 2.3 and 2.4 mg. These increases in the urinary excretion of taurine accounted for 0.10% and 1.60%, respectively, of the cysteine loading dose (McAfee and Williams, 1962).

Similar results were obtained by Merrow et al. (1966). Rats fed a diet deficient in vitamin B_6 for four or five weeks excreted 5.9 μg of taurine per g of body weight before a load dose of methionine and 10.1 μg per g after the administration of 14 mg of DL-methionine. The controls excreted 22.2 μg of taurine per g of body weight and 26.2 μg per g before and after methionine, respectively. After the rats deficient in vitamin B_6 and their controls were fed a diet containing 2200 μg % of vitamin B_6 for two weeks, the deficient rats excreted 24.5 μg of taurine per g of body weight before methionine and 41.1 μg per g after methionine, while the controls excreted 24.5 μg per g and 37.4 μg per g, respectively.

No consistent trend in the urinary excretion of taurine was found among adult human subjects consuming diets low in vitamin B_6 for short periods of time. Swan et al. (1964) fed six men, 23 to 36 years old, a partially purified diet containing 0.16 mg of vitamin B_6 and 100 g of protein daily. After eight days of vitamin B_6 depletion the subjects excreted from 791 to 1318 μmoles (99.2 to 165.3 mg) of taurine per 24 hours. The urinary excretion of taurine by the subjects after 23 or 36 days of vitamin B_6 depletion was the same or had changed only slightly since the eighth day of depletion. When the subjects ingested five g of L-cysteine-hydrochloride-monohydrate, urinary taurine accounted for 0.2 to 1.0% of the load dose after eight days of vitamin B_6 depletion, and 0.0 to 0.8% after 26 or 36 days. When the subjects were given a supplement of 50 mg of pyridoxine daily for three days, the subjects excreted from 639 to 1294 μmoles (80.1 to 162.3 mg) of taurine before cysteine loading. After the administration of cysteine, the repleted subjects excreted from 1.0 to 1.7% of the load dose as taurine.

Merrow et al. (1966) fed a diet containing 0.30 mg of vitamin B_6 and one g of protein per kg of body weight to nine men. No consistent trend in the urinary excretion of taurine by the subjects depleted of vitamin B_6 was observed either before or after the administration of 6 g of DL-methionine every eight days

during the 50 days of depletion. Merrow et al. also reported that the vitamin B_6 depleted subjects excreted more taurine after a test dose of L-cysteine than after a test dose of DL-methionine when equimolar amounts of these amino acids were given. McAfee and Williams (1962) reported similar results in vitamin B_6-deficient rats.

Plasma Levels of Taurine

Merrow et al. (1966) found that the level of plasma taurine in subjects depleted of vitamin B_6 decreased even though no consistent change in the urinary excretion of taurine was observed.

Effect of Vitamin B_6 Antimetabolites on Urinary Taurine

The antimetabolites of vitamin B_6, isoniazid (INH) and deoxypyridoxine (DOP), do not cause a decrease in the urinary excretion of taurine. At the end of 60 days, four men who received a daily diet containing 0.54 mg of vitamin B_6 excreted a decreased amount of taurine, while four men who were given INH, in addition to the diet low in vitamin B_6 showed no change in the urinary excretion of taurine (Johnston and Donald, 1964). Four girls, 9 to 14 years of age, were given 200 mg of DOP per day for 10 consecutive days. Their urinary excretion of taurine increased above their base line on the first day of

administration, decreased on the second day, and rose above the base level by the tenth day of administration (McCoy and Wehrle, 1966).

Other Dietary Factors Affecting the Urinary Excretion of Taurine

The dietary intake of free taurine can affect the urinary excretion of this metabolite (Goodman, 1967). Free taurine is found in foods containing animal protein (Awapara, 1955b; Scharff and Wool, 1964; Kurtz and Luck, 1935; Holden, 1962). Beef, lamb, pork, chicken and fish muscle contain more taurine than milk, and beef, lamb and pork liver or kidney. Oysters and clams contain more free taurine than animal muscle. In the preparation of meat or fish, more taurine is retained by baking than by boiling in water (Roe and Weston, 1965).

The effect of the level of dietary protein on the urinary excretion of taurine was studied by Pentz, Moss and Denko (1959). The average daily urinary excretion of taurine by a subject consuming his usual diet was 161 mg. When this subject consumed a high protein diet (twice as much protein as he normally consumed), he excreted an average of 193 mg of taurine. The high protein intake did not, however, cause a significant change in the ratio of urinary taurine to creatinine.

"Large amounts" of alcohol caused an increase in the urinary excretion of taurine (Pentz et al. 1959).

Non-Dietary Factors Affecting the Urinary Excretion of Taurine

Several non-dietary factors also influence the excretion of taurine. Decreased amounts of taurine appeared in the urine of mongoloids (Thomas et al.,1965; King, Goodman and Thomas, 1966; Goodman, King and Thomas, 1964; Wainer et al., 1966; McCoy and Wehrle, 1966); of rats (Williams and Passman, 1960) and children (Berry, 1960) with healing wounds; and of adults with adrenocortical malfunction (Pentz et al., 1959) and Hartnup syndrome (Evered, 1956).

Increased urinary excretion of taurine was found in children with anaphylatic purpura, pneumonia, febrile convulsions, galactosemia, leukemia (Berry, 1960), infantile scurvy (Jonxis and Huisman, 1954) or following major surgery (Berry, 1960), and in an adult with diabetes insipidus (Pentz, 1959). The excretion of taurine increased sharply after the administration of ACTH or corticoids (Pentz et al., 1959; Pentz, 1964) and after exposure to irradiation (Katz and Hasterlik, 1955; Kay, Harris and Entenman, 1956; Kay, Early and Entenman, 1957; Kay and Entenman, 1959; Pentz, 1958; Cavalieri et al., 1960; Angel and Noonan, 1961; Goyer, Yin and Bowen, 1964). Rats deficient in vitamin B_6 do not excrete increased amounts of urinary taurine after irradiation (Boquet and Fromageot, 1965b).

Irradiation does not change the concentration of taurine in muscle, liver or spleen of rats (Stern and Stim, 1959; Schram, 1961; Boquet and Fromageot, 1965b).

Urinary Excretion of Taurine by Normal Human Subjects

Humans excrete relatively large amounts of taurine in urine. Stein (1953) reported that taurine along with glycine, histidine and methylhistidine accounted for 70% of the amino acids in urine. Bigwood et al. (1959) found that taurine made up 14% of the urinary amino acids. Most of the urinary taurine was excreted in the free form (Stein, 1953).

The daily urinary excretion of taurine by normal individuals is summarized in table 1. The amount of taurine excreted in urine varies considerably among individuals. Stein (1953) found that the excretion of taurine by seven men ranged from 86 to 294 mg. Evered (1956) reported that three men excreted from 35 to 81 mg of taurine, and one woman excreted 80 mg. The urine specimens of the 19 men studied by Pentz et al. (1957) contained from 44 to 514 mg of taurine; of these 19 men, 17 excreted a range of 120 to 287 mg. Hydrolyzing the urine with acid did not greatly alter the taurine content (Pentz et al., 1957).

The ranges of taurine excretion by the six men and nine women reported by Soupart (1959) were 53 to 189 mg, and 27 to

Table 1. Daily Urinary Excretion of Taurine by Normal Humans

Author	Subjects	Age	Taurine excretion		Days studied
			Average	Range	
	Number	Years	mg/24 hours		Number
		Men			
Stein, 1953	8	25-40	156	86 - 294	1
Evered, 1956	3	27-45	59	35 - 81	1
Pentz et al., 1957	19	20-40	212	44 - 514	1
	17[a]			120 - 287[a]	1
Soupart, 1959	6	28-38	104	53 - 173	1
Pentz et al., 1959	1	34	161	132 - 187	4
			89[b]	75 - 95[b]	
Cavilieri et al., 1960	5	-	138[b]	62 - 320[b]	6
Swan et al., 1964	6	23-35	169	99 - 272	1
		Women			
Evered, 1956	1	22	80	-	1
Pentz et al., 1959	20	17-54	196	115 - 331	1
Soupart, 1959	9	20-36	87	27 - 161	1
Soupart, 1960	5	-	104	35 - 224	1
		Children			
Jonxis and Huisman, 1954	2	1	-	8 - 9	1
Pentz, et al., 1959	20	4-11	-	41 - 127	1

Continued

Table 1. Daily Urinary Excretion of Taurine by Norman Humans --Continued

Author	Subjects	Age	Taurine excretion		Days studied
			Average	Range	
	Number	Years	mg/24 hours		Number
Carver and Paska, 1961	12	3-13	-	8 - 121	1

[a] Range of taurine excretion if the two extreme values are excluded.

[b] Values reported in mg of taurine/g of creatinine.

161 mg. respectively. Soupart (1960) reported a range of 35 to 244 mg for five women. Pentz et al. (1959) reported a range of 115 to 331 mg of taurine for 20 women, and Swan et al. (1964), a range of 99 to 272 mg for six men.

A few values for the urinary excretion of taurine by normal children are available. The urine of 20 children, 4 to 11 years old, contained from 41 to 127 mg of taurine (Pentz et al., 1959). Carver and Paska (1961) found that 12 children, 3 to 12 years old, excreted from 8 to 121 mg of taurine; and Jonxis and Huisman (1954) reported that two one-year old infants excreted 8 and 9 mg, respectively. In these studies the amount of taurine excreted in the urine was not related to sex.

Daily variation in the urinary excretion of taurine has been reported by Pentz et al. (1959) and Cavalieri et al. (1960). Pentz found that the daily excretion of taurine by one man for five consecutive days was 132, 187, 236, 160 and 164 mg. The increased excretion of taurine on the third day was attributed to the consumption of "generous quantities of alcohol in any form" that the subject chose. Cavalieri et al. reported a study in which five men collected urine for six consecutive days. Their urine contained an average of 138 ± 52 mg of taurine per g of creatinine.

"The values ranged from 62 to 320 mg per g, and the day to day variation in each of three subjects was greater than the variation in the entire group. There was no diurnal pattern in taurine excretion." (Cavilieri et al., 1960, p. 187).

Methods to Determine Taurine

In the early studies taurine was assayed along with several other amino acids in urine. Dent (1948), the first to report that taurine is a normal constituent of urine, used phenol collodine filter paper to study the chromatography of animo acids and other ninhydrin-reacting substances in urine. Moore and Stein (1951) separated a mixture of synthetic amino acids with a column of Dowex 50-X8 (H^+), a strongly cationic exchange resin. After the amino acids were eluted from the column, they were estimated by measuring their ninhydrin derivatives. Better separation of a mixture of synthetic amino acids was later obtained by using Dowex 50-X4 (H^+) and a gradient elution (Moore and Stein, 1954). Stein (1953) and Evered (1956) used the method of Moore and Stein (1951) to measure the amino acids in human urine; Soupart (1959) used the procedure of Moore and Stein (1954).

Methods were later developed to measure only taurine. The inability of taurine to adhere to the acid form of strongly cationic

exchange resins was utilized in several procedures. Pentz et al. (1957) developed a method based on the measurement of the dinitrophenol derivative of taurine after taurine was separated from other amino acids in urine with Dowex 50-X8. Ling (1957) devised a method similar to the one by Pentz et al., but used Amberlite IR-112 in place of Dowex 50. In a column chromatographic procedure using Dowex 50W proposed by Sorbo (1960), taurine was collected in the effluent and was determined as a derivative of ninhydrin. According to Sorbo, the specificity of this method was comparable to the method by Ling, but more specific than the procedure by Pentz et al. An additional advantage of the procedure by Sorbo is the elimination of the use of dinitrofluorozenzene which causes a pronounced skin reaction.

A method to determine the taurine content of tissues was devised by Garvin (1960). In this column chromatographic procedure, Dowex 1 (Cl^- form), a strongly anionic exchange resin, was used in addition to Dowex 50 (H^+). Taurine was measured after color development with ninhydrin.

EXPERIMENTAL PROCEDURE

Subjects

The daily urinary excretion of taurine was studied in six women. Five subjects collected 24-hour urine specimens for 10 days, and another subject for 37 days. In addition to taurine, the daily urinary excretion of creatinine by these subjects was determined.

The subjects' ages ranged from 22 to 36 years. The subjects were free from any known metabolic disease. They consumed their regular diet.

Handling of Urine Specimens

During the day of collection the urine was stored in the refrigerator under toluene in amber bottles. The following day the 24-hour urine specimen was measured in a stoppered graduated cylinder, the bottles were rinsed with redistilled water, and the rinsings were added to the urine. The urine was diluted to a convenient volume, usually 1000 or 2000 ml, and mixed thoroughly. Two-40 ml aliquots of each 24-hour urine specimen were stored at -10^{o}C. until analysis.

Determination of Taurine

The taurine content of urine was determined by a modification of the method by Pentz et al. (1957). In this method the dinitrophenol derivative of taurine was measured. Taurine was separated from the other urinary amino acids by treating the urine with Dowex 50W (H^+), a strongly cationic exchange resin. Taurine, unlike other amino acids, does not adhere to this resin (Crokaert et al., 1951, as cited by Pentz et al., 1957). Dinitrophenol taurine was obtained by reacting taurine with dinitrofluorobenzene (DNFB). Ammonia and other nitrogenous substances in the concentrations found in urine did not interfere with the determination of taurine (Pentz et al., 1957).

Equipment

1. Graduated cylinders with stoppers, 25, 50, 1000, 2000 ml.
2. Volumetric pipets, 1, 2, 3, 4, 5, 10, 15, 20 ml.
3. Funnels, short stemmed, 8 cm in diameter at the upper edge.
4. Erlenmeyer flasks, 25, 50 ml.
5. Volumetric flasks, 25, 2000 ml.
6. Centrifuge tubes with stoppers, Maizel-Gerson type, to hold approximately 40 ml.
7. Griffin beakers, 5, 50, 100, 200, 400, 600, 2000 ml.

8. Syringe pipets

   Hypodermic syringe, 1, 2, 5, 10, 20 ml.

   Syringe holders, adjusted to repeatedly deliver the same amount of solution. These were made by Northern Tool and Instrument Company, Flushing, New York 11358.

9. Tincture bottles, with screw caps, 50 ml.

10. Reagent bottles with stoppers, 200, 500, 1000, 2000 ml.

11. Whatman filter paper, No. 40, 11 cm.

12. Weighing paper, glassine.

13. Funnel support, six-place, adjustable, wood.

14. Stopwatch with holder.

15. Pipet filler, "Propipette".

16. Spatulas, micro, stainless steel.

17. Scoopulas, stainless steel.

18. Wash bottles, polyethylene.

19. Pump, water aspirator.

20. Spectrophotometer, Beckman, Model DU.

21. Analytical balance.

22. Torsion balance.

23. Trip balance.

24. pH meter, Beckman, Model G.

25. Magnetic stirrer.

Reagents

1. Dowex 50W-X8, 200 to 400 mesh, (H^+ form).

   Resin from the same lot was used in all determinations. The resin was used as received.

2. Potassium acid phthalate-sodium hydroxide buffer, pH 4.5, (phthalate buffer).

   4.0 g of sodium benzoate were placed in a beaker and 500 ml of 0.2 M potassium acid phthalate were added. The mixture was stirred to dissolve the sodium benzoate. 0.2 M sodium hydroxide was added gradually to the solution until pH 4.5 was obtained with the Beckman pH meter, Model G. During the addition of the sodium hydroxide, the solution was mixed with a magnetic stirrer. The buffer was quantitatively transferred to a 2000 ml volumetric flask and diluted to volume with redistilled water.

3. Sodium bicarbonate, 8% solution (w/v).

   160 g of sodium bicarbonate were placed in a 2000 ml volumetric flask and diluted to volume with redistilled water. In order to completely dissolve the sodium bicarbonate, the flask was allowed to stand for a while in a pan of hot tap water.

4. Dinitrofluorobenzene, (1-fluoro-2, 4-dinitrobenzene, DNFB). Eastman Organic Chemicals.
5. Hydrochloric acid, 6 N.
6. Hydrochloric acid-potassium chloride buffer, pH 1.25 (HCl-KCl buffer).

   500 ml of 0.4 M KCl were placed in a beaker and 0.4 M HCl was added gradually until pH 1.25 was obtained with the Beckman pH meter, Model G. During the addition of HCl, the solution was mixed with a magnetic stirrer. The buffer was quantitatively transferred to a 2000 ml volumetric flask and diluted to volume with redistilled water.
7. Chloroform, reagent grade.
8. Taurine, Sigma Chemical Company, Lot 83b-1500.

   Taurine stock standard. 120.0 mg of taurine were dissolved and diluted to 100 ml with phthalate buffer (1 ml = 1.20 mg).

   Taurine working standard. One ml of the stock standard was diluted to 100 ml with the phthalate buffer (1 ml = 12 μg). The standards were stored in the refrigerator.

Method for the Determination of Taurine in Urine

1. 5 ml of urine were diluted to 25 ml with phthalate buffer in a 25 ml stoppered graduated cylinder. The urine and buffer were mixed by inversion.
2. For each urine sample, 5 g of Dowex 50W were placed in a 50 ml Erlenmeyer flask.
3. 10 ml of diluted urine were added to the resin. At the same time a reagent blank was prepared by adding 10 ml of the phthalate buffer to 5 g of Dowex 50W.
4. The urine or buffer was allowed to stand with the resin at room temperature for five minutes with occasional swirling.
5. At the end of five minutes the mixture was filtered through Whatman filter paper, No. 40. The filtrate was collected in a 25 ml Erlenmeyer flask. The used resin was discarded.
6. 1 ml of urine filtrate was placed in each of two-25 ml volumetric flasks. For the reagent blank, 1 ml of buffer filtrate was placed in a 25 ml volumetric flask. Sometimes 2 ml of urine filtrate were used to obtain a higher reading in absorbance. In this case a reagent blank of 2 ml was also prepared.

7. 5 ml of 8% sodium bicarbonate were added.

8. 0.10 ml of DNFB was added. Initially 0.28 ml of DNFB was used. It was possible to use the smaller amount without altering results. See "Preliminary Studies", which follows.

9. Samples were protected from light and were shaken 16 hours (overnight) at 280 oscillations per minute on a mechanical shaker.

10. After removing the flasks from the shaker, 1 ml of 6 N HCl was added to each flask. The flasks were swirled to release some of the CO_2 formed.

11. 2 ml. of HCl-KCl buffer were added. The flasks were again swirled until the escape of CO_2 was no longer apparent.

12. 10 ml of chloroform were added.

13. Flasks were then filled to the 25 ml mark with redistilled water.

14. Contents of each 25 ml volumetric flask were transferred to a correspondingly labeled Maizel-Gerson centrifuge tube.

15. The centrifuge tubes were stoppered and shaken vigorously for one minute. The tops of the tubes were

covered with tissue to absorb any solution that would leak from the tubes during shaking.

16. 10 ml of the aqueous layer from each tube were transferred to a second centrifuge tube.

17. 15 ml of chloroform were added and the tubes were shaken vigorously 1 minute. During shaking the tops of the tubes were covered with tissue.

18. The chloroform layer, which formed at the bottom, was withdrawn with an aspirator.

19. 3 ml of the aqueous layer were read in a Beckman Spectrophotometer, Model DU, at 360 mμ. The absorption spectra obtained to determine the wavelength of maximum absorption of dinitrophenol taurine are presented below in "Preliminary Studies".

Preliminary Studies

Absorption Spectra of Dinitrophenol Taurine. To select the appropriate wavelength for spectrophotometric assay, the absorption spectra of dinitrophenol taurine were determined for three levels of taurine. To obtain dinitrophenol taurine the "Method for the Determination of Taurine in Urine" was followed starting at step 6. One, 2 and 3 ml of taurine standard (12, 24 and 36 μg, respectively) that had not been treated with resin

were reacted with 0.28 ml of DNFB.

After the derivatives were prepared, absorbance was obtained with the Beckman spectrophotometer, Model DU. Since Pentz et al. (1957) reported that the maximum absorption of dinitrophenol taurine was at 355 mμ, readings were made every 2 mμ from 340 to 370 mμ. From 300 to 340 mμ, and 370 to 450 mμ, readings were taken every 10 mμ. The absorption spectra obtained for three levels of taurine are presented in figure 2. Maximum absorption occurred at 360 mμ. This wave length was used throughout the study to measure taurine.

Amount of Dinitrofluorobenzene Used. After having determined the taurine content of approximately one-fifth of the urine specimens, it seemed desirable to reduce the amount of DNFB used in the analysis. This reagent was extremely irritating to the writer's skin.

Originally 0.28 ml of DNFB was used. To determine if smaller amounts of DNFB could be used, 0.28 ml of DNFB and several levels less than 0.28 ml were reacted with 3 ml (36 μg) of untreated taurine standard. To obtain dinitrophenol taurine the "Method for the Determination of Taurine in Urine" was followed, starting at step 6. The results were as follows:

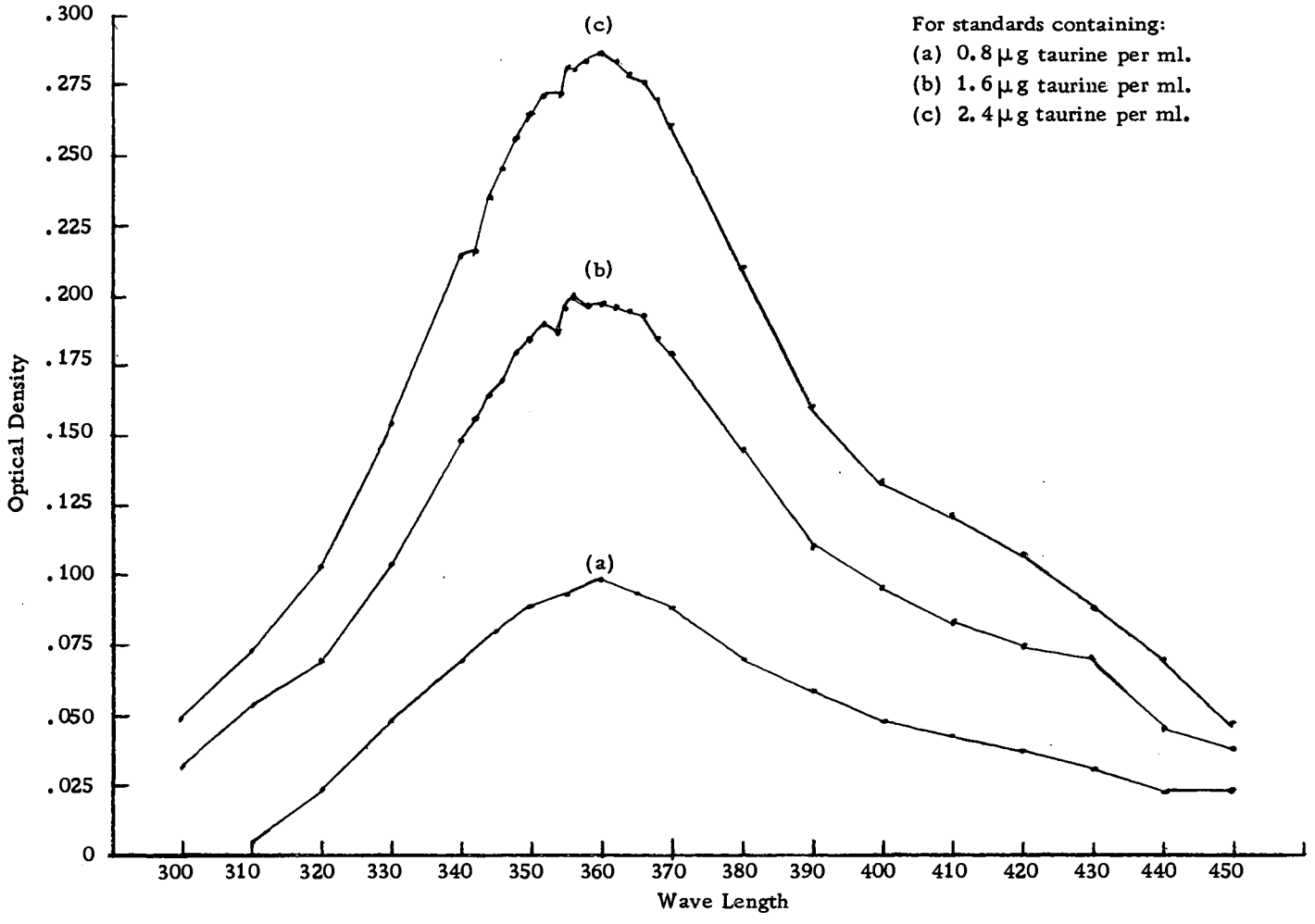

Figure 2. The Absorption Spectra for Three Levels of Dinitrophenol Taurine.

Amount of DNFB ml	Absorbance of sample 1	2
0.28	0.305	0.300
0.20	0.304	0.303
0.15	0.302	0.302
0.10	0.298	0.299
0.05	0.299	0.299
0.01	0.301	0.296

All readings compared well to those obtained when 0.28 ml of DNFB was used.

After 16 hours of shaking, a small amount of unreacted DNFB remained as a globule in all flasks except in the ones containing 0.01 ml of DNFB. To be sure that an excess amount of DNFB was available for formation of dinitrophenol taurine, the amount chosen for the remaining determinations was 0.10 ml, one-third of the suggested amount. When 0.10 ml of DNFB was used in subsequent determinations, the values obtained for the three levels of taurine standard, untreated and treated with resin used to construct the calibration curves, were similar to those obtained when 0.28 ml of DNFB was used.

Precautions

DNFB is a vesicant. Care should be taken to prevent contact of the skin with it. After having used DNFB for a short period of time, the writer's hands became blistered, swollen, calloused and cracked. Decreased dexterity and sensitivity occurred in the fingers and hands.

Chemical-resistant vinyl gloves gave the greatest protection against contact with DNFB. Since an allergic reaction to the rubber as well as to the DNFB was possible due to the irritated condition of the writer's hands, plastic surgical gloves were worn inside the vinyl gloves. After each use the vinyl gloves were thoroughly washed and the surgical gloves were discarded. Disposable or heavy plastic gloves were inadequate in protecting the skin. In addition, when there was any possibility of contact with vapors containing DNFB, work was carried out under a hood.

Formation of CO_2 caused the samples to leak from the centrifuge tubes during shaking. Several precautions in technique were taken to prevent further contact of the skin with DNFB. The volumetric flasks were swirled to release as much CO_2 as possible after the addition of 6 N HCl in step 10, and HCl-KCl buffer in step 11. In steps 15 and 17 the chloroform and aqueous

mixture was shaken in Maizel-Gerson centrifuge tubes. Less leakage occurred from the Maizel-Gerson centrifuge tubes than from the 50 ml stoppered graduated cylinders which were originally used. Also in steps 15 and 17, the tops of the Maizel-Gerson centrifuge tubes were covered with paper toweling to absorb any solution that leaked from the tubes during shaking.

Preparation of Calibration Curves for the Determination of Taurine

Two calibration curves were prepared with each set of determinations: one with taurine standard treated with Dowex 50W, and another with taurine standard not treated with the resin (figure 3).

Taurine Standard Treated with Resin. The procedure for constructing the calibration curve of taurine treated with resin was as follows:

1. 10 g of Dowex 50W were placed in a 50 ml Erlenmeyer flask.
2. 20 ml of taurine standard (12 μg per ml) were added to the resin. Because the resin contains a colored material, a reagent blank of phthalate buffer treated with resin was also prepared. The preparation of the reagent blank was the same as that for the standard except 7.5 g of Dowex 50W and 15 ml of

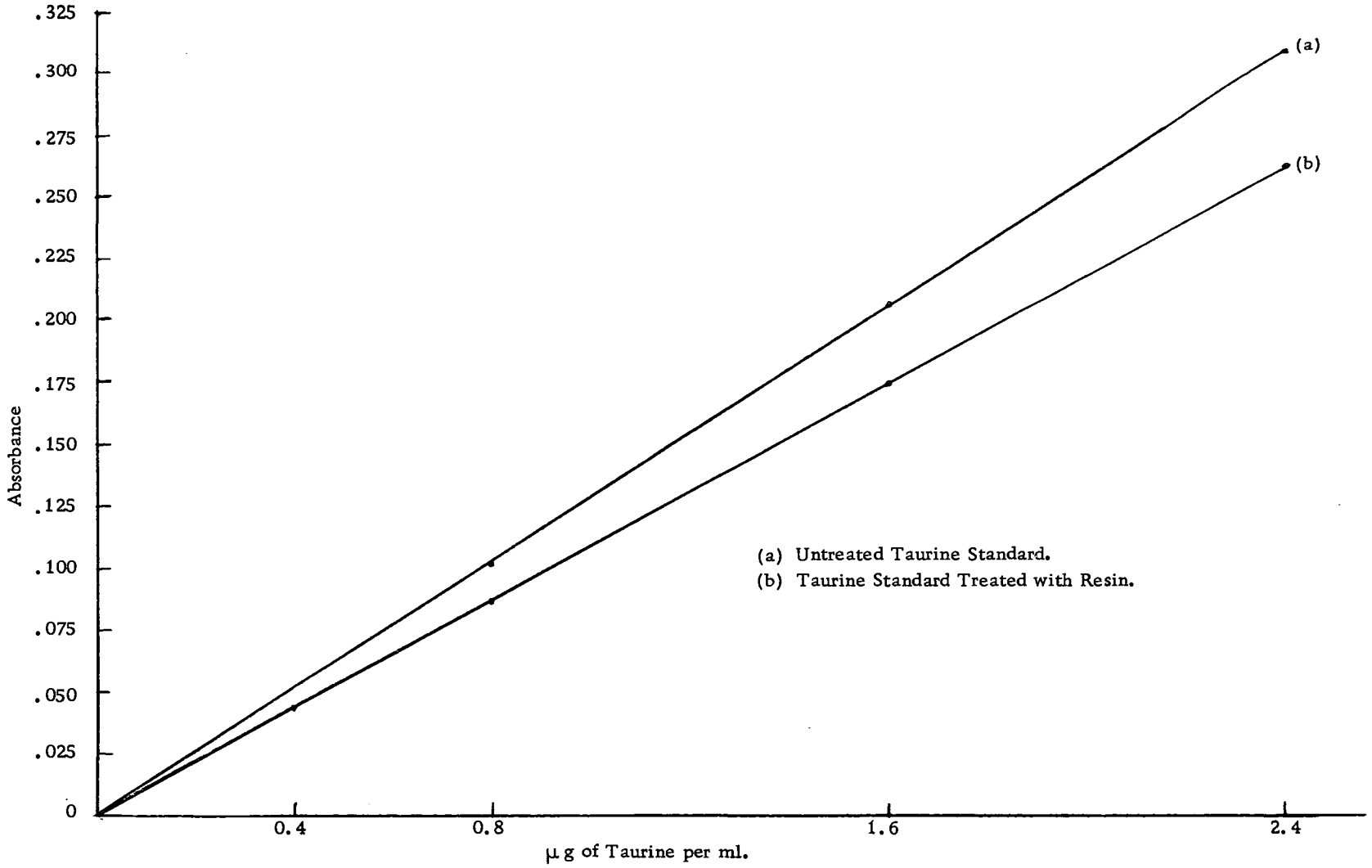

Figure 3. Standard Calibration Curves for Taurine.

phthalate buffer were used.

3. The taurine standard or buffer was allowed to stand with the resin at room temperature for five minutes with occasional swirling.
4. At the end of five minutes each mixture was filtered through Whatman filter paper, No. 40, and the filtrate was collected in a 25 ml Erlenmeyer flask. The used resin was discarded.
5. 1, 2 and 3 ml aliquots of filtrate containing taurine standard were added to each of a series of duplicate 25 ml volumetric flasks. Also 1, 2 and 3 ml of filtrate containing buffer were added to a series of volumetric flasks. The blanks were not prepared in duplicate.

The procedure for the standards and blanks was then followed exactly as outlined under "Method for the Determination of Taurine in Urine", steps 7-19 inclusive.

To obtain the corrected reading for each level of taurine, the absorbance of the reagent blank was subtracted from that of the corresponding standard.

Untreated Taurine Standard. To prepare the calibration curve of taurine standard not treated with Dowex 50W, steps 1-4 inclusive, given above for "Taurine Standard Treated with Resin" were omitted. At step 5, 1, 2 and 3 ml aliquots of taurine standard (12, 24 and 36 μg of taurine, respectively) were pipetted into a series of duplicate 25 ml volumetric flasks. A reagent blank of phthalate buffer was also prepared for each level of taurine; 1, 2 and 3 ml of phthalate buffer not treated with Dowex 50W were introduced into a series of 25 ml volumetric flasks. To obtain corrected readings used to construct the calibration curve, the absorbance of the reagent blanks were subtracted from those of the corresponding standards.

Calculation of Taurine Content in Urine

The absorbance of the phthalate blank treated with resin was subtracted from the absorbance of each urine sample. This corrected reading was used to obtain the taurine content of urine from the calibration curve prepared with taurine standard treated with resin. To calculate the taurine content of a 24-hour urine specimen, the following formula was used:

$$\frac{(\mu g \text{ of taurine found per ml})\ (5)\ (15)\ (\text{urine volume in ml})}{1000}$$

= mg of taurine per 24-hours.

5 = original dilution of urine with buffer.

15 = dilution of urine in the procedure.

Percent Recovery of Taurine

To determine the recovery of taurine added to urine, the procedure as outlined under "Method for the Determination of Taurine in Urine" was followed, except that: at step 3, 5 ml of the taurine standard (1 ml = 12 μg) and 5 ml of diluted urine (1 ml of urine diluted to 5 ml with phthalate buffer) were added to the resin; at step 6, 2 ml of the filtrate from the diluted urine plus added taurine were introduced into a 25 ml volumetric flask; and a reagent blank of 2 ml of phthalate buffer that had been treated with Dowex 50W was used. The procedure was then followed, steps 7-19 inclusive.

The absorbance of the phthalate blank treated with resin was subtracted from the absorbance of each recovery sample. The percent recovery was calculated according to the following formula:

$$\frac{(\mu g \text{ of taurine in recovery sample}) - (\mu g \text{ of taurine in urine})}{0.8\ \mu g \text{ of taurine}} \times 100 = \text{percent recovery.}$$

0.8 μg of taurine = final concentration of the added taurine.

The percent recovery of taurine added to urine generally

ranged from 83 to 113%, with a median of 98%.

Determination of Creatinine

Urinary creatinine was determined by the "micro" modification (Hawk, Oser and Summerson, 1954) of the method by Folin (1914a, b). This method is based on the ability of creatinine to form a red tautomer of creatinine picrate in an alkaline picric acid solution (Jaffe reaction). The production of this tautomer depends upon the formation of a salt, a keto-enol change within the creatinine molecule, and a change in the picric acid molecule involving the hydrogen atoms in the meta position and probably all three nitro groups (Hawk, Oser and Summerson, 1954). The amount of creatinine excreted in urine was calculated from a calibration curve.

Equipment

1. Automatic burette, 50 ml, with a three-way stopcock, and attached to a two-liter storage bottle. Both the burette and the two-liter storage bottle were made of amber glass.
2. Volumetric pipets, 0.5, 1, 2, 5 ml.
3. Graduated pipets, 2, 5 ml.
4. Volumetric flasks, 10, 50, 100, 200, 500, 2000 ml.
5. Suction flask, 1000 ml.

6. Buchner funnel.
7. Analytical balance.
8. Torsion balance.
9. Stopwatch with holder.
10. Photoelectric colorimeter, Klett-Summerson with filter No. 54 with a flow-through cell.

Reagents

1. Hydrochloric acid, 0.1 N.
2. Creatinine standard (1 ml = 1 mg).

   200 mg of creatinine were dissolved and diluted to 200 ml with 0.1 N HCl.
3. Sodium hydroxide, 10% (w/v).

   50.0 g of NaOH were placed in a 50 ml volumetric flask. Redistilled water was added in small amounts and the contents of the flask were swirled until the NaOH was in solution. When cool, the solution was diluted to volume.
4. Picric acid, saturated solution.

   24 g of picric acid were diluted with redistilled water to 2 liters. To effect solution the flask was placed in warm tap water. The solution was then allowed to cool to room temperature and the undissolved picric

acid was removed by filtration through a Buchner funnel. The saturated solution of picric acid was stored in an amber bottle.

Method for the Determination of Urinary Creatinine

The following procedure was used to determine the creatinine content of the urine specimens:

1. 1.0 ml of urine was added to a 100 ml volumetric flask. For less dilute urine 0.5 ml of urine and 0.5 ml of redistilled water were placed in a 100 ml volumetric flask. Duplicates of each urine sample were made. A reagent blank was prepared in duplicate by placing 1.0 ml of 0.1 N HCl in each of two 100 ml volumetric flasks.
2. 20 ml of saturated picric acid were added.
3. 1.5 ml of 10% NaOH were added and the contents of the flasks were thoroughly mixed.
4. The flasks were allowed to stand 15 minutes with occasional swirling.
5. The contents of each volumetric flask were diluted to volume with redistilled water and mixed by inversion.
6. Samples were read in the Klett-Summerson Photoelectric colorimeter with filter No. 54. The

instrument was set at zero with redistilled water.

Preparation of Calibration Curve

1. 0.0, 2.5, 5.0, 7.5 and 10 ml of creatinine standard (1 ml = 1 mg) were added to a series of 10 ml volumetric flasks. The flasks were diluted to volume with 0.1 N HCl. These solutions contained 0.0, 0.25, 0.50, 0.75 and 1.00 mg of creatinine per ml, respectively.
2. 1 ml of each diluted standard was placed in each of a series of duplicate 100 ml volumetric flasks.
3. The procedure under "Method for the Determination of Urinary Creatinine" was then followed, starting at step 2.

The reading obtained for the reagent blank prepared with 0.1 N HCl was subtracted from that obtained for each standard. The calibration curve was constructed with these corrected readings (figure 4).

The calibration curve was not repeated each time urinary creatinine was determined. With each set of analyses, however, one standard was prepared to check the validity of reading the samples on the calibration curve previously prepared. A new curve was constructed each time a new reagent was made.

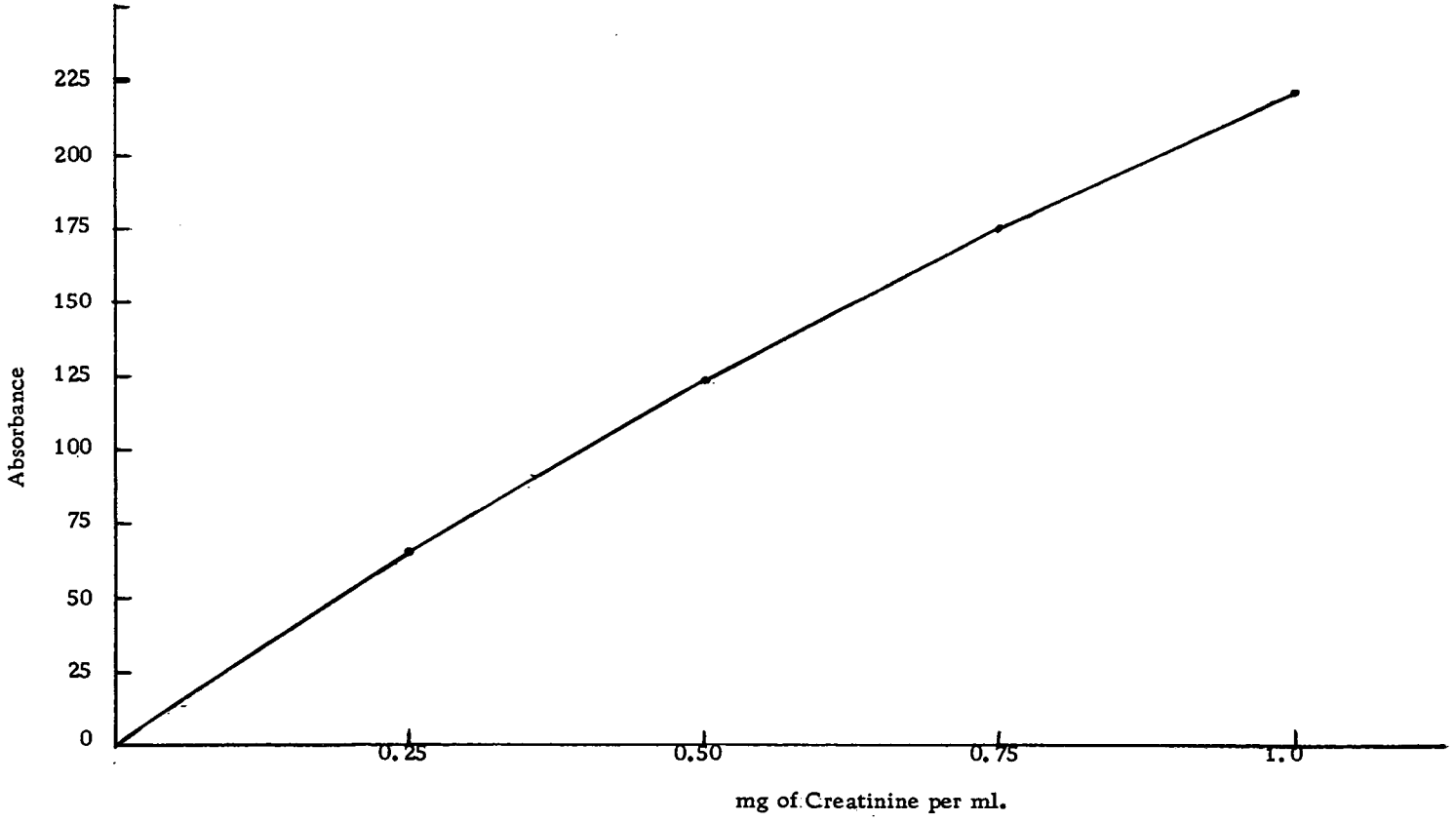

Figure 4. Calibration Curve for Creatinine.

Calculation of Creatinine Content of Urine

The absorbance of the reagent blank was subtracted from that obtained for each sample of urine. The amount of creatinine in each sample was determined from the standard curve. The formula used to calculate the creatinine content of a 24-hour urine specimen was:

$$\frac{(\text{mg creatinine per ml of urine sample})(\text{urine volume in ml})}{1000}$$

= g of creatinine per day.

The size of the sample was varied so that readings would be within the range of the standard curve. One ml of urine was used when the total urine volume was 2000 ml or more, and 1/2 ml of urine when the total volume was 1000 or near 1000 ml. Calculations were based on 1 ml of urine.

RESULTS

Averages and ranges for the daily urinary excretion of taurine and creatinine by the six subjects during ten days are presented in table 2. From these data the following observations on the urinary excretion of taurine were made:

The average daily excretion of taurine varied widely among the subjects, ranging from 60.9 (subject 5) to 196.0 (subject 6) mg per 24 hours. The average daily urinary excretion of taurine was not related to the subject's age, height, weight or excretion of creatinine.

The excretion of taurine by each subject varied from day to day. Three subjects excreted relatively constant amounts of taurine during the 10-day period. The ranges of urinary taurine excretion by subjects 3, 4 and 5 were 123.0 to 163.5 mg, 66.0 to 105.0 mg and 51.0 to 83.3 mg per 24 hours, respectively. The coefficients of variation of the taurine excreted by these subjects were 9.4, 16.5 and 16.9%, respectively. The remaining subjects showed greater variation in taurine excretion. The ranges of taurine excreted by subjects 1, 2 and 6 were 84.8 to 187.5 mg, 79.5 to 145.5 mg and 154.5 to 337.5 mg per 24 hours, respectively. The coefficients of variation were 29.1, 20.1 and 26.6%, respectively. If the daily excretion of taurine is expressed as mg of taurine per g of creatinine this variation in

Table 2. Average Daily Urinary Excretion of Taurine and Creatinine by Six Normal Women

Subject	Age	Height	Weight	Taurine	Creatinine	Taurine/Creatinine
		cm	kg	mg/24 hr.	g/24 hr.	mg/g
1[a]	23	166	55.5	130.1 ± 38.0[b, c] (84.8 - 187.5)[f]	1.08 ± 0.21[b, d] (0.91 - 1.36)	119.6 ± 21.1[b, e] (73.1 - 146.4)
2[g]	22	159	59.1	117.9 ± 23.7 (79.5 - 145.5)	1.36 ± 0.08 (1.14 - 1.42)	87.1 ± 17.3 (58.5 - 107.0)
3[g, h]	25	166	51.8	143.3 ± 13.5 (123.0 - 163.5)	1.07 ± 0.10 (0.96 - 1.27)	133.6 ± 7.4 (120.8 - 145.7)
4	25	163	55.5	77.7 ± 12.8 (66.0 - 105.0	1.23 ± 0.24 (0.82 - 1.76)	64.4 ± 11.8 (49.4 - 89.0)
5[i]	31	163	52.3	60.9 ± 10.3 (51.0 - 83.3)	0.79 ± 0.21 (0.48 - 1.03)	83.7 ± 35.7 (54.3 - 173.4)
6	36	173	59.1	196.0 ± 52.1 (154.5 - 337.5)	1.43 ± 0.14) (1.20 - 1.64)	138.6 ± 42.7 (110.4- 255.7)

a Values represent first 10 days or urine collection.
b Standard deviation.
c Coefficients of variation for the six subjects were: 29.1, 20.1, 9.4, 16.5, 16.9 and 26.6%, respectively.

Continued

Table 2. Average Daily Urinary Excretion of Taurine and Creatinine by Six Normal Women -- Continued

d Coefficients of variation were: 19.4, 5.9, 9.4, 19.5, 27.6 and 9.8%, respectively.
e Coefficients of variation were: 17.6, 19.8, 5.5, 18.4, 42.6 and 31.0%, respectively.
f Range of excretion during the 10 days of urine collection.
g Subjects taking oral contraceptives. Subject 2, Ovulen; subject 3, Ortho-Novum.
h Chinese.
i Korean.

the urinary excretion of taurine was still observed. Data on the daily urinary excretion of taurine and creatinine, and the daily taurine to creatinine ratio for each subject are presented in tables 3 through 8.

The use of oral contraceptives did not appear to affect the excretion of taurine by subjects 2 and 3. They excreted amounts of taurine that were not markedly different from those excreted by the other subjects.

The excretion of taurine by the two Orientals, subjects 3 and 5, did not appear to be different from that of the other subjects who were Caucasians.

Menstruation may affect the urinary excretion of taurine. Subjects 1 (table 3a) and 5 (table 7) started to menstruate on the tenth and ninth day, respectively, on which they were collecting urine. Subject 1 excreted amounts of taurine that were above her average on the seventh day of urine collection and below her average on the eighth day, three and two days before menstruation, respectively. Subject 5 showed a similar pattern on days six and eight of urine collection. Taurine excretion by both subjects rose just above the average value the second day of menstruation. Subject 2 who did not menstruate during the 10-day collection period showed a similar increase and decrease in taurine excretion. Whether the pattern of taurine excretion was due to

Table 3a. Daily Urinary Excretion of Taurine and Creatinine by Subject 1 Before the Administration of Acthar Gel

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
1	110.76	0.99	111.88
2	145.91	1.32	110.54
3	127.50	1.11	114.86
4	187.50	1.36	137.87
5	184.50	1.26	146.43
6	84.75	1.16	73.06
7	157.50	1.16	135.78
8	85.50	0.78	109.62
9	94.50	0.78	121.15
10[a]	123.00	0.91	135.16
Ave.	130.14	1.08	119.64
11	132.00	1.40	94.29
12	165.00	1.28	128.91
13	93.75	1.22	76.84
14	70.50	1.22	57.79
15	225.75	1.22	185.04
16	118.50	1.28	92.58
Ave.[b]	131.68	1.15	114.49

a First day of menstruation.
b Average for days 1-16.

Table 3b. Daily Urinary Excretion of Taurine and Creatinine by Subject 1 During the Administration of Acthar Gel

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
17[a]	189.00	1.31	144.27
18	151.50	1.73	87.57
19	82.50	1.15	71.74
20[a]	106.50	1.30	81.92
21	54.75	1.26	43.45
22	70.50	1.28	55.08
23	70.50	1.32	53.41
24	58.50	1.32	44.32
25[a]	103.50	1.26	82.14
26	68.25	1.27	53.74
27[a]	95.25	1.32	72.16
28	56.25	1.20	46.88
29[a]	160.50	1.66	96.69
30	63.75	1.30	49.04
31	96.11	1.28	75.09
32	119.70	1.31	91.37
33	100.50	1.26	79.76
34	48.00	1.24	38.71
35[a][b]	103.50	1.29	80.23
36	59.25	1.20	49.38
37[a]	145.50	1.27	114.57
Ave.[c]	95.42	1.31	71.98
Ave.[d]	78.58	1.29	59.96

a Received 60 units of acthar gel intramuscularly.
b First day of menstruation.
c Average of days 17-37.
d Average excluding the values from days when acthar gel was received.

Table 4. Daily Urinary Excretion of Taurine and Creatinine by Subject 2

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
1	105.00	1.14	92.11
2	145.50	1.42	102.46
3	145.50	1.36	107.00
4	141.00	1.36	103.68
5	123.00	1.40	87.86
6	97.50	1.41	69.15
7	99.00	1.41	70.21
8	79.50	1.36	58.46
9	105.00	1.36	77.21
10	137.87	1.34	102.89
Ave.	117.89	1.36	87.10

Table 5. Daily Urinary Excretion of Taurine and Creatinine by Subject 3

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
1	153.02	1.05	145.73
2	142.50	1.04	137.02
3	129.94	1.01	128.65
4	129.75	0.97	133.76
5	142.50	1.18	120.76
6	123.00	0.96	128.12
7	140.25	1.02	137.50
8	161.70	1.27	127.32
9	147.26	1.06	138.92
10	163.50	1.18	138.56
Ave.	143.34	1.07	133.63

Table 6. Daily Urinary Excretion of Taurine and Creatinine by Subject 4

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
1	105.00	1.18	88.98
2	81.90	1.24	66.05
3	75.00	1.20	62.50
4	66.00	0.82	80.49
5	87.00	1.76	49.43
6	66.00	1.13	58.41
7	72.45	1.13	64.12
8	67.50	1.18	57.20
9	67.50	1.18	57.20
10	88.50	1.48	59.80
Ave.	77.69	1.23	64.42

Table 7. Daily Urinary Excretion of Taurine and Creatinine by Subject 5

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
1	57.75	1.02	56.62
2	60.00	1.00	60.00
3	51.00	0.94	54.26
4	51.00	0.85	60.00
5	66.75	1.03	64.81
6	69.75	0.76	91.78
7	83.25	0.48	173.44
8	53.25	0.55	96.82
9[a]	52.50	0.56	93.75
10	63.75	0.75	85.00
Ave.	60.90	0.79	83.65

a First day of menstruation.

Table 8. Daily Urinary Excretion of Taurine and Creatinine by Subject 6

Day	Taurine	Creatinine	Taurine/Creatinine
	mg/24 hr.	g/24 hr.	mg/g
1	201.60	1.62	124.44
2	189.00	1.50	126.00
3	186.45	1.55	120.29
4	337.50	1.32	255.68
5	154.50	1.40	110.36
6	174.90	1.34	130.52
7	162.00	1.39	116.55
8	168.30	1.34	125.60
9	184.50	1.20	153.75
10	201.00	1.64	122.56
Ave.	195.98	1.43	138.58

menstruation or to normal variation is not known. Excretion of urinary creatinine by subjects 1 and 5 also showed a reduction two days before the subjects began menstruating, and the values for creatinine by these two subjects increased the second day of menstruation. Subject 2 did not show this change in the excretion of creatinine.

A preparation of ACTH, acthar gel, appeared to cause an increase in the urinary excretion of taurine. Between the 17th and 37th day of urine collection subject 1 received seven injections of acthar gel to control an allergic reaction to DNFB (table 3b, figure 5). Taurine excretion on the day ACTH was administered was greater than on the day preceding or following the injection. The average urinary excretion of taurine by subject 1 during days 1 through 16 decreased from 131.7 mg to 95.4 mg during days 17 through 37, or 78.6 mg if the taurine excreted on the days the injections were received were not included in the average.

The urinary excretion of creatinine by all subjects varied from day to day.

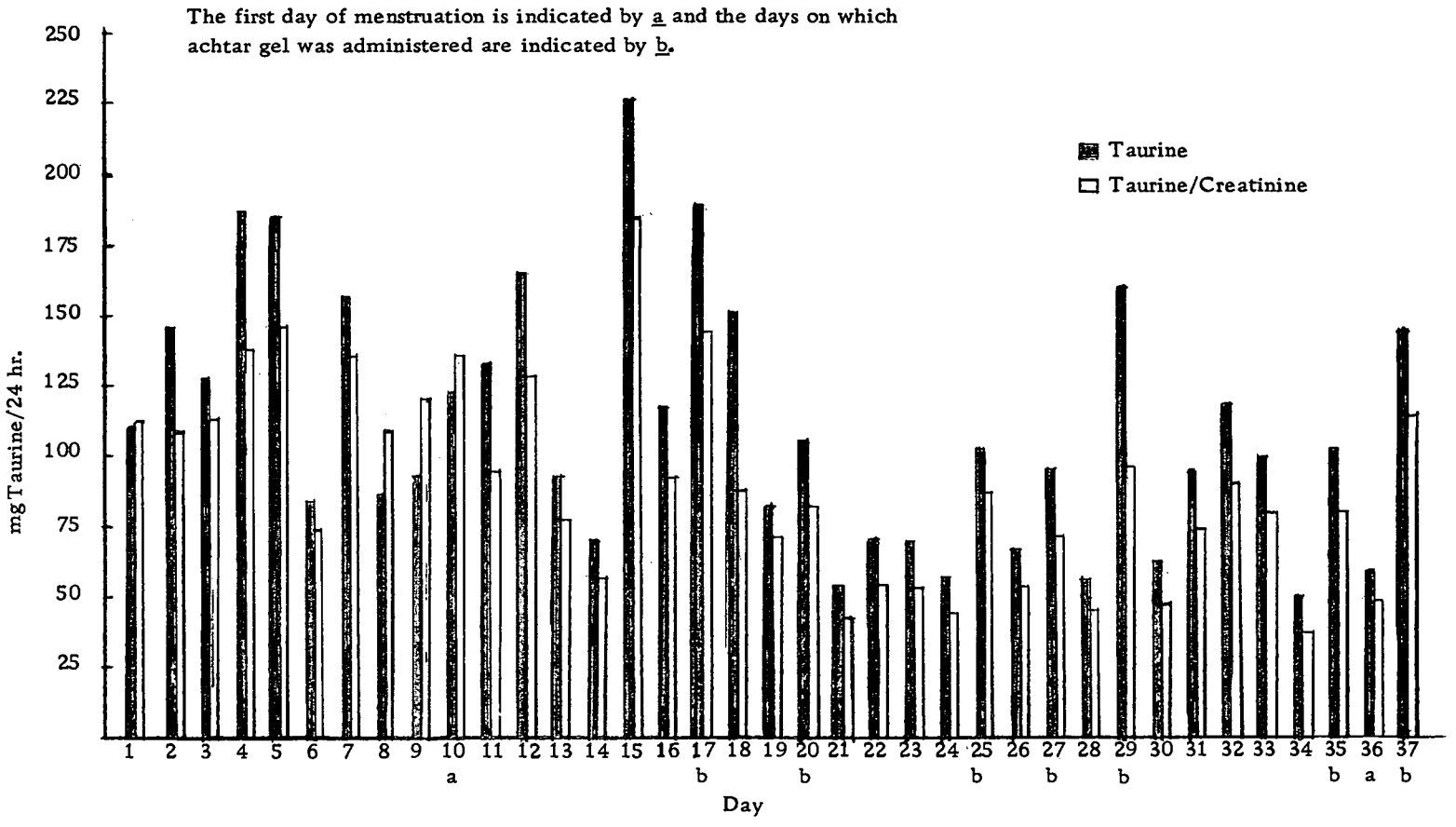

Figure 5. Daily Excretion of Taurine and Taurine to Creatinine Ratio by Subject 1.

DISCUSSION

A wide range in the daily urinary excretion of taurine by normal human subjects has been reported by several workers (Stein, 1953; Evered, 1956; Pentz et al., 1957, 1959; Soupart, 1959, 1960; Swan et al., 1964; Jonxis and Huisman, 1954; Carver and Paska, 1961; Cavilieri et al., 1960). The over-all range in the daily excretion of taurine by the six women in the present study during a period of 10 or more days, 51.0 to 337.5 mg of taurine, is similar to the levels of urinary taurine of normal women reported by other workers (Evered, 1956; Pentz et al., 1959; Soupart, 1959, 1960).

Although the urinary excretion of taurine by each subject in the present study varied, some subjects showed more constancy in taurine excretion than others. Cavilieri et al. (1960 p. 187,) noted that the day to day variation over a six-day period "in each of three subjects was greater than the variation in the entire group" (five subjects). The coefficient of variation (CV) in the excretion of taurine by a subject reported by Pentz et al. (1959) was 14.0% during the four days the subject consumed his normal diet, and 7.7% during the eight days the subject consumed a "high protein diet". The CV in the excretion of taurine by the subjects in the present study ranged from 9.4 to 29.1% during the ten days.

The variation in the average excretion of taurine by the subjects and in the daily excretion by each subject could have been caused by several dietary factors. Because the diet of the subjects was not controlled, differences in the composition of the subjects' diet could have affected the urinary excretion of taurine.

Since methionine and cysteine can be converted to taurine, the type and quantity of dietary protein consumed by the subjects could have affected the urinary excretion of taurine. A subject reported by Pentz et al. (1959) excreted an average of 161 mg of taurine during a four-day period while consuming his normal diet. During the period of eight days when the subject's diet contained an amount of protein "twice that he normally consumed" he excreted an average of 193 mg of taurine per day.

In addition to the amount of protein consumed in the diet, the urinary excretion of taurine could have been affected by: individual variations in the requirements for amino acids (Williams and Passman, 1960), the ability to reabsorb amino acids, and the activity of enzymes directly or indirectly related to the synthesis and degradation of amino acids (Meister, 1965).

The free taurine content of the diet could also have influenced the daily urinary excretion of taurine. The taurine content of animal tissues varies considerably. Fresh clams, for example,

contain five times as much free taurine as an equal amount of most muscle meats, and 16 times as much as homogenized milk (Roe and Weston, 1965). Consumption of shellfish can increase the excretion of taurine among normal subjects from five to ten fold (Goodman, 1967).

Stress appears to cause increased urinary excretion of taurine (King et al., 1966). During the 10-day period the subjects in the present study collected urine, they participated in their normal activities. The observed daily variation in taurine excretion could have been caused by stresses normally experienced by each individual.

To eliminate the variation in urinary excretion of taurine caused by diet, and to more clearly define the effect of stress on taurine excretion, the subjects should be fed a constant diet of known protein, amino acid, taurine and vitamin B_6 content. The subject should record activities and "stresses".

The urinary excretion of taurine in humans is increased after the administration of ACTH (Pentz, et.al., 1959) and corticosteroids (King et al., 1966). Subject 1 who received seven injections of acthar gel, an ACTH preparation, between the 17th and 37th day of urine collection, excreted more taurine on the days ACTH was administered than on the day before or after each injection. No injection of acthar gel was received on day 32, but the amount

of taurine excreted on that day by subject 1 was also greater than the preceding or following day. Thus, the variation in the urinary excretion of taurine that occurred could be due to a normal variation as well as to the administration of ACTH.

Soupart (1960) observed no consistent difference in the amount of taurine excreted by five women in one 24-hour urine specimen collected during the early part of the luteal phase, and another collected during the follicular phase of the menstrual cycle. In the present study, the excretion of taurine by subjects 1 and 5 increased above and then decreased below the subjects' averages during the late luteal phase of the menstrual cycle.

When studying the metabolic pathways in women which require pyridoxal phosphate-dependent enzymes, Price et al. (1967) recommended that the use of steroid hormones to control ovulation must be considered. Price et al. found that women ingesting the oral contraceptive Enovid E excreted normal amounts of tryptophan metabolites in the urine before tryptophan loading, but excreted more xanthurenic acid, 3-hydroxykynurenine, kynurenine, kynurenic acid and acetylkynurenine than the controls after a loading dose of 2 g of L-tryptophan. Supplementation with 100 mg of pyridoxine daily for two days almost completely corrected the abnormal tryptophan metabolism in the women ingesting the contraceptive. The use of oral contraceptives by

the two subjects in this study did not appear to affect the urinary excretion of taurine. To more completely determine the effect oral contraceptives have on the urinary excretion of taurine, taurine excretion should be studied before and during the use of oral contraceptives, and before and after a loading dose of L-cysteine, with and without supplementation with vitamin B_6.

Constancy in creatinine excretion is frequently used to determine completeness of urine collection. The variation in the excretion of creatinine observed in this study could indicate irregularity in the collection of urine. Vestergaard and Leverett (1958) found that some individuals were constant in the excretion of creatinine, the CV of creatinine excretion ranging from 0 to 3.75%. However, others were irregular in excretion of creatinine; the CV was 10.1% or greater. According to this standard no subject in the present study excreted a constant amount of creatinine. Subjects 2, 3 and 6 excreted moderately constant amounts; CV in the excretion of creatinine for these subjects were 5.9, 9.4 and 9.8%, respectively. In contrast the excretion of creatinine by subjects 1, 4 and 5 was quite inconstant, the CV were 19.4, 19.5 and 27.6%. Dietary intake, according to Bleiler and Schedl (1962), could alter the urinary excretion of creatinine. The creatinine available for excretion not only reflected the day's turnover of creatinine but also the

subject's previous protein intake, meat appreciably increasing the creatinine pool. Creatinine excretion could be reduced as much as 30% when the pool size was adjusted toward a supply and demand situation by ingestion of a diet free from creatine and creatinine.

Creatinine has not been reported to vary with the menstrual cycle, but creatine has. Creatine is not excreted in the urine of normal men and is normally absent from the urine of women. Creatine appears in the urine during menstruation, pregnancy and for several days after delivery (Krause, 1911, as cited by Mathews, 1939). The variation in the excretion of creatinine by subjects 1 and 5 before menstruation in the present study can not be attributed to the increased excretion of creatine, because the method to determine creatinine devised by Folin (1914a, b) measures only creatinine.

Results from recent studies reported by Merrow et al. (1966) indicate that taurine in plasma may be more indicative of vitamin B_6 nutriture than taurine excreted in urine. In subjects deprived of vitamin B_6, the taurine in plasma decreased while no consistent change was observed in the urinary excretion of taurine. The plasma of rats deficient in vitamin B_6 was lower in taurine than that of the controls. In view of this a study on the relationship between plasma taurine and the urinary excretion

of taurine by adequately nourished individuals and ones deficient in vitamin B_6 would be of considerable interest.

Since dinitrofluorobenzene (DNFB) caused considerable skin irritation, a method to determine taurine other than one using DNFB would be preferable. Sorbo (1960) has developed a method based on the measurement of the ninhydrin derivative of taurine which appears to be more specific than the one devised by Pentz (1957). Pentz (1967) found that high levels of urinary glucose gave artifically high taurine values. An unknown substance which sometimes appears in human urine and is not ninhydrin positive, also appears to interfere with her method.

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