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In pyridoxine-deficient rats, the decreased urinary excretion of taurine has been attributed to the decreased activity of cysteinesulfinic acid decarboxylase, a pyridoxal phosphate-dependent enzyme involved in the conversion of cysteine to taurine (McAfee and Williams, 1962). In humans depleted of pyridoxine, the level of taurine excreted in urine did not change (Swan, Wentworth, and Linkswiler, 1964; Merrow et al., 1966) while that in plasma decreased (Merrow et al., 1966). This suggests that the determination of this amino acid in plasma may be a means by which pyridoxine nutriture of an individual can be measured.

Although column chromatographic procedures for the determination of amino acids in plasma are in common use, a procedure for the determination of taurine alone is not available. Therefore, this study was undertaken to develop one.

A procedure was adapted from those by Garvin (1960) and Sörbo (1960). The separation of taurine from other amino acids was based on the selective passage of the zwitterion of taurine through cationic and anionic exchange resins. A protein-free extract of plasma or blood was adjusted to pH 5, the isoelectric point of taurine, and passed through a column containing separate layers of Dowex 50W (H^+) and Dowex 1 (Cl^-). Taurine was determined in the effluent according to the ninhydrin procedure of Sörbo. To check the specificity of the method, concentrates of the effluent were subjected to ascending one-dimensional paper chromatography with two solvents, butanol-acetic acid and butanol-pyridine. Taurine was identified with ninhydrin and Erlich reagents.

The procedure was applied to 8 blood samples from 5 healthy women in the post-absorptive state. The taurine concentration of blood of 4 of these subjects ranged from 27.9 to 40.2 $\mu g/ml$, and of blood taken from one subject on 4 consecutive mornings, 26.1 to 34.2 $\mu g/ml$. The recovery of taurine added to these samples ranged from 88.6 to 101.9%. Paper chromatography of each concentrated effluent demonstrated only one component, and this component had the same R_f as the taurine standard and gave a positive reaction with ninhydrin and Erlich reagents.

Although this procedure can be used to determine taurine in human blood, it is not sensitive enough to measure the small quantities of taurine in human plasma.

The Determination of Taurine
in Human Blood

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TABLE OF CONTENTS

INTRODUCTION -----	1
REVIEW OF LITERATURE -----	3
Biosynthesis of Taurine in Mammalian Tissues -----	3
Two Pathways Including Cysteinesulfinic Acid ----	3
Three Other Pathways -----	7
Metabolism of Taurine in Mammalian Tissues -----	8
Effects of Pyridoxine Deficiency on the Activity of Enzymes Involved in the Synthesis of Taurine -----	10
Effects of Pyridoxine Deficiency on the Taurine Content of Mammals -----	11
Effects on Taurine Content of Tissues -----	11
Effects on Taurine Content of Plasma -----	12
Effects on Urinary Excretion of Taurine -----	12
Other Dietary Factors that May Affect the Taurine Content of Mammals-----	14
Dietary Taurine -----	14
Dietary Protein -----	15
Protein Malnutrition -----	16
Pantothenic Acid -----	17
Vitamin B ₁₂ -----	18
Non-Dietary Factors -----	18
Taurine Content of Blood -----	18
Taurine Content of the Four Compartments of Blood -----	18
Leukocytes -----	19
Platelets -----	19
Erythrocytes -----	19
Plasma -----	19
Precautions with Blood Collection and Cell Separation -----	22
Methods to Determine Taurine -----	23
EXPERIMENTAL -----	26
Procedure -----	27
Description of Samples for Preliminary Studies-	27
Plasma from Stored Blood -----	27
Plasma from Freshly Drawn Blood -----	27
Stored Whole Blood -----	28
Preparation of Samples for Ion Exchange Chromatography -----	29

Reagents -----	29
Procedure -----	30
Ion Exchange Chromatography -----	31
Preparation of Ion Exchange Resins -----	31
Dowex 50W X4 (H ⁺) (200-400 mesh)-	31
Dowex 1 X8 (Cl ⁻) (200-400 mesh)---	32
Ion Exchange Chromatography of the	
Samples -----	32
Equipment -----	32
Packing the Columns -----	33
Application of Sample to Column --	34
Determination of Taurine in the Effluent	
by Ninhydrin Reaction -----	35
Equipment -----	35
Reagents -----	36
Procedure -----	37
Standard Curve -----	38
Preparation of the Standard	
Curve -----	38
Paper Chromatography -----	40
Reagents -----	41
Equipment -----	42
Procedure -----	42
Preliminary Ion Exchange Chromatography Studies ---	44
Experiments to Determine the Quantities of	
Dowex 50W (H ⁺) and Dowex 1 (Cl ⁻). Necessary	
to Remove Ninhydrin-Positive Substances that	
Interfere with the Determination of Taurine in	
Blood and Plasma -----	44
Recovery of Taurine from Dowex 50W (H ⁺) and	
Dowex 1 (Cl ⁻) -----	46
Paper Chromatography of Effluents -----	46
Determination of Taurine in Whole Blood of Healthy	
Women -----	47
Procedure -----	47
Leukocyte Counts -----	50
Equipment -----	50
Reagents -----	51
Procedure -----	51
Platelet Counts -----	52
RESULTS AND DISCUSSION -----	53
Preliminary Studies-----	53

General Comments on the Procedure -----	53
Results of Preliminary Ion Exchange Chroma- tography Studies -----	55
Quantities of Dowex 50W (H ⁺) and Dowex 1 (Cl ⁻) Necessary to Remove Ninhydrin- Positive Substances that Would Interfere with the Determination of Taurine in Blood and Plasma -----	55
Studies with Plasma -----	59
Studies with Blood -----	63
Recovery of Taurine from Dowex 50W (H ⁺) and Dowex 1 (Cl ⁻) -----	64
Paper Chromatography of Effluents ----	64
Taurine in Whole Blood of Healthy Women -----	65
 BIBLIOGRAPHY -----	 71

LIST OF TABLES

1	Taurine content of plasma of normal individuals-----	20
2	Summary of experiments to determine the amount of water needed to wash the columns after application of sample -----	56
3	Summary of experiments to determine the quantity of Dowex 50W (H^+) and Dowex 1 (Cl^-) necessary to remove ninhydrin-positive substances in plasma and blood that interfere with the determination of taurine -----	57
4	Taurine content and leukocyte and platelet counts of blood of five women -----	66
5	Recovery of taurine in blood and reproducibility of procedure -----	69

LIST OF FIGURES

1	Pathways related to taurine -----	4
2	Standard curve for taurine -----	39
3	Passage of taurine through a 6-cm column of Dowex 50W (H ⁺). -----	60
4	Passage of taurine through a column of 6-cm of Dowex 50W (H ⁺) and 3 cm of Dowex 1 (Cl ⁻) -----	61

The Determination of Taurine in Human Blood

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is synthesized from the sulfur-containing amino acids, cysteine and methionine. Several of the enzymes involved in the formation of taurine from these amino acids require the presence of the coenzyme, pyridoxal phosphate (Robinson, 1966). When rats are fed a diet deficient in pyridoxine, cysteinesulfinic acid decarboxylase is one of the first enzymes to be inactivated (Tower, 1956), and the low levels of urinary taurine excreted by these animals has been ascribed to the decreased activity of this enzyme (Hope, 1955). During the time human subjects were deprived of pyridoxine, their urinary excretion of taurine remained relatively constant (Swan, Wentworth and Linkswiler, 1964; Merrow et al., 1966) but their plasma taurine decreased (Merrow et al., 1966). This suggested to Merrow that the nutritional status of pyridoxine in the human may be better reflected by the concentration of taurine in plasma than in urine.

Although procedures are currently available for the determination of taurine alone in urine and tissues, none has been developed for the determination of this amino acid in plasma or whole blood. The purpose of this thesis is to review the literature for factors affecting the taurine content of blood and plasma, and to

present the studies done toward the development and application of a procedure for the determination of taurine in human plasma and blood.

REVIEW OF THE LITERATURE

Biosynthesis of Taurine in Mammalian Tissues

At least 5 metabolic pathways (Figure 1) have been indicated for the synthesis of taurine in mammalian tissues.

Two Pathways Including Cysteinesulfinic Acid

Taurine can be synthesized from cysteine by two pathways that include cysteinesulfinic acid as an intermediate. To oxidize cysteine to cysteinesulfinic acid, an enzyme system studied by Sörbo and Ewetz (1965) required oxygen, ferrous ion, and reduced nicotinamide adenine dinucleotide phosphate. The addition of hydroxylamine prevented the immediate decarboxylation of cysteinesulfinic acid (Ewetz and Sörbo, 1966; Wainer, 1965).

In one pathway cysteinesulfinic acid is converted to hypotaurine. In 1955, Chapeville and Fromageot isolated labeled cysteinesulfinic acid and hypotaurine in the livers of rats injected with ^{35}S -cysteine (Jacobsen and Smith, 1968). Later, Sumizu (1962) reported that hypotaurine was oxidized to taurine by a dehydrogenase that required nicotinamide adenine dinucleotide as a cofactor.

Cysteinesulfinic acid can also be converted to cysteic acid, as was shown by Awapara and Doctor (1955). They isolated

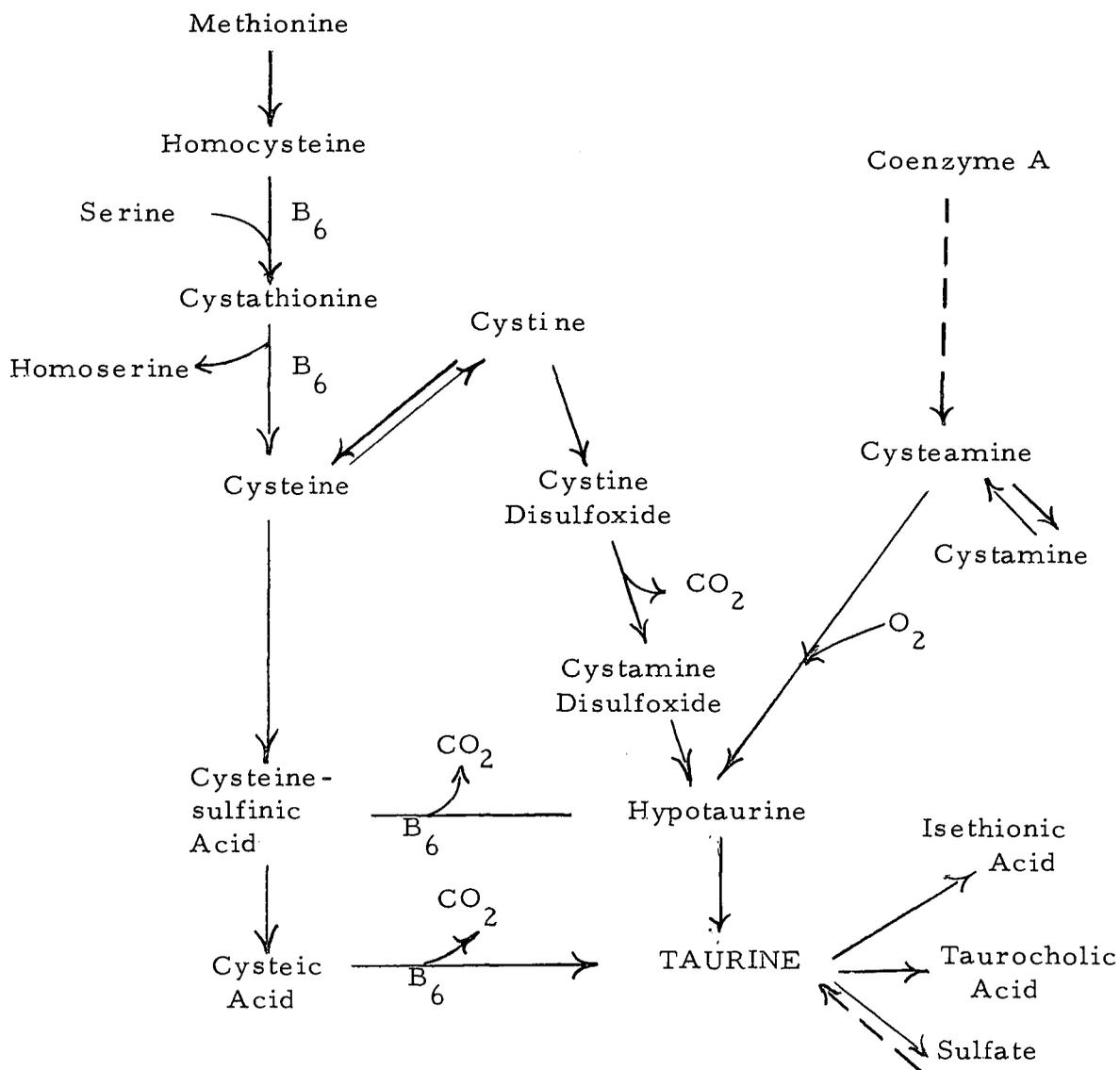


Figure 1. Pathways related to taurine (adapted from Jacobsen and Smith, 1968). Reactions in which pyridoxal phosphate is of particular importance are indicated with the symbol (B₆) adjacent to the arrows. Arrows with broken lines indicate postulated pathways.

labeled cysteinesulfinic acid and cysteic acid from a reaction mixture that contained an enzyme isolated from a rat liver homogenate, ³⁵S-cysteine, magnesium ion, cytochrome c, adenosine triphosphate, and diphosphopyridine nucleotide. Earlier Blaschko, Datta, and Harris (1953) had demonstrated the formation of taurine in a rat liver homogenate that had been incubated with cysteic acid.

Both cysteinesulfinic acid decarboxylase and cysteic acid decarboxylase require pyridoxal phosphate as a coenzyme (Jacobsen and Smith, 1968). Hope (1955) at one time suggested that they are the same enzyme. Later studies, however, revealed several differences between the decarboxylase in rat brain and the one in liver. The activity of the decarboxylase in rat brain increased when pyridoxine was added to the reaction mixture, whereas the activity of the one in liver did not (Bergeret, Chatagner, and Fromageot, 1955; Jacobsen, Thomas, and Smith, 1964). The liver decarboxylase was more active than the one in brain, but the brain decarboxylase was more resistant to pyridoxine deficiency (Jacobsen et al., 1964; Hope, 1957). Since these enzymes have dissimilar characteristics but catalyze the same reactions, they were classified recently as isozymes (Jacobsen et al., 1964; Jacobsen and Smith, 1968).

In the tissues that were studied, cysteinesulfinic acid was decarboxylated faster than cysteic acid (Hope, 1955; Davison, 1956;

Jacobsen et al., 1964; Jacobsen and Smith, 1963). This may indicate that the preferred pathway for the synthesis of taurine from cysteine is through cysteinesulfinic acid to hypotaurine (Hope, 1955; Bergeret et al., 1955).

Jacobsen and Smith (1963) compared the in vitro decarboxylation of cysteinesulfinic acid-1-¹⁴C and cysteic acid-1-¹⁴C by liver and brain decarboxylase of the human, dog, and rat. Enzyme preparations from dog and rat liver decarboxylated both cysteinesulfinic acid and cysteic acid, whereas preparations from human liver decarboxylated neither. There was significant decarboxylase activity in human brain when either substrate was used, but it was less than that in dog or rat liver. Since free taurine was found in the human liver, Jacobsen and Smith (1963, 1968) speculated that taurine is synthesized in this organ by a pathway that does not require either decarboxylase.

Methionine, which can be converted to cysteine, is also a precursor of taurine. According to Mudd et al. (1965), methionine is activated by adenosine triphosphate and is subsequently demethylated to form homocysteine. Cystathionine synthetase combines homocysteine and serine to form cystathionine which in turn is converted to cysteine and homoserine by cystathionase. Both cystathionine synthetase and cystathionase require pyridoxal phosphate as a coenzyme (Matsuo and Greenberg, 1958a, 1958b,

1959; Binkley, Christensen, and Jensen, 1952).

When rats were injected with ^{35}S -methionine, labeled cystathionine, cysteic acid, and taurine were identified in the livers and other organs (Tabachnick and Tarver, 1955). Later, Peck and Awapara (1967) demonstrated the transsulfuration pathway of methionine and the biosynthesis of taurine from cysteine via cysteinesulfinic acid and hypotaurine in rat brain. Some of the taurine was converted to isethionic acid.

Three Other Pathways

Cystamine and cysteamine can be converted to taurine in a number of tissues. The enzyme in horse kidney which catalyzed this reaction required elemental sulfur or sulfide for activity. One mole of oxygen was consumed in the enzymatic conversion of one mole cysteamine to hypotaurine. Sulfur also catalyzed the nonenzymatic oxidation of cysteamine to cystamine (Cavallini, Scandurra, and DeMarco, 1963, 1965). The physiological significance of this pathway has not been established. Jacobsen and Smith (1968) suggested that it may be important in man because there is no cysteinesulfinic acid decarboxylase activity in human liver. Since taurine in brain is not reduced in pyridoxine deficiency, this pathway may also be of significance in that organ. Coenzyme A may be a precursor of cysteamine (Eldjarn, 1954).

The incorporation of sulfate into taurine and its precursors was studied by Green and Robinson (1960) who noted the presence of radioactive taurine in brain extracts from rats injected with tagged sulfate. The present consensus is that in the mammal only intestinal bacteria utilize sulfate to form significant amounts of taurine or other sulfur-containing compounds (Jacobsen and Smith, 1968).

Mendes and Floyd (1942) proposed that cystine is converted to hypotaurine through cystine disulfoxide and cystamine disulfoxide. The existence of this pathway in mammalian tissues has not been studied further.

Metabolism of Taurine in Mammalian Tissues

Although taurine was considered at one time to be only an end product of metabolism, it is now evident that this amino acid has several metabolic functions (Figure 1).

Taurine is conjugated with the bile acids: cholic, chenodeoxycholic, and deoxycholic acids. Free bile acids and those conjugated with taurine (or glycine) facilitate the intestinal absorption of fat by contributing to lipolysis, micelle formation, and re-esterification of fatty acids within the mucosal cells of the intestine (Jacobsen and Smith, 1968).

When an excess of taurine was added to a rat liver homogenate, the conjugation of cholic acid with taurine increased (Bergström and

Gloor, 1954). Bremer and Gloor (1955) later reported that the combination of taurine with cholic acid by the microsomal fraction of a rat liver homogenate was stimulated by the addition of nicotinamide, diphosphopyridine nucleotide, adenosine triphosphate, magnesium, versene, and possibly, triphosphopyridine nucleotide. The ingestion of 0.5 gm of taurine three times daily by normal human subjects increased the proportion of bile acids conjugated with taurine (Sjovall, 1959).

The presence of the deamination product of taurine, isethionic acid, in mammalian tissues was identified by Welty, Read, and Shaw (1962). They postulated that taurine and isethionic acid form a complex that regulates the efflux of potassium ions from cardiac cells and the excitability of cardiac tissue. Peck and Awapara (1967) later demonstrated the conversion of ^{35}S -taurine to ^{35}S -isethionic acid in rat brain. That man also has the enzymatic means for converting taurine to isethionic acid was shown by the detection of isethionic acid in the urine of patients with nonmuscular diseases (Jacobsen et al., 1967).

Orally administered taurine was excreted in urine as an inorganic sulfate by the rat and rabbit (Schram and Crokaert, 1957; White, Lewis, and White, 1937). Since subcutaneously injected taurine was excreted in urine as organic neutral sulfur by the rat, Maw (1953) concluded that urinary inorganic sulfate is due to

the action of intestinal bacteria.

The conversion of taurine to carbamyltaurine (Schram and Crokaert, 1957) and taurocyamine (Blass, 1960) in mammalian tissue has not been confirmed definitely (Jacobsen and Smith, 1968).

Effects of Pyridoxine Deficiency on the Activity
of Enzymes Involved in the Synthesis of Taurine

Pyridoxine deficiency reduces the activity of many pyridoxal phosphate-dependent enzymes. These enzymes, however, are impaired at different stages of pyridoxine depletion. According to Tower (1956), the enzyme most susceptible to pyridoxine deficiency is cysteinesulfinic acid decarboxylase, which is involved in the synthesis of taurine from cysteine. Pyridoxine deficiency also depresses the activity of cystathionase (Robinson, 1966), an enzyme necessary for the conversion of methionine to cysteine.

Blaschko and Hope (1954) and Chatagner, Tabechian, and Bergeret (1954) found that the activity of liver cysteinesulfinic acid decarboxylase was completely lost in rats fed a diet deficient in pyridoxine for one week, and could not be stimulated by the in vitro addition of pyridoxal phosphate. However, during the first week of pyridoxine deficiency, the activity of cysteinesulfinic acid decarboxylase was partially lost but could be restored by large doses of pyridoxal phosphate. Greengard and Gordon (1963) showed

that puromycin blocked the increase in cysteinesulfinic acid decarboxylase activity when pyridoxal phosphate was added to liver preparations from rats deficient in pyridoxine. They concluded that pyridoxal phosphate not only regulated the activity, but also the de novo synthesis of this enzyme. Since the addition of pyridoxal phosphate to liver preparations from normal rats caused little or no increase in the activity of cysteinesulfinic acid decarboxylase, it was suggested that this enzyme is normally saturated with pyridoxal phosphate (Hope, 1955; Bergeret et al., 1955; Jacobsen et al., 1964).

Bergeret et al. (1955) reported that the activity of cysteinesulfinic acid decarboxylase in rat brain was not depressed by pyridoxine deficiency. Furthermore, brain cysteinesulfinic acid decarboxylase is not saturated with pyridoxal phosphate, since the in vitro addition of this cofactor enhanced the activity of this enzyme in preparations from controls and animals deficient in pyridoxine (Bergeret et al., 1955; Davison, 1956; Jacobsen, 1964).

Effects of Pyridoxine Deficiency on the Taurine Content of Mammals

Effects on Taurine Content of Tissues

Since a deficiency of pyridoxine in rats caused no change in the taurine content of spleen, liver, muscle (Jacobsen and Smith,

1968), and brain (Hope, 1957), Jacobsen and Smith (1968) suggested that taurine may be synthesized by a pathway that does not require pyridoxal phosphate-dependent enzymes. On the other hand, Merrow et al. (1966) reported that the concentration of taurine was higher in muscle and spleen, and lower in liver, of pyridoxine-deficient rats than of controls.

Effects on Taurine Content of Plasma

The taurine content of plasma of rats deficient in pyridoxine was lower than that of the controls. Taurine in human plasma also appears to reflect pyridoxine nutriture. The taurine concentration, expressed as leucine equivalents, in plasma of 9 subjects who had received a diet deficient in pyridoxine for 7 days was 5.0 $\mu\text{g/ml}$; and for 47 days, 3.9 $\mu\text{g/ml}$; and after supplementation with 10 mg of pyridoxine daily for 4 days, 4.9 $\mu\text{g/ml}$ (Merrow et al., 1966).

Effects on Urinary Excretion of Taurine

The urinary excretion of taurine by the rat decreases in pyridoxine deficiency. This has been ascribed to the decrease in activity of cysteinesulfinic acid decarboxylase (Chatagner et al., 1954; McAfee and Williams, 1962) or cysteic acid decarboxylase (Blaschko, Datta, and Harris, 1953a, 1953b). Hope (1957) found that control rats excreted an average of 18.6 mg of taurine in urine

daily, whereas rats fed a pyridoxine-deficient diet for 14 weeks excreted an average of 1.1 mg of taurine. Mercer, Bowen, and Johnston (1966) reported that the urinary excretion of taurine, in terms of 100 gm of body weight/day, for rats deficient in pyridoxine was 1-2 mg; and for pair-fed controls, 7 mg.

The urinary excretion of taurine by man has not been shown conclusively to decrease with pyridoxine depletion. Swan, Wentworth, and Linkswiler (1964) found no change in urinary taurine in 6 men who were fed a diet containing 0.16 mg of pyridoxine and 100 gm of protein for 36 days. When the subjects were given an oral loading dose of 3.4 gm of L-cysteine after 26 or 36 days of depletion, they excreted from 0.0 to 0.8% of the cysteine as taurine. After the subjects had been given 50 mg of pyridoxine daily for three days, they excreted amounts of urinary taurine that were no different from those they had excreted during pyridoxine depletion. Following cysteine loading, however, the repleted subjects excreted from 1.0 to 1.7% of the amino acid as taurine. Merrow et al. (1966) also found no consistent trend in the urinary excretion of taurine by 9 young men who received a diet containing 1 gm of protein/kg of body weight and 0.30 mg of pyridoxine for 50 days.

After receiving a load dose of L-cysteine, rats deficient in pyridoxine excreted less urinary taurine than the controls. McAfee

and Williams (1962) reported that about 0.1% of a supplement of 200 mg of L-cysteine hydrochloride hydrate was excreted as taurine by rats deficient in pyridoxine, while 1.6% to 7.0% of the cysteine supplement was excreted as taurine by the controls. When the deficient rats were fed 50 µg of pyridoxine along with the cysteine supplement, the urinary excretion of taurine did not increase. McAfee and Williams suggested that pyridoxine deficiency may have caused a lack of some of the apoenzymes involved in taurine formation.

Oral loading doses of methionine caused no increase in urinary taurine in man or the rat (McAfee and Williams, 1962; Merrow et al., 1966; Block, Markovs, and Steel, 1965).

Other Dietary Factors that May Affect the Taurine Content of Mammals

Dietary Taurine

In the late 19th century Salkowski found that the ingestion of free taurine caused an increase in the urinary excretion of this amino acid. He reported that 87% of a 5-gm oral dose of taurine was eliminated in urine "in an organic form" (Schmidt, von Adelung, and Watson, 1918).

All animal protein foods contain taurine. Invertebrates that are particularly rich in taurine are clams and scallops. These

contain 252.5 and 238.3 mg of taurine/100 gm of raw wet weight, respectively. Oysters and shrimp (73.8 mg and 63.3 mg/100 gm, respectively) are higher in taurine than cod and haddock (38.0 mg and 28.9 mg/100 gm, respectively). Beef, lamb, pork, chicken, and milk also contribute significant amounts of taurine to the diet; these foods contain, respectively, 30.2, 19.5, 51.4, 32.4, and 31.3 mg/100 gm (Roe and Weston, 1965; Roe, 1966). The more active muscles contain more of the amino acid than the less active ones. Although some taurine is lost in cooking, more is retained by baking than by boiling (Roe and Weston, 1965). The distribution of taurine in plants appears to be very limited (Jacobsen and Smith, 1968).

Dietary Protein

Pentz, Moss, and Denko (1959) reported that the doubling of protein intake by normal males did not cause a significant increase in urinary taurine. The protein-containing foods that the subjects consumed during the control period or during the period of high-protein intake were not reported.

Block et al. (1965) fed two human subjects, a man and a woman, two levels of the same dietary proteins. When consuming a diet containing 54 gm of protein, they excreted, respectively, an average of 94.6 mg and 20.9 mg of urinary taurine/day. When they were

given a daily supplement of 9 gm of L-methionine, their 24-hour urine specimens contained an average of 101.8 mg and 34.7 mg of taurine, and their plasma when they were in the post-absorptive state contained 1.35 and 1.31 mg of taurine/100 ml, respectively. The protein intake was increased to 104 gm, and the average daily urinary excretion of taurine by the subjects was 99.8 mg and 80.7 mg. When they received a daily supplement of 9 gm of L-methionine in addition to the diet high in protein, they excreted an average of 91.2 and 67.8 mg of taurine in urine, and their plasma when they were fasting contained 1.43 and 1.09 mg of taurine/100 ml.

Stein, Bearn, and Moore (1954) found that two hours after a subject had ingested 50 gm of a taurine-free mixture of casein and carbohydrate, the taurine content of plasma remained constant while the concentrations of all other amino acids increased.

Protein Malnutrition

In the early stages of protein deprivation in children, the urinary excretion of taurine (and β -aminoisobutyric acid) increases. As the condition becomes more severe, the hyperaminoaciduria extends to other amino acids. Soupart (1962) suggested that this pattern is a "reflection of a trend to adaptation of the intracellular pool of free amino acids to electro-osmotic variations." The

urinary excretion of taurine returned to normal within 24 hours after the administration of a diet high in milk (Soupart, 1962).

Protein-depleted rats injected with ^{35}S -taurine retained more radioactivity than the control animals. Radioactivity was heavily concentrated in the musculoskeletal system, but there was also considerable radioactivity in the liver and kidney (Portman and Mann, 1956; Roe, 1967).

Pantothenic Acid

Marks and Berry (1962) reported that pregnant and weanling rats deficient in pantothenic acid excreted more taurine than the pregnant and weanling controls. Koyanagi, Tanaka, and Takasaki studied Japanese children who were consuming diets suboptimal in vitamins A and B complex. When the diet was supplemented with B complex vitamins including pantothenate, the average urinary excretion of taurine dropped from 900 μmoles to 400 $\mu\text{moles/day}$ (Jacobsen and Smith, 1968). Since pantothenic acid is necessary for the synthesis of coenzyme A, Jacobsen and Smith (1968) suggested that these results may indicate taurine is indirectly synthesized from cysteamine.

Vitamin B₁₂

An abnormally large amount of urinary taurine was excreted by patients with megaloblastic anemia due to vitamin B₁₂ deficiency (Fowler et al., 1960; Todd, 1959).

Nondietary Factors

Other factors that affect the presence of taurine in tissues and urine of mammals are age, adrenocorticotrophic and adrenocortical hormones, radiation, trauma, inborn errors of sulfur metabolism, mental retardation, psoriasis, and certain disorders of the muscle, liver, kidney, and bone. These factors have been reviewed comprehensively by Jacobsen and Smith (1968).

Taurine Content of Blood

Taurine Content of the Four Compartments of Blood

Taurine is distributed unevenly among the 4 compartments of blood: plasma, erythrocytes, leukocytes, and platelets. Platelets and leukocytes are especially high in taurine. Soupart (1962) found that, in terms of 100 gm of wet weight, leukocytes are 521 times higher in taurine than plasma; and platelets, 420 times higher. The other free amino acids are 4 to 60 times as

high in these cells as in plasma.

Leukocytes

McMenamy and coworkers (1960) found that human leukocytes contained an average of 27,800 μ moles of taurine/kg of water. The taurine content of leukocytes of an adult man studied by Soupart (1962) was 2,603 μ moles/100 gm of wet weight.

Platelets

The taurine content of platelets in an adult man, as reported by McMenamy and Lund (1960), was 22,800 μ moles/kg of water; and as found by Soupart (1962), 2,100 μ moles of taurine/100 gm of wet weight. Frendo, Koj, and Zgliczyński (1959) reported that the mean taurine concentration of platelets of 21 normal persons was 1.5 mg/gm of platelets.

Erythrocytes

The mean taurine content of erythrocytes from 8 subjects studied by McMenamy et al. (1960) was less than 70 μ moles/kg of water.

Plasma

The taurine content of plasma of normal adults that has been reported in the literature ranges from 0.13 to 1.73 mg/100 ml (Table 1). McMenamy and coworkers (1957, 1960) suggested that

Table 1. Taurine Content of Plasma of Normal Individuals.

Reference	Subjects	Number of subjects	Age (years)	Taurine (mg/dl)
Ackermann & Kheim (1964)	men	16	16-39	0.78
	women	17	20-41	0.69
	older men	17	52-86	0.71
	older women	17	56-96	0.69
Brigham <u>et al.</u> (1960)	men	3		0.46, 0.56 0.53
Childs & Nyhan (1964)	children	17		0.83
Cusworth & Dent (1960)	men	2		0.88, 0.79
	women	2		0.96, 1.30
Dickenson <u>et al.</u> (1965)	infants	31		0.93-2.70
	adults	8		0.57-1.73
Everud (1955)	men	2		1.24, 0.74
	women	1		0.34
McMenamy <u>et al.</u> (1957)	men	2		0.13
Scriver & Davies (1965)	children	9	3-10	0.71-1.45
Stein & Moore (1954)	men	5		0.41-0.82
Taguchi (1964)		10		4.5 ^{1/}
Zinneman <u>et al.</u> (1963)	men	11		0.68
Zinneman <u>et al.</u> (1967)	men	5		0.54
	pregnant women	7		0.43
	women	7		0.58

^{1/} In serum.

these values may be high because the plasma might have been contaminated by the disintegration of taurine-rich platelets and leucocytes during the manipulation of the sample. Using procedures to prevent the lysis of leukocytes and platelets, McMenamy and coworkers found, respectively, 0 and 20 μ moles of taurine/100 ml of plasma of two subjects (1957), and no detectable taurine in plasma of 12 subjects (1960).

The general pattern of free amino acids in the plasma of a person who is fasting approximately simulates the amino acid composition of the tissue proteins (Soupart, 1962; Westall, 1962). This may not be true of taurine since the plasma clearance of this amino acid by the kidney, as calculated by Soupart (1962), is 3 hours, the fastest of all amino acids. Taurine accounts for a relatively large proportion of the amino acids in human urine. McCague (1967) reported that the daily urinary excretion of taurine by 6 women who were consuming self-selected diets ranged from 51.0 to 337.5 mg.

After a meal high in protein, the plasma levels of most amino acids increased (Westall, 1962) but the changes in plasma taurine were not consistent (Frame, 1958). Frame suggested that the variable results might have been due to the lysis of platelets and leukocytes during the separation of cells from plasma.

The effects of pyridoxine deficiency on plasma taurine has been reported under "Effect on Taurine Content of Plasma."

Hormonal factors have not been shown as yet to affect taurine in plasma. Ackermann and Kheim (1964) (Table 1) reported that young and older men and women have essentially the same levels of plasma taurine. However, the taurine content of the plasma of infants 1 or 2 weeks of age may be as high as 2.70 mg/100 ml (Dickenson, Rosenblum, and Hamilton, 1965). Schreier (1962) suggested that this may result in the neonate from either an immaturity of the pathways degrading taurine or from an increased synthesis of the amino acid.

To the knowledge of this reviewer the taurine content of whole blood has not been reported in the literature.

Precautions with Blood Collection and Cell Separation

Since platelets and leukocytes are much higher in taurine content than plasma, several precautions need to be taken to prevent the disintegration of these cells during the manipulation of blood.

The use of non-wettable surfaces, such as polyethylene, nylon, Teflon, or glass treated with liquid silicone or methyl chlorosilane, are essential because platelets and leukocytes are preferentially wet by water and are easily fractured when their wetness is attracted to a glass surface. Erythrocytes, in contrast, are wet by oil (Tullis and Rochow, 1952; Wintrobe, 1961).

The method of preventing blood coagulation also determines the survival of cells. McMenemy et al. (1960) obtained a better yield of leucocytes when the blood was drawn with a syringe that contained a decalifying resin than when the blood was treated with heparin or citrate. The structure of the erythrocyte is better preserved with ethylenediamine-tetraacetic acid than with oxalate (Wintrobe, 1961).

To prevent contamination of the plasma, it should be removed from the cells as soon as possible after the blood has been drawn. The blood should be transferred to plastic test tubes and centrifuged at 2°C. For centrifugation at this temperature, McMenemy et al. (1960) used a force of 1750 x g.

Methods to Determine Taurine

Most of the procedures for the determination of taurine in biological materials utilize strongly cationic exchange (polystyrene sulfonic acid) resins. Taurine, unlike most other amino acids, is not adsorbed by these resins. The attraction of the amino group of taurine to the negatively charged sulfonic groups of the resin is reduced because, within the taurine molecule, the negative charge of the sulfonic group ($pK_a = 1.4$) neutralizes the positive charge of the amino group ($pK_b = 8.7$).

Stein and Moore (1954) have developed column chromatographic

procedures with strongly cationic exchange resins (Dowex 50 and 50W) to separate taurine and other amino acids in plasma and urine. After the deproteinized sample has been applied to the column, the column is developed with appropriate solutions. As the amino acids emerge, they are reacted with ninhydrin and are estimated by the development of color (Moore and Stein, 1946) or the liberation of carbon dioxide (Hamilton and van Slyke, 1943). Taurine is found in the first few fractions that issue from the column.

Procedures specific for the determination of taurine have been developed. Awapara (1956, 1957) determined taurine by applying a protein-free extract of tissues to a column of Dowex 50 X8 (H^+). The amino acid was isolated from other compounds in the eluate by paper chromatography, and after elution from the chromatogram, taurine was quantified with ninhydrin. Pentz et al. (1957) removed the interfering amino acids in urine by batch treatment with Dowex 50W X8 (H^+) and determined taurine in the supernatant as a dinitrophenol derivative. Taguchi (1964) also used Dowex 50W X8 (H^+) batchwise to separate taurine from most other amino acids in urine and serum. Taurine, after separation from interfering ninhydrin-positive substances by paper electrophoresis, was determined with ninhydrin. Ling (1957) removed amino acids, amines, and peptides from urine by batch treatment with Amberlite IR 112 (H^+), another polystyrene sulfonic acid-type resin, and estimated taurine as the dinitrophenol

derivative. Sörbo (1960) applied urine to a column of Dowex 50W X8 (H^+) and determined taurine in the effluent with ninhydrin. Determining taurine in identical samples by several procedures, Sörbo found that the values obtained with the one he developed and the one developed by Ling were close but lower than the values obtained with the procedure by Pentz. Sörbo suggested that the specificity of his procedure could be improved by applying the samples to a column of Dowex 2 (Cl^-), an anionic exchange resin, in addition to a column of Dowex 50W X8 (H^+). Garvin (1960) passed the deproteinized tissue extract through two identical columns that contained separate layers of Dowex 1 (Cl^-) and Dowex 50W X8 (H^+). Taurine was quantified with a ninhydrin reagent that had been modified to reduce interference by urea. By using the column procedure developed by Garvin and the ninhydrin procedure by Sörbo, Roe and Weston (1964), and Mercer (1963) estimated taurine in selected foods and rat urine, respectively.

Taurine can also be determined by paper (Smith, 1960) and thin layer (Pataki, 1966) chromatography. Dent (1947) originally identified taurine as a normal constituent of urine by paper chromatography. Taurine on the paper or thin layer chromatogram is usually estimated by the development of a purple-colored product with ninhydrin or as a dinitrophenol derivative.

EXPERIMENTAL

The purpose of this study was to develop a procedure for the determination of taurine in plasma and whole blood. The procedures of Sörbo (1960) and Garvin (1960) were combined and adapted. To separate taurine from the other amino acids, a protein-free extract of plasma or whole blood at about pH 5 was applied to a column similar to the one used by Garvin which contained separate layers of Dowex 50W (H^+), a cationic exchange resin, and Dowex 1 (Cl^-), an anionic exchange resin. Taurine, which is not adsorbed by either resin, was estimated in the effluent by the ninhydrin procedure of Sörbo.

To adapt the column chromatographic procedure of Garvin, it was necessary to find (1) the amount of each resin needed to remove the ninhydrin-positive substances which would interfere with the determination of taurine in the effluent, and (2) the amount of redistilled water required to wash taurine from the column. The efficiency of the resins in removing interfering amino compounds was checked with one-dimensional, ascending paper chromatography. Two solvents and two location agents were used to identify taurine.

Taurine was determined in 8 samples of whole blood collected from 5 women in the postabsorptive state. Platelet and leukocyte counts were made on each sample for comparison to the

concentration of taurine in whole blood. Recovery of added taurine from blood, and paper chromatography of the column effluents, served to check the efficiency of the procedure.

Procedure

Description of Samples for Preliminary Studies

Plasma from Stored Blood

Blood that had been collected 12 days earlier and stored in a blood "bank" was obtained from the clinical laboratory of Good Samaritan Hospital. The plastic bag in which it had been stored initially contained 450 ml of blood and 67.5 ml of anticoagulant Acid Citrate Dextrose (Solution A U.S.P.). The blood could no longer be used for transfusion because the bag had been opened the day before. After the blood was transported on ice to the laboratory, it was transferred to large plastic test tubes, and centrifuged at 2500 rpm at 2^o C for about 50 minutes. The plasma was cloudy and pink, indicating some hemolysis had taken place. Three-ml portions of plasma were placed in tubes that were subsequently covered with parafilm and stored at -10^o C.

Plasma from Freshly Drawn Blood

About 200 ml of venous blood were drawn from each of two healthy women by a laboratory technician at Good Samaritan

Hospital. To prevent lysis of platelets and leukocytes the blood was drawn into plastic donor bags using silicone-treated steel needles and plastic tubing. Before the blood was collected 30 mg of heparin in 2 ml of solution had been placed in each bag. The blood was promptly carried on ice to the laboratory, transferred to large plastic test tubes, and centrifuged at 2500 rpm at 2° C until the plasma was clear, about 50 minutes. The plasma from both subjects was transferred to a 400-ml beaker and mixed thoroughly. Three-ml portions were placed in tubes that were subsequently capped with parafilm and stored at -10° C.

Stored Whole Blood

Citrated whole blood was obtained from the laboratory at Good Samaritan Hospital. The blood, Rh negative type A, had been collected one month earlier and could no longer be used for transfusion. The sample was transported on ice to the laboratory where it was transferred to an 800 ml beaker and mixed. The blood was hemolyzed by adding 475 ml of blood to an equal volume of redistilled water. After thorough mixing, 5- and 7-ml portions of the diluted blood were pipetted into tubes which were subsequently capped with rubber stoppers or parafilm, and stored at -10° C.

This blood was used in some of the preliminary studies,

and as a control sample when the procedure was applied to blood collected from several individuals.

Preparation of Samples for Ion Exchange Chromatography

Reagents

1. Trichloroacetic acid, 10% (w/v).

Prepared fresh daily from a stock solution of 100% (w/v) trichloroacetic acid.

2. Ether, purified grade, water-saturated.

Equal volumes of ether and redistilled water were shaken together. The water was removed by aspiration after the two phases had separated.

3. Sodium hydroxide, 0.05, 0.2, and 0.8 N.

4. Methyl red, 0.02% (w/v) in 95% ethanol.

The solution was stored at 4° C.

5. Taurine (2-aminoethanesulfonic acid) standard, Sigma Chemical Co., St. Louis, Mo., Lot No. 83B-150.

0.1000 gm of taurine was dissolved and diluted to 100 ml with redistilled water (1 ml = 1 mg). The standard was stored at 4° C.

Procedure

One volume of plasma was diluted with one volume of water, or water was added to blood so that the final dilution was one volume of blood to two volumes of water. In experiments to determine the recovery of added taurine from blood, a known volume of taurine standard replaced part of the water. Blank samples to be chromatographed were prepared by substituting redistilled water in the same volume as the diluted plasma or blood; and taurine standard to be chromatographed, by substituting a known concentration of taurine in the same volume. To each of the preceding an equal volume of 10% trichloroacetic acid was added slowly with stirring. Each sample was covered with parafilm and warmed in a water bath at 50° C for 15 minutes. After the sample had been centrifuged at 3000 rpm for 10 minutes, the supernatant was filtered through Whatman No. 42 paper into a 38-ml or 42-ml stoppered centrifuge tube (Maizel-Gerson type). To remove the trichloroacetic acid, the supernatant was extracted 4 times with an approximately equal volume of water-saturated ether.

To a known volume of the extract, 0.02 ml of 0.02% methyl red was added, and the pH was adjusted with 0.8, 0.2, and 0.05 N NaOH until the appearance of the first faint yellow color, about pH 5. The sample was then ready for ion exchange column chromatography.

Ion Exchange Chromatography

Preparation of Ion Exchange Resins

Dowex 50W X4 (H^+) (200-400 mesh)

To remove the fines the resin was suspended in equal volume of redistilled water. After the resin had settled the supernatant was removed by suction. This procedure was repeated 6 to 8 times, until the supernatant was clear. The resin was allowed to stand under water until further treatment.

The resin was generated in a 4.25 (outer diameter) x 75 cm column. A short piece of rubber tubing was attached to the outlet so that the flow rate could be controlled with a screw clamp. Since considerable heat is produced when the resin is generated, the column was wound with masking tape to prevent breakage.

A piece of folded cotton gauze was placed at the bottom of the column, and the gauze and inside of the column were wetted with redistilled water. The outlet was closed and a slurry of resin was poured through a funnel into the column. After the resin had settled by gravity, the outlet was opened and the water was allowed to drain down to the surface of the resin.

To prepare the resin for chromatography, it was washed successively with: 2 N NaOH until the effluent was blue to litmus, redistilled water until the effluent was neutral, and

4 N HCl until the effluent was red to litmus. The resin was washed again with redistilled water until the effluent was neutral, and the procedure was repeated. After the last addition of 4 N HCl the resin was washed exhaustively with redistilled water. To generate one lb of Dowex 50W, the following volumes of reagents were used: 1.25 l of 2 N NaOH, 1.25 l of redistilled water, 5 l of 4 N HCl, 4 l of redistilled water, 1.25 l of 2 N NaOH, 1.25 l of redistilled water, 5 l of 4 N HCl, and 7 l of redistilled water. The prepared resin was stored under water in a closed glass jar.

Dowex 1 X8 (Cl⁻) (200-400 mesh)

The procedure for removing the fines from Dowex 1 was the same as that for Dowex 50W. The resin was stored under water in a closed glass jar and used without further treatment.

Ion Exchange Chromatography of the Samples

Equipment

1. Ion exchange columns, 1.2 (outer diameter) x 20 cm.

A 200-ml round bottom flask, which served as a reservoir, was fused to the top of the column, and a glass tube ending with a fine tip and containing a Teflon stopcock with a needle valve was fused to the bottom of the column.

2. Funnels with capillary stems.

The stems of small glass funnels were heated and pulled into a capillary.

3. Graduated conical centrifuge tubes, with lip, 12 ml.

4. Conical centrifuge tubes, 40 ml (calibrated 40-ml tubes).

These were calibrated by pipetting 3 ml of redistilled water into the tube. The meniscus was marked with a diamond pencil.

Packing the Columns

The stopcock was closed and the column was filled with redistilled water. A plug that had been prepared under water by loosely folding a square of cotton gauze around a small piece of cotton was placed at the bottom of the column. Some of the water in the column was drained, the stopcock was closed, and a slurry of resin was transferred by pipette to the column and released under the water. The resin was allowed to settle by gravity. The final height of the column of resin varied according to the experiment.

When a column of both Dowex 50W and Dowex 1 was used, the layer of Dowex 50W was always placed at the bottom. A slurry of Dowex 1 was placed carefully by pipette on top of the Dowex 50W when it had completely settled. The second resin was also allowed

to settle by gravity. It was essential that the Dowex 50W had completely settled before the Dowex 1 was added and that the bed of Dowex 50W was not disturbed by the addition of Dowex 1. Failure to do this resulted in the formation of small "curds" where the two resins mixed together.

Each column was washed with 50 ml of redistilled water before use. At this time the flow rate of the column was adjusted with the needle valve to less than 1 ml per minute, as was suggested by Garvin (1960).

Application of Sample to Column

The stopcock was closed and the sample was applied to the previously prepared column with a pipette, or transferred quantitatively through a funnel with a capillary stem. The resin was permitted to settle before the effluent was allowed to issue from the column. The meniscus of the sample was brought just down to the surface of the packing.

The resin was washed with portions of redistilled water, the total volume depending on the height of the resin and the experiment. Any sample that had adhered to the inner wall of the column was washed down with the first few portions of water. The effluent was collected either in twenty-four 0.8-ml fractions in graduated 12-ml centrifuge tubes or in a batch in a calibrated 40-ml tube.

The resin was discarded after use.

Determination of Taurine in the Effluent by Ninhydrin Reaction

Equipment

1. Evaporation equipment.

Because of the low concentration of taurine in plasma and blood, the effluent collected in a batch had to be reduced in volume. The effluent was evaporated in the calibrated 40-ml tube in which it had been collected. Each tube was fitted with a two-holed rubber stopper. A Pasteur pipette with the capillary tip extending about 5 cm into the tube served as an air inlet. Air intake was controlled by clamping a piece of rubber tubing attached to the top of the pipette so that the stream of air just rippled the surface of the liquid. A bent piece of glass tubing inserted in the other hole of the stopper was attached to a water suction manifold. Each manifold could accommodate up to 6 tubes; however, too many tubes reduced the suction and lengthened the time required for evaporation. The effluents were evaporated in a 50° C water bath. To reduce an effluent of 15 ml to one to 1.5 ml took from two to 4.5 hours, depending

on how many tubes were attached to the system.

2. Coleman Jr. Spectrophotometer, Model 6A, with adaptor 6-108 for 10 x 75 mm cuvettes.
3. Bent-rod mixer.

A bent rod was attached to a 1/20 hp motor. The sample was mixed by touching the tube to the rotating bent rod.

Reagents

1. Sodium hydroxide, 0.05, 0.2, and 0.8 N.
2. Ninhydrin (triketohydrindene hydrate) stock solution, 3% (w/v) in 2-methoxyethanol.

The 2-methoxyethanol was chromato-quality reagent grade from Matheson, Coleman and Bell, Rutherford, N. J.

3. Sodium citrate buffer, 0.2 M, pH 5.7.
42.03 gm of citric acid monohydrate were added to 560 ml of N NaOH and diluted to 1000 ml. The pH was adjusted to 5.7 with 0.2 M citric acid. The buffer was stored at 4° C.

4. Stannous chloride solution.
4.51 gm $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ were dissolved in 1.7 ml of concentrated HCl in a 10-ml volumetric flask and diluted to the mark with redistilled water. This reagent

was stable for about one month when stored at 4° C.

5. Ninhydrin reagent.

0.10 ml of stannous chloride solution, 25 ml of 3% ninhydrin stock solution and 25 ml of 0.2 M sodium citrate buffer were mixed together just before use.

6. Ethanol, 50% (v/v).

7. Methyl red, 0.02% (w/v) in 95% ethanol.

Procedure

To the effluent that had been evaporated in the calibrated 40-ml tube, 0.01 ml of 0.20% methyl red was added. The pH was adjusted with 0.8, 0.2, and 0.05 N NaOH until the first faint yellow color appeared, at about pH 5. Redistilled water was added to bring the volume to 3 ml. In mixing, care was taken to rinse the sample from the sides of the tube.

Duplicate one-ml portions of the effluents were placed in each of two 15 x 125 mm test tubes. With each determination duplicate reagent blanks were prepared by using one ml of re-distilled water in place of the effluent. Two ml of ninhydrin reagent were added to each tube and the contents of the tube were mixed briefly on the bent rod mixer. The tubes were capped with aluminum foil, heated for exactly 20 minutes in a boiling water bath, and cooled. The aluminum caps were removed, 5 ml of 50% ethanol were added, and the contents of the tubes were mixed for

approximately 10 seconds on the mixer. Just before reading the absorbance at 570 $m\mu$ with the Coleman Jr. Spectrophotometer, the samples were mixed again and transferred to 10 x 75 mm cuvettes. The instrument was set at zero with 50% ethanol.

To each tube that contained an 0.8-ml fraction of the effluent were added: 0.01 ml of 0.02% methyl red, NaOH to the first faint yellow color, redistilled water to the one-ml mark, and two ml of ninhydrin reagent. The procedure was continued as described above, except that these tubes had to be mixed by hand.

Standard Curve

After the absorbance of the chromatographed sample had been corrected by subtracting the absorbance of the column blank, the concentration of taurine was determined by reference to a standard curve. With the ninhydrin reaction, there was a linear relationship between absorbance and concentration of taurine, ranging from 5 to 40 $\mu\text{g/ml}$ (Figure 2). The standard curve was prepared from standards that had not been chromatographed.

Preparation of the Standard Curve

The taurine standard containing 1 mg of taurine/ml was diluted with redistilled water to give concentrations of 50, 100, 150, 200, 300, and 400 μg of taurine/ml. To each of six 10-ml volumetric flasks were added one ml of each standard and 0.01 ml

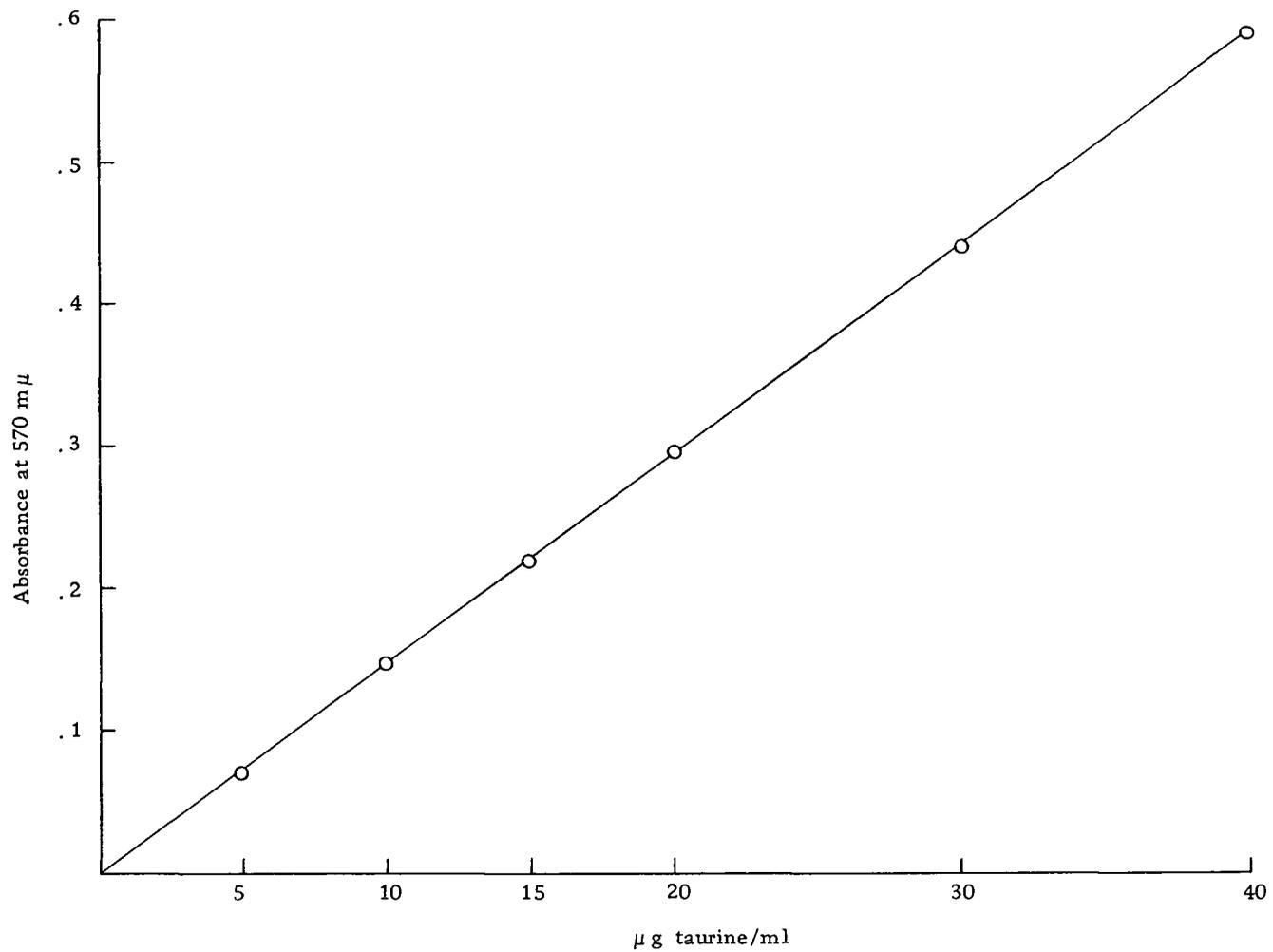


Figure 2. Standard curve for taurine.

of 0.02% methyl red. The standards were brought to the first faint yellow color with 0.05 N NaOH and diluted to the mark with redistilled water. The concentrations of taurine in these standards were 5, 10, 15, 20, 30 and 40 $\mu\text{g/ml}$, respectively.

Duplicate one-ml portions were removed from each flask, and the ninhydrin procedure described above was followed. For the blank, 0.01 ml of methyl red was diluted to 10 ml, and duplicate one-ml portions were removed for the ninhydrin procedure. The absorbance of the blanks and standards was read at 570 $\text{m}\mu$. The standard curve was plotted after the readings for each standard were averaged and corrected by subtracting the blank.

Paper Chromatography

Paper chromatography was used to identify taurine and to check the efficiency of the columns in removing ninhydrin-positive substances in the effluent that interfered with the determination of taurine.

Preliminary studies indicated that several solvent systems^{1/} were satisfactory for the identification of taurine by paper

^{1/} Butanol-acetic acid-water (4:1:1); phenol-water (4:1); butanol-pyridine-water (1:1:1); pyridine-water (4:1); and ethanol-0.88 N ammonia-water (18:1:1).

chromatography. Two solvents chosen for the analysis of the column effluents were butanol-acetic acid-water (4:1:1) and butanol-pyridine-water (1:1:1). These solvents separated taurine and urea, a possible interfering agent (Garvin, 1960; Sörbo, 1960). Furthermore, different ratio of fronts (R_f) values were obtained for these two compounds with each of these two solvents. Taurine was resolved as a more compact spot with the butanol-acetic acid solvent than with the other solvent.

Taurine was located with ninhydrin and Erlich reagent. Amino compounds that would interfere with the quantitative determination of taurine were detected with ninhydrin test (Smith, 1960). Urea was located with Erlich reagent. As little as 1 μ g of taurine and urea could be located with ninhydrin and Erlich reagents, respectively.

Reagents

1. n-Butanol-acetic acid-water solvent, (v/v) (133:33:33).
2. n-Butanol-pyridine-water solvent (v/v) (66:66:66).
3. Ninhydrin solution, 0.2% (w/v) in acetone.

The solution was stored at 4° C.

4. p-Dimethylaminobenzaldehyde, 10% (w/v) in concentrated hydrochloric acid (Erlich reagent).

Immediately before use, 10 ml of this reagent were mixed with 40 ml of acetone.

Equipment

1. Whatman No. 1 paper, 20 x 24 cm.

Plastic gloves were worn when cutting the sheets from 18 x 22 inch sheets. The grain was parallel to the 24-cm sides.

2. Glass jars, one gallon size, wide mouth with screw lids (chromatography jars).

The inside of the lids were coated with paraffin to protect the metal from volatile solvents.

3. Plastic dip tray.

This was used for dipping chromatograms in color reagents.

4. Plastic clips.

5. Microliter syringes, 10 μ l.

Hamilton Co., Whittier, California.

6. Chromato-Vue, Model C-3.

Ultra Violet Products, Inc., San Gabriel, California.

Solvent fronts of dried chromatograms were marked under ultraviolet light.

Procedure

The origin was ruled with a pencil 2.5 cm from the bottom 24-cm edge of two 20 x 24 cm Whatman No. 1 papers. To each paper 10 to 50 μ l of evaporated effluent were applied with a microliter syringe. The spots were dried in between each

application so that they were never bigger than 0.5 cm. At least 2 cm were allowed between spots. The upper 24-cm edge was bent to form a cylinder, overlapped about 2 cm and fastened with a plastic clip. Each paper was placed in one of two chromatography jars: one containing 200 ml of butanol-acetic acid-water; and the other, 200 ml of butanol-pyridine-water. These solvents had been placed in the jars at least one hour before the papers were placed in them and the lids had been tightly closed so that the air was in equilibrium with the vapors of the developing solvent. The chromatograms were developed at room temperature for 4 hours, after which they were removed from the jars and hung to dry in a hood.

When the chromatograms were dry, they were dipped in approximately 50 ml of 0.2% ninhydrin solution and again hung to dry. Taurine was indicated by the appearance of a purple spot after the chromatograms were heated for three to 4 minutes in an oven at 100° C. Because the purple spot faded with further testing, each spot was outlined with pencil.

The same papers were then dipped in Erlich reagent and allowed to dry in the hood for at least 20 minutes. The presence of taurine was identified with a yellow spot, and the presence of urea with a yellowish-green spot.

After the application of the location agents, the solvent front was marked under ultraviolet light, and the R_f values were determined.

Preliminary Ion Exchange Chromatography Studies

Experiments to Determine the Quantities of
Dowex 50W (H^+) and Dowex 1 (Cl^-) Necessary
to Remove Ninhydrin-Positive Substances That
Interfere with the Determination of Taurine in
Blood and Plasma

Before the amount of resin needed to remove interfering ninhydrin-positive substances could be determined, it was necessary to know the amount of redistilled water that was required to wash the resin.

The passage of taurine through the column was studied by applying identical 4-ml portions of plasma extract containing 30 μg of added taurine to Dowex 50W columns of different heights. To obtain identical samples the plasma had been deproteinized and prepared for chromatography in bulk. After the samples had been applied to the column, each column was washed with portions of redistilled water. Twenty-four 0.8-ml fractions were collected and taurine was determined with ninhydrin as described previously.

In similar experiments, identical 6-ml portions of blood extract that contained 180 μg of added taurine were chromatographed on each of 4 columns of different heights of Dowex 50W or Dowex 1. Finally, 6 ml of sample containing 180 μg of taurine and no blood or plasma was applied to a column of 6 cm of Dowex 50W and 3 cm of Dowex 1.

Protein-free plasma and blood extracts were also prepared in bulk for experiments to determine the height of Dowex 50W and Dowex 1 necessary to remove interfering ninhydrin-positive substances. Four columns of Dowex 50W of different heights were used to chromatograph 4 ml of plasma extract (equivalent to 1 ml of plasma) or 6 ml of blood extract (equivalent to 1 ml of blood). Similar identical blood samples were prepared for 4 Dowex 1 columns of different heights. After the sample had been applied to the column, redistilled water was added in appropriate volumes to wash the resin.

Blanks were obtained for each column by applying 6 ml of blank sample that had been prepared with water instead of blood, plasma, or standard. The sample was applied to the column after the blank sample had been chromatographed and the column had been washed with 50 ml of redistilled water.

When the effluent had to be evaporated, the first few fractions from longer columns of Dowex 50W, that were shown to contain no taurine, were discarded. Before applying the ninhydrin reaction the effluents from columns of Dowex 1 were collected in batch and diluted to 25 ml; those from columns of Dowex 50W were evaporated to about 1.5 ml.

Recovery of Taurine From Dowex 50W (H⁺) and Dowex 1 (Cl⁻) and From Blood

The recovery of taurine from the resins and from blood was studied by using columns that contained 6 cm of Dowex 50 W and 3 cm of Dowex 1.

For each experiment separate columns were prepared for a blank, a sample of deproteinized blood, a sample of deproteinized blood with added taurine, and a taurine standard. All samples were described previously under "Preparation of Samples for Ion Exchange Chromatography." After the samples had been applied to the columns, the columns were washed with a suitable volume of redistilled water, as had been determined in previous experiments. The initial three ml of effluent from the columns were discarded, and the following effluent was collected in a batch. The effluents from the columns were evaporated to less than 1.5 ml. An unchromatographed standard and blank was included with the ninhydrin determination.

Paper Chromatography of Effluents

After the samples had been removed from the evaporated effluents for the ninhydrin reaction, the remaining effluent was evaporated further and analyzed with paper chromatography as described previously. A taurine standard that had not been

chromatographed was used as a reference.

Determination of Taurine in Whole Blood of Healthy
Women

Procedure

Venous blood from 5 healthy young women in the post-absorptive state was drawn by the medical technician at the Oregon State University Student Health Center. One of these women had samples taken on 4 consecutive mornings. Each woman consumed a self-selected diet. Approximately 8 ml of blood were collected with Vacutainer disposable needles into Vacutainer tubes containing sodium heparin (Becton, Dickinson, and Co., B-D). After the technician had removed sufficient blood for the platelet count, the sample was transported immediately to the laboratory and refrigerated for 15 minutes to 2 hours, until diluted and frozen. Three of the samples (A, B, and E-1) were diluted in duplicate by placing 3.5 ml of blood in a glass tube that contained 7.0 ml of redistilled water (diluted 1:2). The remaining samples were diluted in duplicate by pipetting 3.5 ml of blood into a glass tube that contained 3.5 ml of redistilled water (diluted 1:1). The contents of the tubes were mixed thoroughly, and the tubes were capped with rubber stoppers and stored at -10° C. The remaining portion of whole blood was used for counting the leukocytes.

Each set of determinations included a blank, a standard, a control sample which was described under "Description of Samples", and a recovery for each blood sample, except three. No more than 7 columns were operated at one time. The diluted blood was thawed just before analysis. The taurine standard containing 90 $\mu\text{g}/\text{ml}$ was prepared from the standard that contained 1 mg/ml .

Quantities used to prepare the samples for chromatography were as follows:

Sample	Blood (diluted 1:1) (ml)	Taurine standard (ml)	(μg)	Redistilled water (ml)	10% Trichloroacetic acid (ml)
Subject	9 ^{1/}				9
	6			3	9
Recovery	6	1.5	135	1.5	9
Control	6			3	9
Standard		1	90	5	6
Column blank				6	6

^{1/} For the three samples that were diluted 1:2.

All samples were treated as described under "Preparation of Samples for Ion Exchange Chromatography." After the supernatant had been extracted with water-saturated ether for the fourth time, exactly 6 ml of each sample were transferred to a 10 x 80 mm tube. To each sample were added 0.01 ml of 0.02% methyl red, and 0.8, 0.2, and 0.05 N NaOH until the first faint yellow color appeared. Less than 0.2 ml of NaOH was required to adjust the pH of each sample. The difference in volume between the

tubes was adjusted by adding an appropriate quantity of redistilled water so that each tube contained 6.2 ml.

The samples were applied to previously prepared columns containing 6 cm of Dowex 50W and 3 cm of Dowex 1. Just before use, the columns had been washed with 50 ml of redistilled water. Each sample was transferred quantitatively to a column with the aid of a funnel with a capillary stem. The effluent was allowed to issue from the column until the sample reached the surface of the resin. The tube and funnel were rinsed with four 0.5-ml portions of redistilled water and the washings were placed on the column. Then 2-, 3-, and 4-ml portions of redistilled water were added by pipette to each column in order to wash the resin. Each column was washed with a total of 10 ml of redistilled water. The first three ml of effluent were collected in a conical 12-ml graduated centrifuge tube and discarded. The remainder of the effluent was collected in a calibrated 40-ml tube, and evaporated at 50° in this tube to a volume of one to 1.5 ml.

After the effluent had been cooled, 0.01 ml of 0.02% methyl red was added, and 0.8, 0.2, and 0.05 N NaOH were added until the appearance of the first faint yellow color. The effluent was diluted to the three-ml mark and the contents of the tube were mixed thoroughly. Duplicate one-ml portions were removed for the ninhydrin determination of taurine as described under

"Determination of Taurine in the Effluent by Ninhydrin Reaction".

To calculate the taurine content of one ml of blood, the corrected reading (absorbance of sample minus absorbance of column blank) was used to determine the concentration of taurine from the standard curve (Figure 2):

$$\mu\text{g of taurine/ml of blood} = (\mu\text{g of taurine/ml from standard curve}) \times 3$$

In this equation "3" represents the number of milliliters of effluent which contain taurine from one ml of blood and from which one ml of effluent had been removed for reaction with ninhydrin.

The remaining one ml of effluent was evaporated to approximately 0.5 ml, and about 10 μl of each effluent were subjected to paper chromatography. Taurine was identified with two solvents and two location agents as described under "Paper Chromatography".

Leukocyte Counts

Equipment

1. Blood diluting pipette for white cell counts.
2. Spencer Bright-line Hemacytometer, American Optical Company, Buffalo, New York.

3. Microscope, general purpose binocular, American Optical Company, Buffalo, New York.

Reagents

1. Diluting fluid.

3 ml of glacial acetic acid were diluted to 100 ml with redistilled water. Methylene blue was added until a deep blue color was obtained.

The solution was stored at 4° C.

Procedure

The leukocytes were counted within 4 to 6 hours after the blood had been drawn. The anticoagulated blood was mixed, diluted 1 to 20 in a blood diluting pipette, and shaken for exactly two minutes. The capillary part of the pipette was drained before both chambers of the hemacytometer were filled with the diluted blood. After the cells had settled, those in the 4 corners and the central squares of both sides of the hemacytometer, a total of 10 mm squares, were counted with the low-power objective of the microscope. Two dilutions and counts were made for each sample and the counts were averaged. The number of cells in each count was calculated:

$$\text{No. of leukocytes/mm}^3 \text{ of blood} = \frac{\text{No. of cells in 10 squares} \times 20}{1}$$

In this equation, "20" represents the dilution of blood, and "1" represents the total volume of squares counted (length x width x depth x number of squares counted = 1 mm x 1 mm x 0.1 mm x 10).

Platelet Counts

The platelets were counted by the laboratory technician at the Student Health Center at Oregon State University.

RESULTS AND DISCUSSION

Preliminary studies

General Comments on Procedure

In this procedure the separation of taurine from other amino acids is based on the principle that the zwitterion of taurine is not adsorbed by strongly cationic and anionic exchange resins. For the pH adjustment of the protein-free extract, methyl red was chosen as an indicator because its pK_{In} is close to the isoelectric point of taurine, pH 5. Garvin (1960) suggested that NaOH be added to the protein-free extract to the phenolphthalein endpoint, which is about 10. It was found, however, that if the pH of the sample applied to the column was too high, less taurine was recovered from the column. For this reason methyl red, an indicator with an endpoint at a lower pH, was chosen.

To remove the excess trichloroacetic acid, Yamada et al. (1966) suggested that the deproteinized sample should be extracted twice with water-saturated ether. It was found that more trichloroacetic acid could be removed with additional extractions. After two extractions, the pH of the protein-free extract was about 2.5, while after 4 extractions it was about 4. It was desirable to remove as much trichloroacetic acid as possible because the trichloroacetate ion is adsorbed by the anionic

exchange resin. The additional extractions also reduced the amount of NaOH needed to bring the protein-free extract to the methyl red endpoint and, thus, fewer sodium ions would be present in the sample to be adsorbed by the sulfonic acid groups of the cationic exchange resin.

When the column effluents were evaporated to complete dryness at temperatures higher than 50 to 60^o C, a brown residue formed which was difficult to dissolve. In addition, results with ninhydrin were inconsistent. This residue may have been due to the browning reaction, a reaction between the aldehyde group of reducing sugars and free amino groups. Since low humidities and high temperatures enhance browning (Lea and Hannan, 1949), the effluents were not allowed to become dry and the temperature for evaporation was kept between 50 to 60^o C. Consistent results with ninhydrin were obtained when the effluents were evaporated to a volume of one to 1.5 ml and the sides of the tube thoroughly rinsed after the effluent was diluted to three ml.

The ninhydrin procedure by Sörbo (1960) was used because no repeatable results were obtained with the ninhydrin procedure by Garvin (1960). Roe and Weston (1965) and Mercer (1963) determined taurine in foods and rat urine, respectively, using the column chromatographic procedure of Garvin and the ninhydrin procedure of Sörbo.

The ninhydrin procedure requires carefully controlled conditions. Because methyl red is colored, the same amount of this indicator was added to each fraction or evaporated effluent. The ninhydrin reagent was prepared just before use and, after its addition, the sample was mixed for only one or two seconds. It also was necessary to place the tubes in actively boiling water for exactly 20 minutes, to cool the tubes immediately, to mix the contents of each tube thoroughly after the addition of diluent (50% ethanol) and before reading, and to begin reading the samples 15 minutes after the tubes were removed from the boiling water bath.

Results of Preliminary Ion Exchange Chromatography Studies

Quantities of Dowex 50W (H^+) and Dowex 1 (Cl^-) Necessary to Remove Ninhydrin-Positive Substances that Would Interfere with the Determination of Taurine in Blood and Plasma

The results of the experiments to determine the quantity of redistilled water required to wash the columns, and the quantities of resins needed to remove interfering ninhydrin-positive substances from one ml of blood or plasma are summarized in Tables 2 and 3, respectively. The passage of taurine through a 6-cm column of Dowex 50W and through a column containing 6 cm

Table 2. Summary of experiments to determine the amount of water needed to wash the columns after application of sample.

Resin	Col. ht. (cm)	Sample applied to each column ^{1/}	Fractions containing taurine ^{2/} (No.)	Water required to remove taurine (ml)	First ml of effluent to discard ^{3/} (ml)
Dowex 50W	6	4 ml of extract	5 - 13	7	-
	9	(= 1 ml of plasma)	6 - 14	9	3
	12	containing 30 µg of added taurine	8 - 15	11	4
Dowex 50W	3	6 ml of extract	3 - 15	6.5	-
	6	(= 1 ml of blood)	6 - 15	6.5	-
	9	containing 30 µg of	7 - 17	9	3
	12	added taurine	8 - 18	11	4
Dowex 1	1	6 ml of extract ^{4/}	2 - 13	6.5	-
	3	containing 180 µg	3 - 14	7	-
	5	of taurine	4 - 16	9	-
	7		7 - 21	12	-
Dowex 50W +Dowex 1	6 3	6 ml of extract ^{4/} containing 180 µg of taurine	7 - 18	10	3

^{1/} Blood or plasma was deproteinized in bulk so that in each experiment an identical sample could be applied to each column.

^{2/} 0.8-ml fractions were collected.

^{3/} These volumes were discarded in subsequent experiments that required evaporation of the effluent. Effluents from Dowex 1 were not evaporated.

^{4/} Sample was prepared without blood or plasma.

Table 3. Summary of experiments to determine the quantity of Dowex 50W (H⁺) and Dowex 1 (Cl⁻) necessary to remove ninhydrin-positive substances in plasma and blood that interfere with the determination of taurine.

Resin	Col. ht. (cm)	Sample ^{1/}	Vol. of wash ^{2/} (ml)	Chromatographed samples ^{3/} (absorbance)	Column blanks (absorbance)	Average corrected reading	
Dowex 50W	3	4 ml of extract (=1 ml of plasma from stored blood)	5	.220	.065	.157	
	6		7	.220	.062	.111	
				.230	.120		
	9				.238	.125	.106
					.245	.140	
	12			11	.250	.142	.110
				.275	.165		
				.280	.168		
Dowex 50W	3	6 ml of extract (= 1 ml of blood)	12.5	.345	.105	.238	
	6			.345	.110	.196	
				.315	.115		
	9				.310	.118	.187
					.315	.130	
	12			17	.318	.130	.218
				.365	.150		
				.370	.150		
Dowex 1	1	6 ml of extract (= 1 ml of blood)	6.5	.330	.050	.279	
	3			.328	.050	.246	
				.295	.050		
	5				.300	.052	.247
					.294	.050	
7		12	.300	.052	.245		
				.295	.050		
				.300	.055		

Table 3--continued

1/ Blood or plasma was deproteinized in bulk so that for each experiment, identical samples could be applied to the columns.

2/ Volume of redistilled water, as determined in previous experiments (Table 2), required to wash columns.

3/ Effluents from columns of Dowex 50W were evaporated to a final volume of 3 ml; those from Dowex 1 were diluted to 25 ml.

of Dowex 50W and 3 cm of Dowex 1 are shown in Figures 3 and 4, respectively.

Studies with Plasma

When 4 ml of protein-free extract (equivalent to one ml of plasma) were applied to each 3-, 6-, 9-, and 12-cm column of Dowex 50W, the average corrected readings of the effluents (Table 3) showed that no additional ninhydrin-positive substances were removed by columns higher than 6 cm.

The concentration of taurine in this sample was estimated at about 24 $\mu\text{g/ml}$, which is high compared to values reported in the literature (Table 1). This high value for plasma from stored blood may have been due to release of taurine during the disintegration of leukocytes and platelets. The blood used in this experiment had been stored for approximately 12 days, and according to Wintrobe (1961), leukocytes are reduced by 50% within 48 hours after blood is drawn. Kugelmass (1959) stated that platelets also have a short survival time in stored blood. The experiments were then continued with plasma from freshly drawn blood that had been collected and handled so that the disintegration of leukocytes and platelets was kept at a minimum.

Since this second sample of plasma contained less taurine, more plasma had to be applied to the column. Thus, 10-, 15-, and

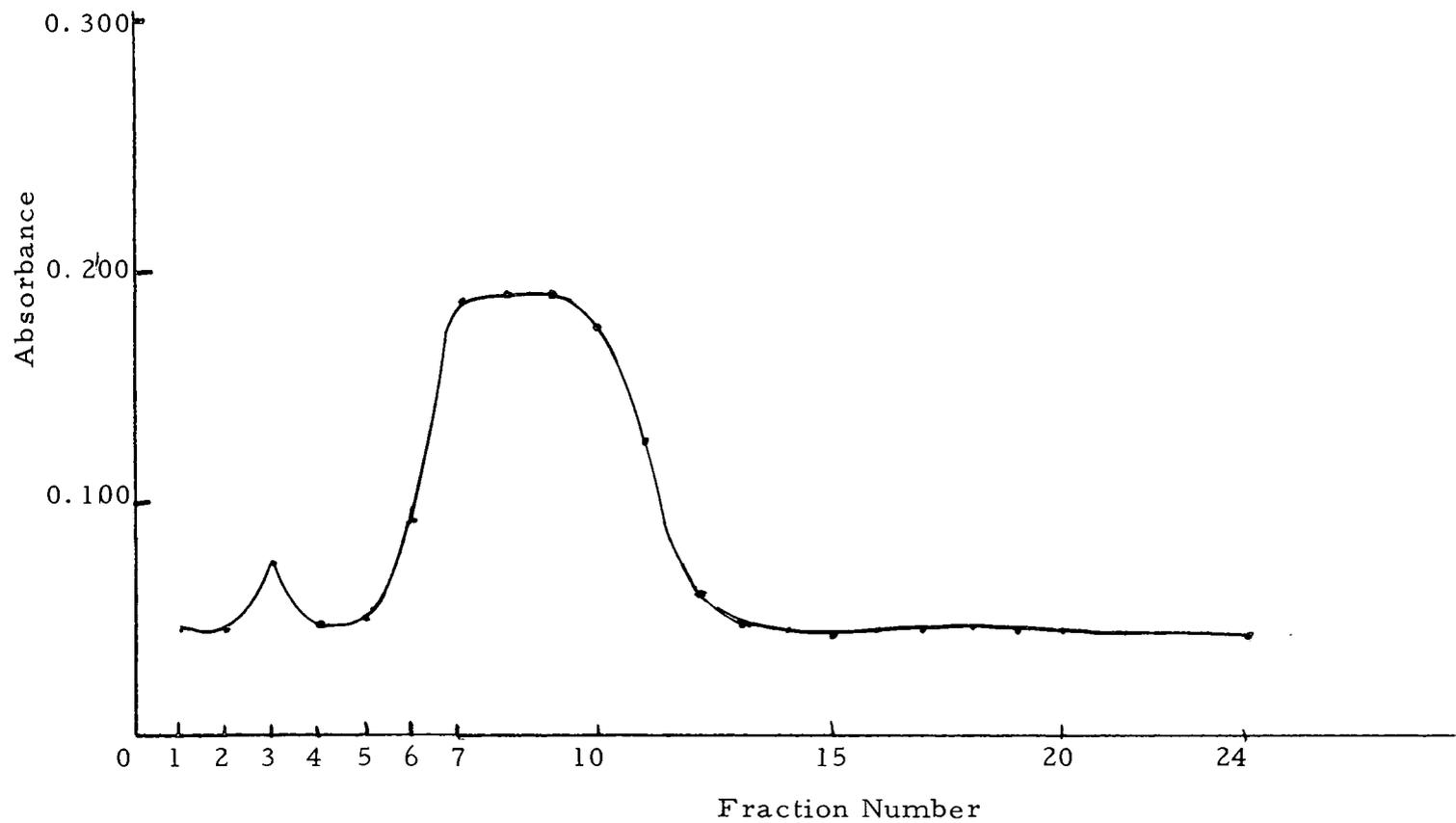


Figure 3. Passage of taurine through a 6-cm column of Dowex 50W (H^+). 6 ml of a protein-free extract of plasma containing 30 μ g of added taurine were applied to the column. 0.8 ml fractions were collected.

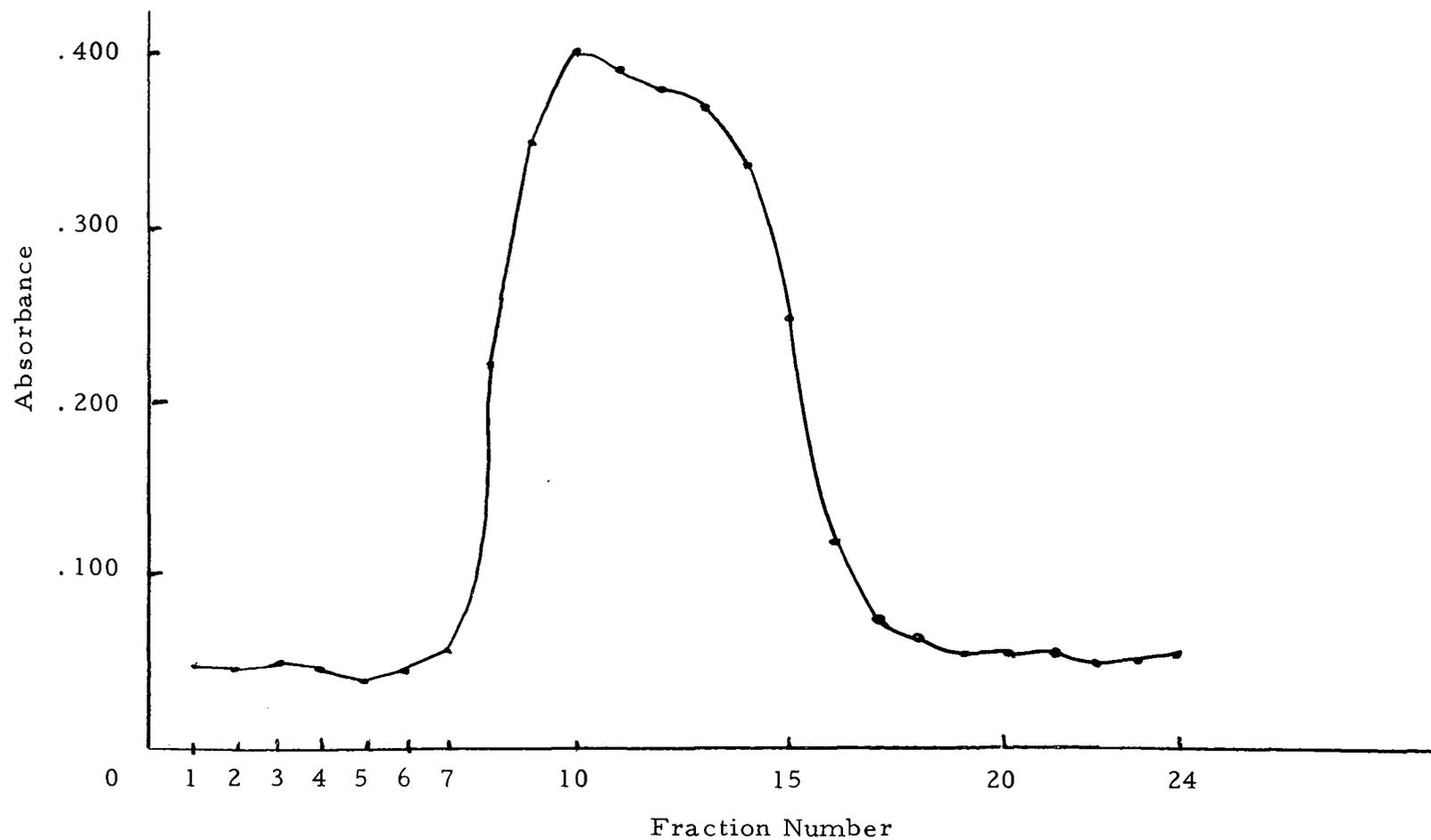


Figure 4. Passage of taurine through a column of 6 cm of Dowex 50W (H^+) and 3 cm of Dowex 1 (Cl^-). 6 ml containing 180 μg of taurine were applied. 0.8 ml fractions were collected.

20-ml portions of the protein-free plasma extract which had been prepared in bulk were evaporated to less than one ml. Each sample was transferred quantitatively with redistilled water to each of three 9-cm columns of Dowex 50W (H^+); the total volume of sample plus washings was approximately 4 ml. The effluents were evaporated and results with ninhydrin were as follows:

Plasma extract (ml)	Equivalent ml of plasma	Absorbance		Averaged and corrected	Taurine ($\mu\text{g/ml}$)
		Blank	Sample		
10	2.5	.110 ^{1/} .102 ^{1/}	.170 ^{1/} .172 ^{1/}	.065	8.8
15	3.8	.150 .155	.235 .240	.084	4.8
20	5.0	.162 .165	.340 .340	.176	6.9

^{1/} Final dilution to 5 ml rather than 3 ml.

The concentration of taurine in plasma is similar to that reported in the literature (Table 1). These values, however, do not indicate the true taurine content of plasma because anionic substances which may also be ninhydrin-positive were not removed by Dowex 50W. It is not understood why the absorbances of the effluents after reaction with ninhydrin were not proportional to the quantities of plasma applied to the columns.

The studies were continued with whole blood rather than plasma for several reasons: a precipitate formed in the

protein-free extract of plasma when it was evaporated; because of high column blanks, the corrected absorbances of the samples fell at the lower limits of the standard curve; and more resin would be needed to adequately remove all interfering ninhydrin-positive substances from these large quantities of plasma.

Studies with Blood

When 6 ml of extract (equivalent to one ml of blood) were applied to 4 Dowex 50W columns of different heights, the averaged corrected readings (Table 3) demonstrated that columns longer than 6 cm did not remove additional cationic ninhydrin-positive substances. A similar sample was applied to each 1-, 3-, 5-, and 7-cm column of Dowex 1, and results (Table 3) indicated that the 3-cm column was sufficient to remove anionic substances which were ninhydrin-positive. The concentration of interfering substances in the effluent from the Dowex 1 column was so high that the effluent had to be diluted to 25 ml in order to obtain reasonable spectrophotometric readings of the product with ninhydrin.

From Table 3 it can be seen that the absorbance of the column blank increased with the height of the Dowex 50W, indicating that some colored substance was leached from the columns. This was not shown with Dowex 1 because the differences, if any, were reduced by the dilution of the effluent to 25 ml.

These experiments also show that Dowex 50W removed more ninhydrin-positive substances than Dowex 1, demonstrating that a protein-free extract of blood at pH 5 contains more cationic amino compounds than it does anionic ones. The difference is greater than Table 3 indicates because, when dilution factors are considered, the absorbances of the effluents from the columns of Dowex 50W and Dowex 1 represent taurine plus anionic ninhydrin-positive substances in 1/3 ml of blood and taurine plus cationic substances in 1/25 ml of blood, respectively.

Recovery of Taurine from Dowex 50W (H^+) and Dowex 1 (Cl^-)

The recovery of 45 μ g of taurine from a column of 6 cm of Dowex 50W (H^+) and 3 cm of Dowex 1 (Cl^-) was 102.8%. The recovery from blood was 100.5%.

Paper Chromatography of Effluents

When effluents from Dowex 50W columns to which extracts had been applied were subjected to paper chromatography in two solvents, there was considerable smearing in the taurine area, indicating that not all interfering compounds were removed. The effluents from the columns of both resins contained only taurine, since chromatograms developed in both solvents had only one ninhydrin-positive spot which had an R_f value similar to that of the taurine standard that had not been subjected to column

chromatography. The Erlich reagent gave similar results to ninhydrin for taurine, and negative results for urea.

Taurine in Whole Blood of Healthy Women

The taurine content and the platelet and leukocyte counts of blood from 5 women in the post-absorptive state are presented in Table 4. The concentration of taurine in 8 samples of blood ranged from 26.1 to 40.2 $\mu\text{g/ml}$. The taurine concentration of samples from 4 individuals ranged from 27.9 to 40.2 $\mu\text{g/ml}$, and taurine in the blood taken on four consecutive mornings from one individual ranged from 26.1 to 34.2 $\mu\text{g/ml}$. For each subject, the number of platelets and leucocytes were within the normal range for adults.

The data presented in Table 4 show that when blood samples were taken from subject E on 4 consecutive days, a decrease in the concentration of taurine was accompanied by a decrease in the number of platelets in blood. At the same time, there was an increase in the number of leukocytes. On the final day of collection, a rise in the concentration of taurine was accompanied by a rise in the number of platelets and a decreased number of leukocytes. Thus it appears that in this subject there was a direct relationship between taurine content and platelet count of blood and an inverse relationship between taurine and leukocyte count.

When the results from the individual subjects are considered,

Table 4. Taurine content and leukocyte and platelet counts of blood of five women.

Subject	Day	Age (yrs)	Leukocytes (per cmm)	Platelets (per cmm)	Taurine ($\mu\text{g}/\text{ml}$)
A		37	6,450	190,000	27.9
B		24	6,600	174,000	38.1
C ^{1/}		42	6,000	270,000	40.2
D ^{2/}		24	6,825	292,000	35.1 ^{4/}
E ^{3/}	1	29	5,000	280,000	34.2
E	2		5,430	244,000	30.9
E	3		5,760	236,000	26.1
E	4		5,330	246,000	32.1

^{1/} Received ferrous sulfate periodically.

^{2/} Received 1 gr of thyroid extract daily.

^{3/} Received sequential contraceptive tablets. Two days prior and during the 4 days of blood collection, she received 0.08 mg of mestranol and 2.0 mg of norethindrone daily. On each of 14 previous days, she had received 0.08 mg of mestranol. The blood was collected from this subject on 4 consecutive mornings.

^{4/} Sample was contaminated during evaporation.

there seems to be no relationship between taurine concentration and platelet and leukocyte counts of whole blood. However, variation from day to day in these subjects is not known. Since there is a probable error of 15% in platelet and leukocyte counts even when both sides of the hemacytometer are counted (Wintrobe, 1961), the number of leukocytes and platelets cannot be reliably correlated with the taurine content of blood.

Since taurine is present in higher concentrations in leukocytes

and platelets than in plasma and erythrocytes, data obtained from whole blood samples must be considered with these differences in mind. Since the chemical composition and enzyme systems of platelets and leukocytes are similar to tissue cells in general (Kugelmass, 1959), the determination of taurine in these cells would be more valuable than the determination of taurine in whole blood.

The taurine concentration of blood of subject E who was receiving an oral contraceptive appeared to be no different from that of the other subjects. However, the taurine content of this subject's blood when she was not using a contraceptive is not known. Price, Thornton and Mueller (1967) recommended that the use of steroid hormones for ovulation control should be considered in metabolic studies with women, especially if pyridoxal phosphate-dependent enzymes are involved. Price and coworkers found that the abnormal tryptophan metabolism evident in women who used these steroid hormones was almost completely corrected by supplementation with pyridoxine.

The taurine concentration of the blood of Subject A was low for this group of women, 27.9 $\mu\text{g}/\text{ml}$. The average daily urinary excretion of taurine by this subject as had been studied earlier by McCague (1967) was 196.0 mg/24 hr (range 154.5 to 337.5 mg/24 hr), the highest of a group of 6 women. The urinary excretion

of taurine by the other five women in the study ranged from 51.0 to 187.5 mg/24 hr.

To lyse the leukocytes, the blood samples collected for this part of the study were diluted and stored at -10° C for 6 to 8 days before analysis. This was the procedure McMenemy et al. (1960) followed to disintegrate leukocytes for the determination of amino acids. The effectiveness of this treatment was not checked by counting the leukocytes in the samples after thawing. Later a sample of capillary blood was diluted with an equal volume of water, stored at -10° C for 4 days and thawed; and the leukocyte count was within the normal range. It cannot be assumed that the leukocytes were disintegrated, even with the addition of 10% trichloroacetic acid in the deproteinization step.

In the preliminary studies inconsistent results were obtained with the stored blood that was used as a control sample. An explanation for this was that the blood might not have been uniformly mixed before portions were removed for freezing. Therefore, before these final studies were done, about 200 ml of the stored blood were thawed and mixed. Mixing was continued with a magnetic stirrer while portions of the diluted blood were removed for refreezing. The taurine values for the control sample (Table 5) were still somewhat erratic and it seems that even with

Table 5. Recovery of taurine in blood and reproducibility of procedure.

Subject	Day	Taurine in 1 ml blood (μg)	Recovery of 45 μg of added taurine (μg)	(%)	Taurine in control <u>2/</u> ($\mu\text{g}/\text{ml}$)	Taurine in standard <u>3/</u> (μg)
B		38.1			43.5	47.4
C		40.2	41.1	97.1	42.3	42.3
D		35.1 <u>4/</u>	37.5	88.6	"	"
E	2	30.9	43.2	99.3	39.0	43.5
E	3	26.1	45.3	98.7	30.9	45.9
E	4	32.1	46.8	101.9	"	"

1/ Recoveries were not done of blood of subjects A, B, and E-1.

2, 3/ Ditto marks indicate that this sample was determined at the same time as the preceding control or standard.

4/ Sample was contaminated during evaporation.

this precaution the control sample was not uniform. Values for the chromatographed standards (Table 5) were more consistent, and those for the recovery of taurine added to blood were acceptable.

When the evaporated effluents from the blood samples and the blood samples containing taurine were subjected to paper chromatography, there was one prominent spot on chromatograms developed in both solvent systems, and it was in the same area as taurine. When two effluents were concentrated to less than 0.25 ml, a faint unidentified spot with ninhydrin that had an R_f value considerably higher than taurine was observed. When the effluents were evaporated just to 0.5 ml. the resulting spots located with both color reagents had R_f values the same as the taurine standard,

and there were no other spots on the chromatograms.

Results from recovery experiments and paper chromatography show that interfering ninhydrin-positive substances were removed by the two Dowex resins, and it can be concluded that this method is specific for the measurement of taurine in blood.

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