CITRATE, SEX AND SKELETAL REMAINS

WHAT'S SEX IN THE EAST IS NOT NECESSARILY SEX IN THE WEST: CITRATE, SEX AND HUMAN SKELETAL REMAINS

By

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ABSTRACT

Human trabecular bone from seven historic cemeteries in southern Ontario and modern cadavers was analysed for chemical content using U.V-enzymatic citrate lyase spectrometry. Citrate was the principal element of interest.

Ninety percent of total body citrate is contained within the adult human skeleton. The amount of citrate present in human bone is known to vary between bone types. Citrate accumulation in trabecular bone also varies between the sexes. This study was carried out in order to address two questions. First, is it feasible to compare trabecular bone citrate levels from different sites within a given region in order to make sex discriminatory inferences? That is, can variation through time in trabecular bone citrate content between the sexes be detected, and if present, does it exist in sufficient quantities in order to be used as a chemical means for the determination of the sex of human skeletal remains. Second, if valid comparisons can be made, what precision and reliability of sex discrimination is possible for incomplete or fragmentary human skeletal remains?

Seven historic cemeteries totalling forty-seven individuals were chosen for this study. The earliest

documented cemetery in the series was the St. Thomas Church cemetery, dating from ca. AD 1821 to ca. AD 1874. The use-dates of four of the cemeteries (ie. Stirrup Court, Breslau, Wise and Harvie) were contemporaneous with the approximately fifty year time span of the St. Thomas Church cemetery. The remaining cemeteries (ie. Waterloo County Gaol and London County Gaol) post-date the rest of the series by only a few decades. Modern cadaver samples totalling twenty-five individuals of known age and sex were also assayed for trabecular bone citrate content.

Both the archaeological and modern human skeletal samples were analysed for trabecular bone citrate content by citrate lyase enzyme spectrometry. All analyses were performed using a DU-7 spectrophotometor. This technique provides good precision and reproducibility, and is specific for citrate only.

Results indicate that observed differences in trabecular bone citrate content of individuals within the seven cemeteries and the modern sample does not stem from testing different anatomical regions of the skeleton. There is also no evidence to show that citrate content varies substantially enough between the sexes within either the cemeteries or modern sample to justify the use of citrate levels as a chemical indicator of the sex of incomplete and/or fragmentary remains.

It is proposed that small sample size, intrinsic age,

sex and geographic distribution, historical time and/or combinations of all of these variables affect adequate assessment of citrate content results derived from archaeological contexts. Finally, it is suggested that it is not possible to compare trabecular bone citrate levels of any individuals in order to determine their sex until more precise information concerning its location and depositon in human bone is known.

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To kill an error is as good a service as, and sometimes *even better than, establishing a new truth or fact.* (Darwin 1903, 2:422) [1]

[1] Darwin, Charles, 1903. More Letters of Charles Darwin. Francis Darwin, ed. 2 vols. New York: D. Appleton Croft & Co.

CHAPTER 1: INTRODUCTION

Conventional methods of skeletal sex determination rely primarily on the qualitative interpretation of morphological characteristics as well as some quantitative measurements (Bass 1987). Success rates attained by most conventional sexing techniques range from correct classifications approaching 100% using the complete skeleton to 70-75% for isolated long bones (Beattie 1982:206). Krogman (1962) further notes it is quite likely that all quantitative estimates should be lowered 5-10%, depending on the relative completeness of the material to be sexed (Ibid:112).

Accuracy in both qualitative and quantitative sex determination is dependent on two major factors: the parts of the skeleton that are present for morphological assessment, and their degree of completeness. Additionally, the following items will influence accuracy rates: observer bias and experience; the degree of skeletal maturation of the material; the poor knowledge of specific population characteristics; and the accuracy of provenience data for the material under investigation. Hence, although quantitative techniques for determining the sex of human skeletal remains decrease the dependence on intuitive

morphological criteria, at the same time they increase the need for well-preserved skeletons and bones.

Fragmented skeletal remains are frequently encountered on archaeological sites. These usually cannot be assigned sex using conventional techniques because they cannot be reconstructed. This problem has stimulated research into developing methods of determining sex based on the compositional properties of bones and teeth. This thesis examines the quantitative determination of sex for incomplete and/or fragmentary skeletal remains. Four recently developed methods involve the compositional analysis of human bones and teeth.

One approach examines the potential of using trace element content as a possible sex discriminator (Beattie 1982). The deposition into bone of various major and trace elements are known to be affected by physiological, environmental and/or behavioural factors (Underwood 1971). Elements selected for Beattie's (1982) study included phosphorus, calcium, lead and strontium. Not only have these elements been shown to be age and sex related, they also occur in the human skeleton in proportionately high concentrations relative to total body stores (Schroeder and Nason 1971).

Samples for Beattie's (1982) study were derived from the crania of modern human cadavers of known age, sex and

health-related status. Xray fluorescence was utilized to obtain 'normalized' concentrations of the selected elements. Beattie's (1982) results indicate that bone lead (higher in males) was the best single sex discriminating element (achieving an accuracy of greater than 70.2%, Ibid:212). The study thus confirmed the assumption that physiological, environmental and/or behavioural differences between males and females do affect the presence and proportion of bone mineral constituents. However, higher lead concentration was traced to behavioural and environmental factors (ie smoking and/or occupational lead exposure). It did not result from any specific physiological difference(s) that existed between the sexes, thus limiting the potential of this approach to stand on its own as a sex discriminating technique.

Two alternate techniques search for sex chromatin and the male histocompatibility-Y or (H-Y) antigen of cell membranes derived from human tooth pulp (Duffy 1989). Sex chromatin is found in all nucleated human somatic cells (Lehninger 1982). Methods for detecting sex chromatin are usually only applicable to tissues in which the cell nuclei are intact. H-Y is a carbohydrate moiety component of cell membranes found in soft tissues of all mammalian species (Wachtell 1983). Furthermore, in some respects, the H-Y technique is analogous to ABO blood group antigenic testing reported for mummified tissues and bones hundreds or

thousands of years old (Allison et al 1978; Lengyel 1984).

Duffy (1989) discovered that it is possible to test for H-Y antigenic molecules in empty tooth pulp cavities. Her preliminary results also confirmed that sex chromatin is present in modern tooth pulp samples stored for as long as one year and possibly longer. However, samples tested from environmentally-specific archaeological contexts (arid versus wet sites) produced less than satisfactory results (Ibid). Further testing is required before any more claims can be made about the sex chromatin and H-Y antigen techniques as potential means for determining the sex of incomplete and/or fragmentary skeletal remains.

The fourth technique, the quantitative analysis of citrate content of human bone mineral, is the subject of this research.

Behaviour of Citrate

Ninety percent of total body citrate is contained within the bone mineral portion of the adult human skeleton (Normon 1980). The total citrate concentration in human bone varies on both sides of 1% (dry weight), being slightly lower in the fetal skeleton (Gedalia et al 1967). The amount of citrate present in human bone varies between trabecular and cortical bone tissue types (Kuyper 1945). Citrate accumulation in trabecular and cortical bone also varies between the sexes (Lengyel 1968). The inequality of bone citrate levels between the sexes has been previously tested

with limited success in both modern and archaeological human skeletal remains (Lengyel 1968; Gibbs 1985; see also Cook et al 1986).

Preliminary results from these studies suggest that bone citrate levels may be affected by several physiological factors such as age and pre-mortem health status (Ibid). It is possible, therefore, that discrimination of sex must be based on more than bone citrate levels alone. Erroneous identifications using bone citrate content as a sex discriminator may also be due to several other factors. These include the concentration of the tissue samples tested; the particular bone type analyzed; and finally the laboratory method of detecting citrate (Gibbs 1985). Theoretically, if these problems could be resolved, or at best, controlled, cautious comparisons of bone citrate levels for sex discrimination can be made.

Statement of Purpose

In this study, trabecular bone samples from seven historic cemetery sites from southern Ontario are analyzed for bone citrate content. In addition, samples of twenty-five cadavers of known sex and age, are also included in the series tested. Two questions are addressed:

1. Is it possible to compare bone-citrate levels of individuals both within and between sites in order to determine sex?

2. If so, what precision and reliability of sex

discrimination by bone citrate levels is possible for incomplete and/or fragmentary remains?

In Chapter 2, the literature on citrate as a possible sex discrimination indicator is reviewed. Chapter 2 also examines the role of citrate in normal biological functioning and more specifically, its relationship to various disease states; and finally, the physiological relationships of citrate between the sexes in both modern and archaeological skeletal contexts.

In Chapter 3 the archaeological sites and modern cadaver samples chosen for this study are described against the background of conventional morphological techniques for assessing sex, age and health-related status. Additional information is provided from historical documentation for several individuals within the archaeological series and for all modern cadaver samples. A discussion of controls in the research design is also included.

In Chapter 4, the details of sample preparation and analysis using U.V.- enzymatic spectroscopy by citrate lyase are described. A discussion of the technical variables of the analysis is presented including recommendations for the use of U.V.-enzymatic spectroscopy by citrate lyase for modern and archaeological bone material.

Results are presented in Chapter 5. Pertinent information from preceding chapters is reviewed in light of these results. The final chapter includes a summary of the

findings and conclusions.

CHAPTER 2: THEORETICAL BACKGROUND AND REVIEW OF THE LITERATURE

2.1- Introduction

Several assumptions form the basis for the use of bone citrate analysis as a possible indicator of sex. This chapter outlines the significance of citric acid within the fields of medical and biological sciences. Current knowledge of the physiological distribution of citrate content in mammalian tissues is reviewed and numerous theories concerning its deposition and maintenance within human bone are detailed. This chapter also examines the role(s) of citrate in normal human biological functioning and its involvement in various disease states. Finally, specific physiological characteristics of citrate between the sexes are discussed in order to assess its potential as a sex discriminator in both modern and archaeological contexts.

2.2- Medical and Biological Importance of Citric Acid

Humans eat a large number of complex plant and animal foods. The processes of digestion and absorption reduce this food to a relatively small number of simpler substances, and further reduction by particular metabolic pathways that are specific for sugars, fats and amino acids results in still simpler products. These products frequently contain from two to six carbon atoms and are largely organic acids or acid

derivatives. Citric acid is a known intermediate in metabolism. Accordingly, it is found widely in plant and animal material.

Medical and/or biological interest stems from the early recognition that citric acid plays a predominant role in all of the terminal metabolic reactions common to major biochemical pathways of energy catabolism (Lehninger 1982). Sir Hans Krebs first elucidated the 'cyclic' and 'catalytic' role of di- and tri- carboxylic acids, in particular, citric acid, that together with its associated enzymes, constitute a chemical system termed the 'common' or 'final metabolic pathway' (Krebs & Johnson 1943). Citric acid is the first tricarboxylic acid formed in this system and hence, today, the names 'Tricarboxylic acid cycle', 'Citric acid cycle' and 'Krebs' cycle' are all used interchangeably (Lehninger 1982).

The Citric acid cycle produces most of the carbon dioxide made in mammalian tissues; it is also the source of a significant part of the reduced coenzyme pool that drives respiratory chains to produce ATP, and it is the means by which excess energy is made available for fatty acid biosynthesis prior to triglyceride formation for fat storage. As well, it provides important precursors for the subunits needed in the synthesis of various molecules, including hemoglobin and the nucleic acids.

In short, it can be described as a veritable

biochemical traffic circle: material comes to it from carbohydrate sources derived from the organism's diet and leaves it to form fat while material coming from amino acids in the diet leaves it to form carbohydrates. Only one 'road' is closed, that leading from fat to carbohydrates.

2.3- Physiological Distribution of Citric Acid

Several bodily fluids and tissues are known to contain citrate. Mammalian milk contains enough citrate to enduce crystalization of calcium citrate from evaporated milk, an occurrence which led to the first identification of citric acid as a normal constituent of mammalian organisms (Dickens 1941). Citric acid was not isolated from human urine until many years later, although it also contains amounts of citric acid of similar order to that present in mammalian milk (20-120 mg/100 ml) (Ibid). High concentrations of citric acid are also present in semen (up to 410 mg/100 ml), and are particularly associated with secretions of the seminal vesicles, which may contain as much as 633 mg/100g (Ibid).

Much less citric acid is present in human cerebrospinal fluid, amniotic and follicular fluids, blood serum, saliva and sweat which contain in diminishing order about 5-0.1 mg/100 ml (Ibid). Skin, hair, liver, kidney, brain and skeletal muscle tissue also contain variable but similarly low concentrations of citrate in many mammalian species (Ibid). The skeleton, however, is by far the site of the

greatest proportion of citric acid produced in the body of any mammal, including human beings.

Dickens (1941) was the first to report that 90% of total body citrate is contained within the adult human skeleton, constituting as much as 1.6 % of dry fat-extracted bone. Since this discovery, however, there has been relatively little or no interest in documenting specific correlations between concentrations of normal bone citrate and other major tissues and fluids of the body.

One relatively recent study reports the distribution ratios of total citrate tissue content to plasma (Dixit et al 1967). The results of this study are presented in Table 2:3:1. As can be seen, in most cases, the citrate tissue content is but a fraction of plasma. The exception is bone (sternum), where normal citrate tissue levels do greatly exceed that of plasma.

Although Dickens' (1941) discovery of such large stores of citrate in bone directed attention to the possibility that citrate must play a definitive role in both calcification processes and in the localization of bone mineral deposits, definitive answers to questions concerning its origins, location and mechanisms of deposition within the skeleton remain largely unresolved.

2.4- Citrate and Bone Deposition

Kuyper (1945) initially investigated the mechanisms of bone deposition of citrate. He noted that citrate percentage

Table 2:3:1- Ratio of Normal Tissue Citrate to Plasma Citrate Values as Reported by Dixit et al (1967).

Ratio: micrograms / gram tissue (wet weight) / micrograms citrate / milliliter plasma		
Tissue	Mean	Range
Skeletal muscle	0.20	0.12 - 0.38
Liver	0.28	0.16 - 0.36
Kidney	0.50	0.42 - 0.64
Diaphragm	0.54	0.45 - 0.71
Spleen	0.75	0.57 - 1.10
Pancreas	1.50	1.19 - 2.25
Sternum	5.54	2.77 - 11.50

in bone not only varied between bone tissue types but that cortical bone citrate, in particular, varied as a direct function of the amount of calcium present. The citrate content of trabecular bone, although lower in concentration than that found for cortical bone, did not depend on its calcium content (Ibid). These results also led Kuyper (1945) to postulate that citrate exists within the skeleton in a soluable form capable of continually substituting with phosphates, carbonates and other calcium salts in order to maintain the skeletal constant.

Henning and Leopold, in 1951, also recognized this depositional relationship between citrate and calcium within cortical bone (Kiszeley 1974). Their study confirmed that in the cortical substance of bone, citrate concentration is a function of the preponderance of calcium phosphate and other calcium salts present (Ibid). This suggests that citric acid must not only participate in calcium metabolism, it also exists in a readily available form. As well, since there is no simple relationship between calcium intake, bone formation and bone mineral maintenance (Garn et al 1969; Pfeiffer & King 1983), any observed variability in the concentration of citrate in cortical bone could be attributed to different dietary histories and their subsequent impact on the health statuses of the individuals sampled.

Dixon & Perkins (1952), on the other hand, were the

first to examine the possibility that active citrate metabolism within the mitochondria of bone cells exists, regardless of the bone type tested. Their study focused on three of the citric acid cycle enzymes (Krebs & Johnson 1943) known to be specifically involved in the formation and/or removal of citric acid within soft tissues, namely citrogenase, aconitase and isocitric dehydrogenase.

The activities of all of these enzymes in any of the hard tissues tested were generally found to be considerably lower than that found in soft tissue mitochondrial preparations (Dixon & Perkins 1952). As well, their results indicated that these enzyme activities were higher in the more active regions of bone growth than in the shaft cortex (Ibid). Since enzymatic activity existed and was still measurable in both bone types, Dixon & Perkins (1952) reasoned that there is a mechanism within bone cells themselves for the *in situ* production of citric acid. It is then coprecipitated within bone mineral lattices during calcification processes. This suggests that the citrate content of either bone type can be attributed to combinations of both dietary and normal hormonal conditions related to age and/or sex dependent relationships. It further suggests that citrate must also exist as a permanent deposit in the lattice of the skeleton.

Armstrong & Singer (1956) insist that the citrate content of cortical bone mineral matrices is only present

adventitiously. According to these researchers, continual replenishment of citrate (viz. heteroionic exchange transport sites) on bone particle surfaces is a sufficient explanation to account for the amount of citrate necessary to supply both the needs of normal citrate excretion from the body and, to maintain a high skeletal citrate constant.

Their experiments demonstrated the presence of a citrate ion within simulated bone plasma ultrafiltrates thus substantiating Kuyper's (1945) original suggestion that citrate in bone must exist in a readily available form in order for calcium homeostasis to be maintained. Brecevic & Furhedi-Milhofer (1979) have since suggested that $Ca(C_6H_5O_7^{-3})$ (calcium citrate) is the prevalent ionic species taking part in this adsorption process, although, to date, it has not been observed in in vivo bone mineral experiments.

The significance of citrate in mineralized tissues has recently been studied by analyzing the relationship between citrate and other elements or groups of chemicals. As noted above, the possibility of substituting phosphate for citrate or vice versa has been interpreted as an indication that citrate is located primarily in the surface layer of bone mineral crystals (Armstrong & Singer 1956; Pak & Diller 1969; Cifuentes et al 1980). The negative correlation between citrate and fluoride in bones and teeth also may be related to crystal surface reactions (Zipkin et al 1960; Gedalia et al 1967).

Knuuttilla et al's (1985) research examined the relationship between citrate concentration and magnesium, calcium, phosphate, zinc, fluoride, chloride and carbonate in human cortical bone. Their results suggest the that either there is competition between citrate and carbonate for the same position in cortical bone matrices or that citrate is able to block the binding of carbonate to apatite structures-- or vice versa (Ibid). The results did not confirm the notion of a substitution between citrate and phosphate and they conclude that the most likely explanation involves the binding of citrate to the lattice itself (Ibid).

Neuman (1980) presents a model that incorporates both a notion of in situ active citrate metabolism and the possibility of a non-skeletal origin of citrate in the calcification process of both bone types. He suggests that, if there is a space around an hydroxy apatite crystal (HAP) and an inadequate supply of an acidic inhibitor, particularly citrate, the HAP crystal in trabecular bone will 'scavenge' calcium and inorganic phosphate ions, which will then complex with citrate from the extracellular plasma fluid.

With fully mineralized bone such as shaft cortex, this is less of a problem. Special sites or 'holes' on the surface exist capable in and of themselves of catalyzing nucleation and maturation of bone mineral crystals. At these

cell sites several inhibitors are secreted, including citrate, whose purpose is to slow the formation and growth of calcium-phosphate aggregates, particularly in the interstitial and non-matrix areas.

This model suggests that citrate provides the stability necessary for early states of calcium phosphate crystalization, agreeing with previous researchers (Armstrong & Singer 1956). Also, through endogenous metabolic activity involving both the regulation and stabilization of pH, citrate provides the necessary environment for demineralization to proceed (Dixon & Perkins 1952).

Citrate, if present in excessive concentrations, will also completely inhibit precipitation of calcium phosphate by spontaneous nucleation (Neuman 1980). This is in line with recent hydroxy apatite crystal growth studies in which citrate is still viewed as a potential inhibitor of crystal growth either on its own (Tew et al 1980) or complexed to various metals that interact with citrate to slow crystal growth and calcium uptake (Thomas 1982, Knuuttilla et al 1985). This latter role also explains its current clinical application in patients suffering from spinal osteoporosis (Fackelman 1989; Pak et al 1989). Citrate administered along with fluoride aids in building structurally stronger and longer lasting bone by slowing the rapid growth induced by fluoride intake on its own.

Since Dixon & Perkins' (1952) and Kuyper's (1945) pioneering research, therefore, most researchers propose that synthesis of citrate whether catalytically produced and/or as a surface solvent for calcium salts in calcium metabolism, occurs either **in vivo** or **in vitro** in the bones of all living vertebrates. Because living bone is in a constant state of metabolic exchange with bodily fluids, citrate concentrations can be expected to vary between the bone types of the skeleton.

Where the least active calcifying / decalcifying processes are occurring, such as in bone cortex, citrate content should be highest. In the more active regions of the skeleton, such as in the metaphyseal and epiphyseal line cartilages, citrate content can be expected to be lower with the metaphyseal regions containing the lowest concentrations. Where no calcium bone salts are 'normally' laid down, such as in the marrow, no citrate deposition should be observed.

Furthermore, the high stores of citrate in the skeleton also exist in a soluble form available for the metabolic requirements of all other bodily tissues, and functions, in general. Theoretically, therefore, differential bone citrate content should be observed as a result of normal hormonal and age-related differences that are known to exist between males and females.

However, different dietary histories could also

possibly affect attempts to assess sex by bone citrate values alone. Since selecting one bone type over the other as the medium for study does not necessarily eliminate this problem, potential cross-cultural dietary differences should be controlled by selecting and comparing bone samples of those individuals derived from archaeological sites of the same ethnic, regional and temporal background.

2.5- Citrate and Normal Biological Functioning

1. Citrate, Menarche and Menopause

Shorr et al (1942) were the first to recognize a correlation between the quantitative fluctuations of urinary citrate excretion and the phase shifts of menstruation. They noted that, in women, the excretion of citrate rises appreciably during ovulation. Furthermore, urinary citrate rejection could be significantly increased by administration of estrogens to both males and females. They also reported that the opposite effect could be produced through the administration of androgenic hormones to the same test group.

Hodgkinson (1962) also noted both fluctuating daily levels of urinary citrate for females and significant differences between mean normal levels of citrate urinary excretion for females and males. However, he declined to speculate on these findings because of lack of comparative data on normal subjects in the published literature in the twenty year lapse since Shorr's and his associates' initial

study.

More recently, Welshman & McGeown (1976) noted significantly different male/female mean levels of citrate excretion among young adult male and female subjects. They suggested that since most of the non-significant female results belonged to the older, post-menopausal groups (ages 40 to 70), the reduction of daily citrate excretion values during ovulation to the level of corresponding males was probably the result of a reduced output of estrogenic hormonal activity, agreeing with Shorr et al's (1942) initial study. This pioneering study thus initiated research into the role played by citrate in the mechanisms of bone loss, its relationship to the aging process, and its potential use as a possible chemical discriminator of sex.

2. Citrate and the Aging Process

Bone loss as the result of aging has long been associated with a variety of cause-effect relationships, although it is now generally assumed that the 'uncoupling of osteoclastic-osteoblastic activity', with osteoclasts predominating, plays a pivotal role in modulating bone loss that attends the aging process (Avioli 1978). Osteocytes play an active part in the transport of substances and function primarily to resorb bone (ie. osteocytic-osteolysis) (Ibid). As noted above, citrate has been implicated as playing a definitive role in osteocytic-osteolysis.

a. Citrate and Estrogens

Lengyel (1968; see also citations in Kiszeley 1974) was the first to postulate that females, in order to compensate for the fluctuating citrate rejection during the phase shifts of menstruation noted in Shorr et al's (1942) study, accumulate higher concentrations of trabecular bone citrate than males, particularly during the reproductive period of their lives. He reported this difference in modern human skeletons as 0.7 gm% for males and 1.16 gm% for females (Ibid). He related the presence of citrate in trabecular bone to heteroionic exchange and described its deposition as follows:

> Bodily fluids contain calcium partly in ionized form (Ca ++). If Citrate-dejection (rejection) decreases in the organism, the citrate-level of blood plasma increases and it causes an increase in the citrate-level of the interstitial fluid. The citrate surplus of the two bodily fluids is (then) built by the interstitial fluid into the bone tissue as the largest citrate depot. (cited in Kiszeley 1974:53)

Correspondingly, if citrate excretion increases, the reverse process would occur and bone citrate would be removed from this reservoir (Ibid).

However, the manner in which the interaction of citrate and estrogens alters cellular function in the aging process

is largely still unknown. It has been proposed that the rapid loss of skeletal mass and subsequent decrease in bone citrate values seen in post-menopausal women results from defects in estrogen binding to cellular receptors in bone (Avioli 1978). It is obvious, however, that changes in other endocrine functions including an increase in circulating parathyroid hormone (PTH), decreases in estrogens and testosterone, and the effects of Vitamin D and its associated metabolites are all intimately involved in what can only be described as a complex metabolic jig-saw puzzle (Matthews 1978).

In order to unravel this puzzle, one begins with established facts and readily perceived corner pieces, and fills in the puzzle only as additional 'bits' begin to fit. The key pieces are the primary bone cells and mineralization sites, themselves, but as indicated below, consideration must be given to both systemic and local factors such as cell-to-cell interactions involved with heteroionic exchange, acid pH relationships, enzyme activation, and suppression and motility factors.

b. Citrate and Vitamin D

The relationship of Vitamin D and citrate was first examined in 1941 in studies of cats (Norman 1980). It was found that the citrate content in bones of Vitamin D-deficient animals was roughly fifty percent that of normal animals. It should be recalled that ninety percent or more

of body citrate is found in the skeleton, in which it is associated with the mineral fraction. Two hypotheses have been put forward to explain the actions of Vitamin D on citric acid metabolism in bone.

One hypothesis suggests that since rachitic bone tissue has reduced levels of citrate, Vitamin D must enhance the biosynthesis of citric acid during osteocytic-osteolysis (Ibid). The second hypothesis proposes that in the presence of Vitamin D there is a diminished rate of metabolism or oxidation of citric acid with a concommitent increase in steady state calcium levels (Ibid).

De Luca and co-workers have shown that administration of Vitamin D lowers the oxidation or utilization rate of citrate and isocitrate but not that of any other Krebs' cycle intermediates (Ibid). As a result of their experiments, it was felt that the diminished oxidation of citrate could be explained in terms of a physical inhibition of citrate penetration into the mitochondria of mobilizing cells.

To confirm this in bone, Norman (1980) found that an in vivo administration of Vitamin D resulted in the exact opposite effect. Vitamin D did not increase the synthesis of citrate in bone. Rather, his results were consistent with the view that the vitamin decreases the rate of conversion of citrate to subsequent intermediates of the Krebs' cycle in bone resorption processes (ie. citrate decarboxylation).

The observation of these relationships between Vitamin D and bone citrate levels suggests to others that increased bone citrate levels might facilitate bone calcium mobilization probably through the chelation of the calcium ion by citrate (Norman 1980). However, recently this hypothesis has been discounted because it is possible to disassociate this effect of increased calcification from either corresponding elevations in serum citrate or bone citrate levels (Ibid).

c. Citrate and Parathyroid Hormone (PTH)

Citrate in bone is also known to accumulate during parathyroid-osteolysis. In 1956, Neuman postulated that parathyroid hormone (PTH) increased the accumulation of citric acid at bone surfaces and effected the dissolution of bone mineral viz. an acid chelating effect (Reynolds 1972). Vaes & Nichols (1961) also reported similar findings. Recently, these acid theories of bone resorption have tended to be discounted in favor of the idea that PTH exerts its main effects on bone resorption by facilitating the transport of calcium ions (Reynolds 1972).

Barzel (1973) postulates that PTH, in mobilizing bone, causes solubilization into the extracellular fluids of potent buffers, the phosphate and carbonate moities of bone. At the bone level, he suggests PTH stimulates glycolysis by the bone cells that in turn leads to the accumulation of citric acid. Generally, this contributes to an increase in

the local hydrogen ion concentration in the interstitial and bone matrices. This increase, which is responsible for solubilization of bone mineral, thus creates the optimal acidic environment discussed previously. The total effect of PTH on hydrogen ion metabolism, therefore, is to increase the extra-cellular load, or pool, of hydrogen ions, both by stimulating increased production and by causing renal retention of this ion (Ibid).

In short, most researchers argue that (citric) acid loading causes bone cells to resorb bone, possibly by a change in their intracellular hydrogen ion concentration. Excess citric acid is then buffered by carbonates and phosphates that are released concurrently (Knuuttilla et al 1985), and whose excretion is also promoted by PTH. The result is re-establishment of normal calcium levels (Ibid).

It is clear from the discussion above that the role of citrate in the regulation of bone loss and its involvement in the aging process involves both local and systemic aspects. Proof that a particular agent such as citrate, PTH, estrogens or Vitamin D is involved, requires demonstration that the agent can be localized to the site of change (Raisz et al 1978).

Wong & Cohn (1978) have differentiated bone cultures that exhibit osteoclast-like functions (CT) from bone cultures, which exhibit osteoblast-like functions (PT) in mechanisms involved in bone loss. Citrate decarboxylation

processes were found to remain active in initially formed CT cells despite PTH stimulation whereas PTH inhibited this action in later formed PT bone cells (Ibid). Both citrate and PTH have also been shown to procure calcium independently from the surfaces of bone (Talmage et al 1978). Estrogens are also known to mediate the effect of PTH on bone surfaces causing a suppression of osteoclastic activity, decrease in bone loss and subsequent increased citrate levels (Jowsey & Offord 1978; Riggs et al 1969).

These observations, taken together, have led many researchers to reject any single cause model that postulates a correlating negative feedback control for any one of these specific agents' independent secretion within a basic equilibrating process between calcium in blood and/or in the bone mineral phases themselves (Jowsey & Offord 1978). In short, relative degrees of estrogen and probably androgens, coupled with additional age-related progressive increments in circulating PTH levels, and 'corresponding' response of citrate levels in bone, all may be implicated in future histological findings of decreased osteoblastic and increased osteoclastic-osteolysis activity observed between the sexes, and pertaining to the aging process.

Hence, although many pieces are beginning to fit, unlike a good puzzle, no clearer metabolic picture emerges to explain the variable roles citrate plays in either the constitution and maintenance of bone mineral and/or the

calcification process, nor its relationship to the aging process itself. It does suggest that abnormal activity by either one or all aspects combined could possibly produce potentially false high/low bone-citrate levels in a study of bone citrate as a discriminator of sex.

2.6- Citrate, Disease and Physiological Differences Between the Sexes

Citrate has been implicated in a variety of disease states, specifically, its altered activity during mineralization processes and/or its metabolism with regard to physiological differences between the sexes. Many of these disease states such as osteoporosis (Pak et al 1989), Vitamin D-deficient rickets (Normon 1980), renal disorders (Hodgkinson 1962; Welshman & McGeown 1976) and PTH abnormalities (Masala et al 1978) have been discussed above. Citrate has also been found to be associated with a variety of other diseases including Paget's (Russell 1984), diabetes meillitus (Type I and Type II) (He et al 1989; Dixit et al 1967) and numerous neurological disorders such as Alzheimer senile and pre-senile dementia, amyotrophic lateral sclerosis, Parkinson dementia of Guam, and dialysis encephalopathy (Slanina et al 1984).

Russell (1984) reports that an increase in both serum and urinary citrate derived from the skeletal reservoir is associated with cases of Paget's disease or 'osteitis deformans'. Theoretically, therefore, an artificial

significant decrease in bone citrate levels could be obtained, as a consequence of testing an individual having this disease. Morphologically, this disease should be apparent in demographically and anatomically complete skeletal series chosen for study.

Dixit et al (1967) and He et al (1989) report that increases in daily plasma and urinary citrates are associated with diabetes mellitus (Type I or Type II). When diabetes is uncontrolled because of deficient insulin, glycolysis decreases leading to increased levels of acetyl CoA and oxaloacetic acid, both of which are precursors for the production of citric acid. As a consequence, urinary citric acid excretion increases and the amount of citrate present in diabetic bone tissue decreases substantially. The possibility thus exists that an indiscriminately low value of citrate could be obtained when testing an individual with this disease.

It is now generally acknowledged, however, that diabetes mellitus Type II, formerly known as the adult form, is a relatively new disease for most Native North and South American populations (Weiss et al 1989; Urdaneta & Krehbiel 1989; Ritenbaugh & Goodby 1989; Szathmary 1989). This suggests that diabetes mellitus should not pose as serious a problem to the interpretation of bone citrate content results within archaeological contexts as it might within modern forensic situations.

The possibility also exists that an accidentally increased bone citrate level would be found in individuals who have suffered from renal disorders, because of the reduction of urinary excretion of citrate associated with these diseases (Welshman & McGeown 1976; Santos et al 1986). Renal disorders would be impossible to discern within archaeological populations unless health-status documentation was available.

The etiological relationship between citrate and neurological disorders has been examined only recently. Since citrate is a strong chelating agent for aluminum, and both chemicals are present in the normal diet of humans, aluminum bioavailability may be enhanced in the presence of citrate. A common characteristic of the neurological disorders outlined above is significantly increased concentrations of aluminum complexed to citrate in target organs including grey matter of the brain and bone tissue (Slanina et al 1984). More research, however, is required before any more claims can be made about how these particular disease states may possibly alter either mineralization processes, their indirect effects on citrate metabolism with regard to physiological differences between the sexes, or their ultimate effect on the use of bone citrate as a sex discrimination indicator.

2.8- Review of Previous Studies of Citrate as a Sex Discriminator

Thunberg (1947) was the first biochemist to report on the bone citrate content of archaeological remains. His interest in the citric acid content of bone stemmed from Dickens' (1941) discovery of high citrate content in fresh human skeletal material, an interest in the then newly discovered Citric acid cycle and, more specifically, from the inherent technical difficulties involved when attempting to extract and measure the citrate content of bone and other proteinous tissues.

Thunberg (1947) utilized an earlier variant of the Hess & White (1955) pentabromide- acetone technique for the determination of citric acid. The pentabromide-acetone technique involves an unusual reaction dependent on the formation of an enolic form of acetonedicarboxylic acid that is supposed to be specific for citric acid. This technique, however, has had a long and problematic technical history within biochemistry because of the inability to isolate a pure sample of citric acid.

Most critical is the cloudiness that may or may not remain in the final stages of the reaction. It is produced from the presence of various interfering substances such as acetone, acetaldehyde and -Keto acids (Moellering & Gruber 1966; Ricq & Buchet 1987). This cloudiness increases optical density transmittance, producing either potentially false

high readings (Gibbs 1985; see also Cook et al 1986) or no readings at all (Dennison 1979) and hence, indiscriminate citrate concentrations. These difficulties are reflected in the results and/or conclusions of most previous researchers' attempts to extract bone citrate from either archaeological or modern contexts employing the pentabromide-acetone technique.

The results of Thunberg's (1947) analysis are presented in Figure 2:8:1. His sample included archaeological specimens of horse, cattle and humans, sex unspecified. Although considerably decreased and highly variable within specimens and between archaeological sites, his results demonstrate that citrate could still be measured in spite of exposure of the bones to decomposing features over hundreds of years. In general, he found that trabecular bone citrate levels were lower than those observed for cortical bone samples agreeing with Kuyper's (1945) observations above.

Thunberg (1947) postulated that differential decompositional factors within and between archaeological sites and/or technical extraction difficulties could possibly account for these differences. He concluded that the citrate content of archaeological bone samples should only be used with the greatest of reservation, until these technical problems could be resolved.

Lengyel (1968) specifically tested his hypothesis about the inequality of bone citrate levels between the sexes in

Figure 2:8:1- Thunberg's (1947) Investigations of Citrate Content of Archaeological Samples.

Table 1

	Age		Ci-content in ppm
Horse	uncertain	.....	1,000
>>	>>	.....	900
Cattle	16th century	.....	2,200
>>	>>	.....	3,400
Man	uncertain	.....	1,800
>>	>>	.....	1,300
>>	AD 1361	"Korsbetning graves", Visby	700
>>	>>	>>	800
Horse	13th century	.....	6,600
>>	>>	.....	9,300
Man	uncertain	"Ragnar Knaphovdes Chapel, Vreta Closter Church"	1,100

Table 2

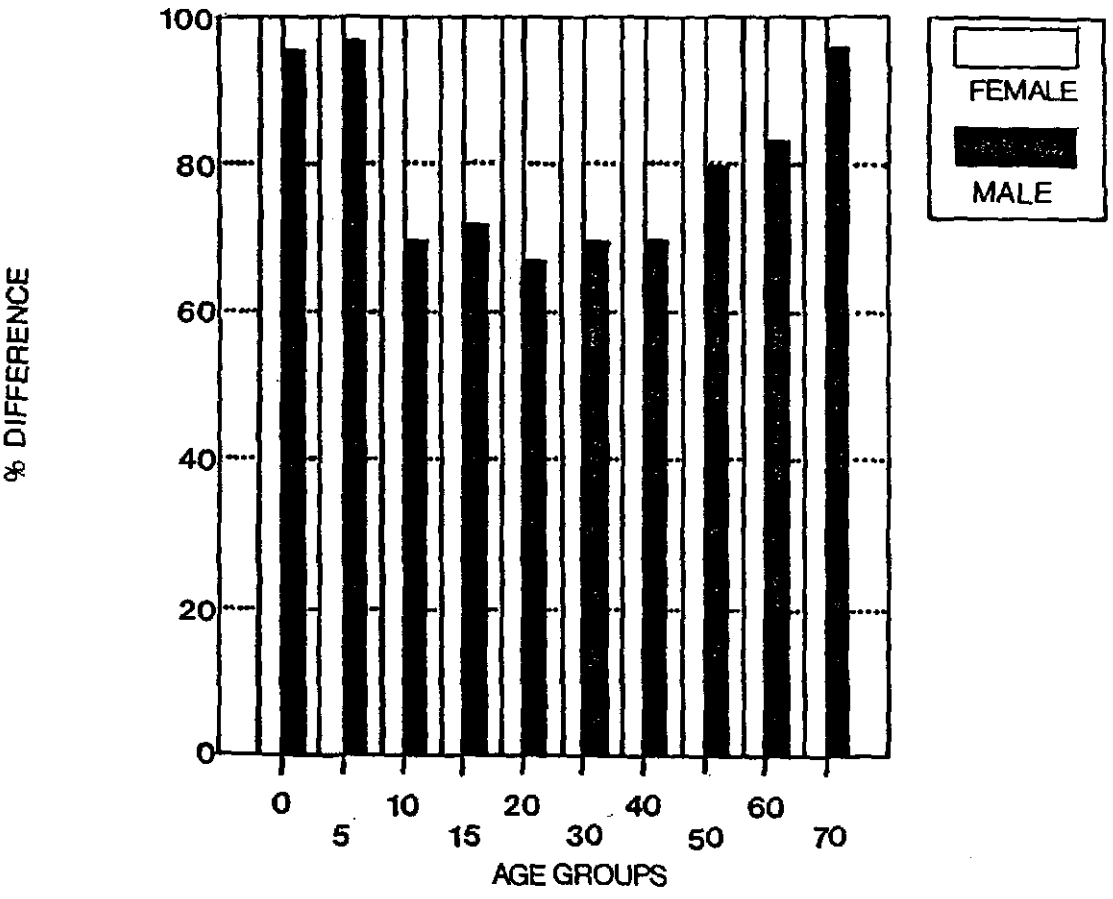
Locality	Approximate age of find in yrs.	Approximate Ci-content in ppm
Lund	750	9,000
Stora Uppaka, (Iron age dwelling)	1500	1,600
Gualov, Mollehusen	4,000	100
Agerod (Stone age dwelling)	7,500-8,000	200

both modern and archaeological human skeletal remains. He also employed the pentabromide- acetone technique for the determination of citric acid, in spite of its technical difficulties (Ibid; see also citations in Kiszeley 1974). His results on modern trabecular bone are presented in Figure 2:8:2.

Until the onset of puberty, there appears to be no significant differences between females and males in the concentration of citrate observed by Lengyel (approximately 4%, Ibid:55). Between the ages of 15 and 60, the citrate concentration in female bone samples was found to be approximately 30% higher than that of corresponding males (Ibid). Between the ages of 40 and 60, Kiszeley (1974) reports that there is a clear trend in Lengyel's data that citrate values of the female samples tested drop at a greater rate with regard to each other and corresponding males (approximately 10%, Ibid). Finally, beyond the age of 70, there again would appear to be no appreciable difference between the bone citrate levels of the two sexes (approximately 4%, Ibid).

Since the bone citrate content of autopsy samples appears to be closely related to the biological age or hormonal state of the individual at the time of death, any conclusions relating to the sex of archaeological cases should also consider age (Lengyel 1968). Furthermore, when comparing these percentages to those obtained for

Figure 2:8:2- Lengyel's Data For Citrate Percentages by Age group and Sex as Discussed by Kiszeley (1974).



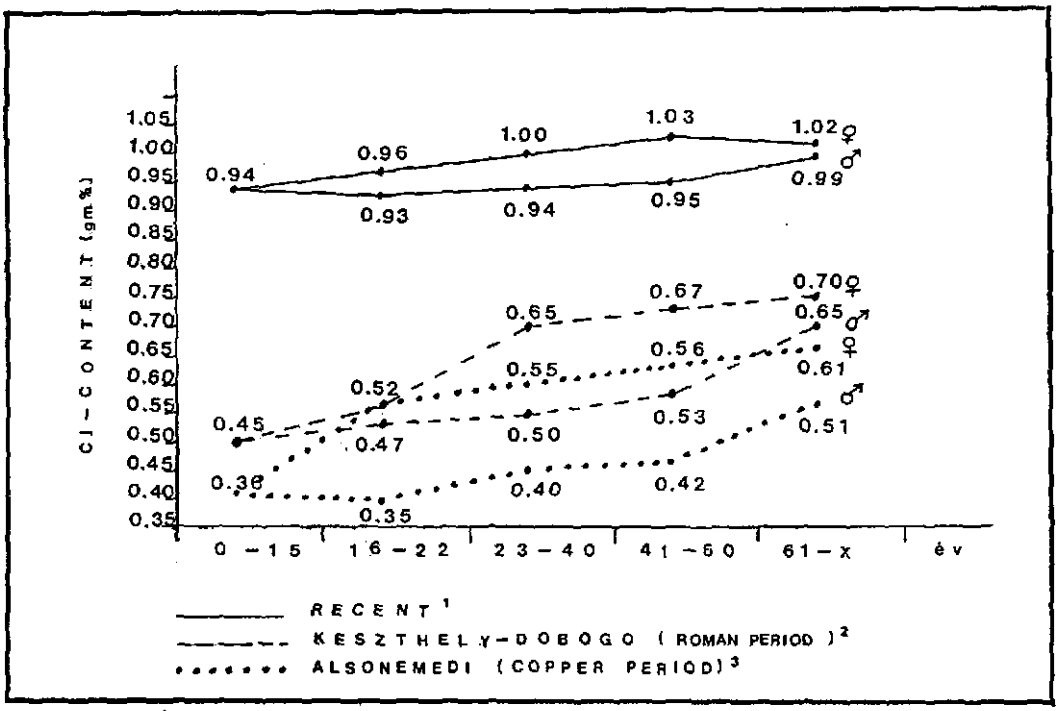
archaeological human skeletal remains, citrate levels in both males and females tested, drop significantly over time (approximately 50%, Ibid:277; see also Figure 2:8:3), agreeing with Thunberg's (1947) pioneering study. This suggests that citrate levels should only be compared between individuals interred within the same historical period.

Dennison (1979) also tested the bone citrate hypothesis on archaeological human skeletal remains employing the pentabromide- acetone technique. His results are presented in Figure 2:8:4. His study confirmed that female cortical bone also contains more citrate than that of males, although these observations were often based on single test samples. Age ranges of the 16 individuals tested were not reported and hence, it is unclear if such differences found in both cadaver and archaeological material by Lengyel (1968) also pertain to cortical bone.

In contrast to previous researchers' analyses, however, his study did not indicate that there were significant differences over time in the bone citrate levels of his sample, which like Lengyel's (1968) study, spanned various archaeological sites well over a 1000 year period. Dennison (1979) did allude however, to problems he encountered when using the pentabromide-acetone technique.

Gibbs (1985; see also Cook et al 1986) also tested the pentabromide- acetone technique on trabecular bone citrate levels of a small historic cemetery sample less than 100

Figure 2:8:3 Lengyel's (1968) Comparison of Ci-content of Modern Versus Archaeological Samples.



[1] MODERN AUTOPSIES; 701 INDIVIDUALS: 317 ♂/384 ♀ (Kiszely 1974)
[2] 4th-5th CENTURY; 20 INDIVIDUALS: 10 ♂/ 10 ♀ (Lengyel 1975)
[3] 3rd MILLENIUM BC; 30 INDIVIDUALS: Sex unspecified (ibid)

Figure 2:8:4- Dennison's (1974:140) Observations of Ci-content of Archaeological Samples.

Table 1

Citrate Gram Percentages in Relation to Nitrogen Levels

N_2%	Male Highest	Male Lowest	Female Highest	Female Lowest
1.57			3.41	**0.00**
1.90			1.84	0.77
1.94			1.84	0.67
2.09	0.24	**0.00**		
2.73	0.28	**0.00**		
4.12			1.58	0.29
4.21			2.00	1.26
4.24	0.83	0.59		
4.28	0.34	0.11		
4.28	1.26	1.03		
4.30	0.07	**0.00**		
4.31			2.63	1.03
4.33			2.18	1.64
4.41	1.09	0.82		
4.43			1.46	1.13
4.46	0.51	**0.00**		

years old from southwestern Ontario. Her results also confirmed previous observations of differing levels of trabecular bone citrate in males and females. However, some erroneous identifications were observed (Cook et al 1986:110). These errors may be due to several factors relating to either the means of determining bone citrate concentration within both modern and archaeological contexts and/or to intrinsic characteristics of the individual samples themselves (Gibbs 1985; Cook et al 1986; Ricq & Buchet 1987).

Although bone citrate levels are known to be affected by physiological factors such as age, and pre-mortem health status, the results of all previous researchers' studies outlined above indicate that using bone citrate content data derived from the pentabromide- acetone technique is highly problematic. The most important variable affecting any interpretation of citrate content as a potential discriminator of sex, to date, is the laboratory method of its detection. If these technical restrictions are eliminated or held constant, bone citrate analysis as a sex discriminator should be possible.

CHAPTER 3: SAMPLE BACKGROUND

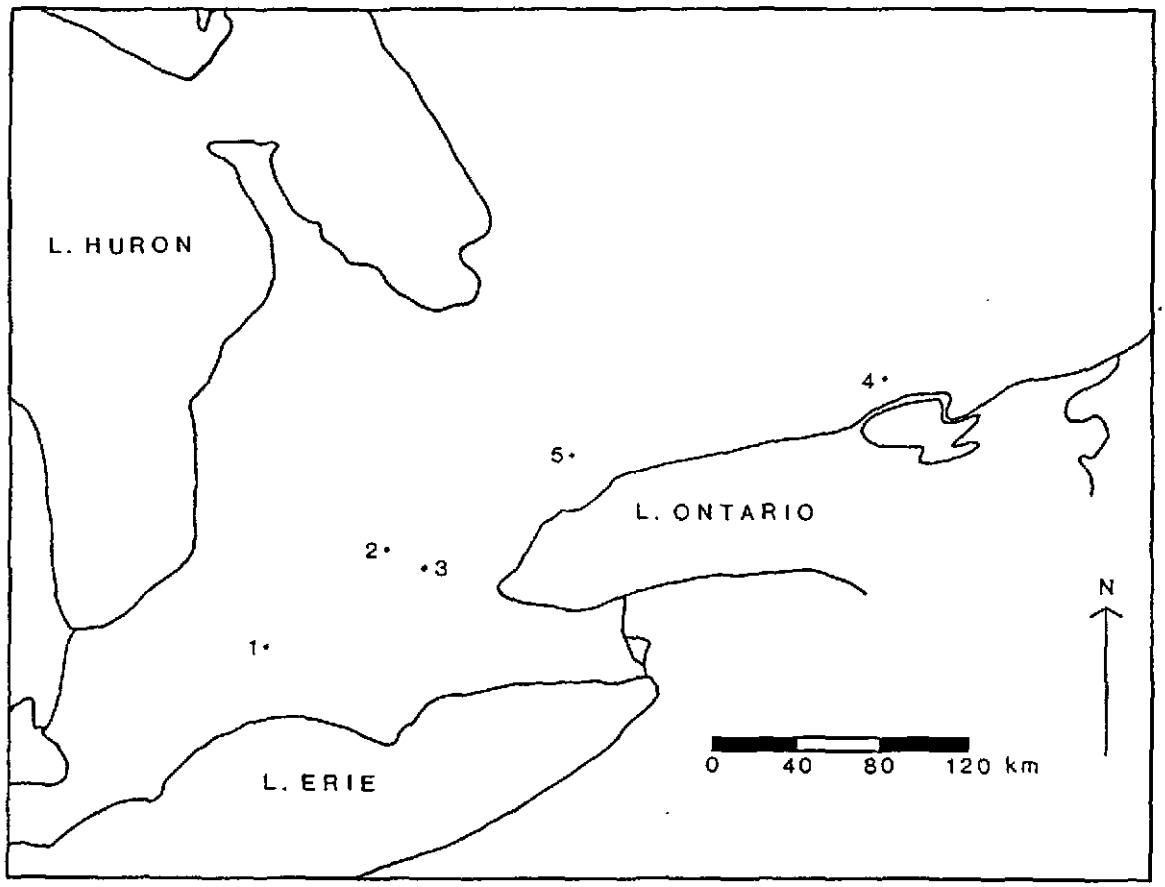
3.1- Introduction

Both modern and archaeological human skeletal remains were sampled for bone-citrate content in this study. Modern cadaver samples, totalling twenty-five individuals of known age and sex, were collected on two subsequent field trips, in the spring and summer of 1989, to the Department of Anatomy, at The University of Western Ontario. The archaeological skeletal remains, totalling forty-seven individuals, were collected from seven historical cemetery sites, recently excavated, in southern Ontario, Canada.

All cemeteries are located within the bounds of Georgian Bay to the north, Lake Huron to the west, Lakes Erie and Ontario to the south and the St. Lawrence River to the east (Figure 3:1:1). Summary information on all of the cemeteries and modern samples selected for analysis is presented in Tables 3:1:1 and 3:3:1, respectively.

Section 3:2 outlines detailed descriptions for each of the cemeteries selected for study. This information is set against a background of conventional osteological techniques for determining sex, age and health-related status. Additional information is provided from historical

Figure 3:1:1- Map of Southern Ontario Showing Locations of Archaeological Series.



Legend

1. London, Ontario
    a.) Stirrup Court Cemetery
    b.) London County Gaol
2. Kitchener-Waterloo, Ontario
    a.) Waterloo County Gaol
    b.) Breslau Cemetery
3. Cambridge, Ontario
    a.) Harvie Family Cemetery
4. Belleville, Ontario
    a.) St. Thomas Church Cemetery
5. Richmond Hill, Ontario
    a.) Wise Family Cemetery

Table 3:1:1- Summary Information on Cemetery Sites.

SITE	LOCATION	DATE	CULTURAL AFFILIATION	No.
Stirrup Court Cemetery	London, Ontario	AD1840-AD1890	English W. European origin	16
Breslau Cemetery	Breslau, Ontario	AD1830-AD1833	Irish/German W. European origin	2
Waterloo County Gaol	Kitchener-Waterloo, Ontario	AD1898; AD1920	British & Bulgarian W. & E. European origin	2
London County Gaol	London, Ontario	AD1899	Black-White/Amerindian American origin	1
Wise Family Cemetery	Richmond Hill, Ontario	AD1832-AD1858	W. European origin	1
Harvie Family Cemetery	Cambridge, Ontario	AD1825-AD1894	Scottish/English W. European origin	8
St. Thomas Church Cemetery	Belleville, Ontario	AD1821-AD1874	W. European origin mainly English	16

documentation for several individuals within the archaeological series and all modern cadaver samples.

Section 3.3 provides a detailed description of the modern cadaver sample including the individual's place of origin where known, sex, age at death, cause of death and the relative date of embalming. The final section, 3.4, describes the research design utilized throughout this study.

3.2- Archaeological Series

1. Stirrup Court Cemetery

The Stirrup Court Cemetery, located in London, Ontario, was excavated in the fall of 1982, by Dr. M. Spence from the Department of Anthropology at The University of Western Ontario and Mr. William A. Fox from the Ontario Ministry of Culture and Communications. Although several families in northwest London had used the cemetery in the latter part of the nineteenth century, all 16 individuals selected for bone-citrate analysis, in this study, are adults of British descent (Cook et al 1986). Also, all individuals tested were interred in the cemetery between ca. AD 1840 and ca. AD 1890 (Ibid).

Six Stirrup Court individuals have been definitely identified on the basis of coffin plaques, historical records and burial location (Dr. M. Spence, personal communication). Census records and other documentary sources have also indicated possible health problems of one

individual within the Stirrup Court population, thus far (Ibid).

Osteological sex identifications were made by Dr. M. Spence (Cook et al 1986), using a variety of conventional measurements and observations of the pelvic bones. These included the presence of the preauricular sulcus, the presence of "parturition" scars either on the dorsal margin of the symphysis pubis or in the preauricular sulcus, the form of the sciatic notch, the sub-pubic angle, and the three criteria of Phenice (1967; see also Houghton 1974; Stewart 1979; Krogman & Iscan 1986). Metric data included a variety of indices: sacral inlet, cotylo-breadth, cotylo-sciatic, ischium-pubis, and anterior pelvic (Washburn 1948; Oliver 1969; Stewart 1979; Krogman & Iscan 1986). The results of Spence's (Cook et al 1986) analysis are summarized in Table 3:2:1.

Ages for the Stirrup Court sample were assessed by Drs. M. Spence and S. Pfeiffer (Personal communication) and Cook (1990) viz. observations of remodelling of the symphysis pubis as described by Suchey-Brooks (1988) and two histological aging techniques (Thompson 1979). The ages presented here, in Table 3:2:1, are those Cook (1990) derived from coffin plaques and documented sources and, in absence of such concrete data from histological measurements of trabecular femoral Mean Wall Thickness (Cook et al 1986), cortical bone assessment (S. Pfeiffer, Personal

Table 3:2:1- Sex, Age and Health-related Status of Stirrup Court individuals.

Burial no.	Osteological Sex	Documented Sex	Estimated Age* 1/ 2/ 3	Documented Age	Health-related Status
1/2 A	F		71/ / /		
Pile A	F	F	74/ / /	63	I
7	F	F	55/72/60	81	A,B
18	F	F	65/ /60	84	A,B,C
3	F		66/77/ /		A,D,E,J
5	F		62/78/ /		A,B,C,H
1/2 X	F		'adult'		
21	M		66/36/45		A,B
14	M		53/65/28		A
10	M	M	61/46/45	45	A,C,G
19	M		58/ /61		A,B,C,F
6	M	M	69/59/61	61	A,B
20	M		59/47/35		A,C
11	M		77/74/45		A,B,G
17	M		57/76/45		A,E
4	M	M	56/80/61	76	A,B,E
1/2 B	M		70/ / /		

A= Osteoarthritis, B= Vertebral Osteophytosis, C= Osteopenia
D= Osteoporosis, E= Bone Metastases, F= Spina Bifida Occulta
G= Peridontal Disease, H= Sinusitis, I= Mental Disorder,
J= Cribra Orbitalia
* decimal points of ages have been rounded down to fit table

Estimated Age 1. Trabecular bone age (Cook 1990)
2. Cortical age (S. Pfeiffer, pers. comm.)
3. Pubic Symphysis (Suchey-Brooks 1988)

communication) and pubic symphysis age estimates (M. Spence, personal communication). The poor representation of individual 1/2X permitted no age identification, other than to say it was adult (Dr. M. Spence, personal communication). Implications for this study concerning noted age assessment problems using traditional age estimation are discussed in Section 3.4. Health status of the Stirrup Court individuals was also assessed by Cook (1989; 1990).

2. Breslau Cemetery

The Breslau Cemetery was uncovered on a construction site near Breslau, Ontario, in the Kitchener-Waterloo area (Spence 1985a). Two unmarked grave shafts containing three individuals were excavated by Mr. William A. Fox from the Ontario Ministry of Culture and Communications and Mr. Jack Redmond, an Ontario Ministry of Culture and Communications Archaeological Conservation Officer. Both graves were found side by side, with one later than (and slightly overlapping) the other (Ibid).

The earlier burial, burial no. 2, held two skeletons: individual 2a, an adult female and individual 2b, an infant held in the crook of her arm. The cause of death of the adult female may have been related to childbirth (Ibid). The stratigraphically later burial, no. 1, contained an adult male whose health was possibly affected by the presence of pyogenic osteomyelitis (Ibid). Both adult individuals were of either British or German descent (Ibid). Sex was assessed

by Spence (1985a) utilizing the variety of criteria outlined above. Age was also determined by Spence (1985a) viz. observations of epiphyseal fusion, cranial fusion, symphysis pubis scores and rib age changes (Todd 1920; 1921; McKern & Stewart 1957; Gilbert & McKern 1973; Iscan et al 1984; Suchey et al 1984). The results of Spence's analysis are summarized in Table 3:2:2.

A newly minted 1831 coin found beneath the head of Burial no. 1 indicated that the burial probably dates to ca. AD 1831- AD 1833 (Spence 1985a). Its close association with Burial no. 2 suggested that they were both probably interred within a decade of each other (Ibid). Hence, for this study, the time span represented by the Breslau Cemetery is tentatively set at ca. AD 1821 - ca. AD 1833.

3. **Waterloo County Gaol**

The Waterloo County Gaol Cemetery was excavated by Dr. Ron Williamson from The Foundation For Public Archaeology. James Allison (Burial no. 2) was hanged in 1898 (Spence 1985b). Burial no. 1, Stokyo Boyeff, was hanged in the Waterloo County jail in 1920 (Ibid). Both individuals were apparently young adult males of British and Bulgarian descent, respectively (Ibid).

Sex and age of both Waterloo County Gaol individuals were assessed by Spence (1985b) utilizing the conventional criteria outlined above. These results are presented in Table 3:2:3 and were further corroborated by historical

Table 3:2:2- Sex, Age and Health-related Status of Breslau Cemetery Individuals.

Burial No.	Osteological Sex	Documented Sex	Estimated Age	Documented Age	Health-related Status
2a	F		21 - 25		Pregnancy
1	M		30 - 40		Possibly pyogenic osteomyelitis

Table 3:2:3- Sex, Age and Health-related Status of Waterloo County Gaol Individuals.

Burial No.	Osteological Sex	Documented Sex	Estimated Age	Documented Age	Health-related Status
				yrs mos.days	
2	M	M	23-28	22 11 20	Hanged
1	M	M	16-19	17	Hanged

Table 3:2:4 - Sex, Age and Health-related Status of London County Gaol Individual.

Burial No.	Osteological Sex	Documented Sex	Estimated Age	Documented Age	Health-related Status
P.L.B	M	M	23-28	25	Hanged

documentation (Ibid). The fact that both individuals died as a consequence of hanging was also osteologically confirmed by the trauma present in the cervical regions of their vertebral columns (Ibid).

Both individuals had been limed, following interment, presumably to induce rapid decomposition (Ibid). Implications for this study of the consequence of this potentially destructive burial practice are discussed in Chapter Five.

4. London County Gaol

The London County Gaol cemetery was partially excavated, in May, 1985, following its accidental discovery by Middlesex Township officials' construction activity at the jail (Spence 1985c). All of the upper half of the skeleton of a single individual had been removed and taken to a fill site in southwest London. Despite intensive efforts by several volunteers, only part of the skeleton was recovered. Furthermore, the recovered material was badly fragmented (Ibid).

This severely limited the data available for Spence's (1985c) osteological assessment of the age, sex and ethnic identification of this individual. The London County Gaol individual appears to be an adult male, of American black-white ancestry (Ibid). Historic references suggest also some Amerindian mixture. Sex was assessed viz. cranial observations, reinforced by Phenice's (1969) criteria which

could only be observed on the left (Spence 1985c). Age was determined by molar eruption, epiphyseal fusion and left *pubis symphysis* scores (Ibid). The results of Spence's (1985c) analysis are presented in Table 3:2:4. The recovery *in situ* of an amputated left leg, however, left no doubt that the disturbed London County Gaol grave was that of "Peg Leg" Brown, described as a mulatto male aged 25 years hanged on May 17, 1899, at the jail (Ibid).

Despite its poor, fragmented condition, what limited osteological observations that could be made on this individual were corroborated by historical documentation. Also, in contrast to the Waterloo County Gaol individuals, this individual was not limed by gaol officials, upon interment (Ibid). Finally, lumbar vertebrae for this individual, essential for one variant of this study, were not recovered. Hence, bone-citrate tests for this individual are not as comprehensive as desired.

5. ***Harvie Family Cemetery***

The Harvie family cemetery, located just outside the present southeastern limits of the city of Cambridge, was excavated, in November, 1988, by Dr. S. Saunders and Mr. Richard Lazenby from the Department of Anthropology, McMaster University. In all, 15 individuals were removed from 14 graveshafts: 9 adults and six infants or children (Saunders et al 1989). The Harvie family cemetery was used from ca. AD 1825 to ca. AD 1894 (Ibid). All Harvie

individuals tested for bone-citrate content are of Scottish or English descent (*Ibid*). Based on skeletal, genealogical and archaeological analysis of coffin plaques, seven of the nine Harvie adults and four of the six sub-adults have been personally identified (Lazenby et al 1989).

Sex determination of the Harvie sample, based on metric and nonmetric morphology of the pelvic girdle, was assessed by Lazenby et al (in press). Age assessments were also made by Lazenby et al (1989; in press) based on Suchey-Brooks (1988) symphysis pubis scores and an histological age estimation technique (Thompson 1979). The results of these analyses for eight of the nine Harvie adults sampled for bone-citrate are presented in Table 3:2:5. Implications for this study of noted discrepencies in Harvie age assessments are discussed in the last section (3:4) of this chapter. Health status of the Harvie Family individuals was assessed by Keenlyside & Clark-Wilson (in press).

6. Wise Family Cemetery

The Wise family cemetery, located in the town of Richmond Hill, Ontario, was excavated, in November, 1988, by Drs. M. Spence of the Department of Anthropology, at The University of Western Ontario and R. Pearce, of the Museum of Indian Archaeology (Spence 1989). Tombstone fragments indicated that the Wise family used the cemetery from ca. AD 1832 to c. AD 1858 (Ibid). Of the six burials exhumed, only one, Burial no. 4 was suitable for testing bone-citrate in

Table 3:2:5 - Sex, Age and Health-related Status of Harvie Individuals.

Burial No.	Osteological Sex	Documented Sex	Estimated Age 1 / 2	Documented Age	Health-related Status
6 (A)	M	M	61.2/50.4	71	A
13 (B)	F	F	38.2/44.6	31	P
11 (C)	F	?	30.7/38.6	?	A
7 (D)	F	F	60.0/62.8	98	A,B
14 (E)	M	?	61.2/66.1	?	A
3 (F)	M	M	28.7/54.1*	31	A,C,D
8 (G)	M	M	35.2/67.5*	71	A
4 (H)	M	M	61.2/58.2	71	A,C

* Probable identity

A= Osteoarthritis; B=Osteopenia; C= Osteophytosis; D= Enamel Hypoplasia; P= Pregnancy

Estimated Age 1. Pubic symphysis age (Suchey-Brooks 1988)
Age 2. Trabecular bone age (Thompson 1979)

this study. Pathology, poor preservation and/or missing skeletal elements prevented sampling of the rest of the adults in the Wise sample (Ibid).

Sex and age of this individual was assessed by Spence (1989) using the variety of conventional osteological techniques outlined above. Burial 4 is apparently a Western European adult female of middle to old age, and is probably the wife of Peter Wise Sr. (Ibid). However, no name, date of burial or age at death could be documented for her (Ibid). The results of Spence's (1989) analysis are summarized in Table 3:2:6. Pathology prevented sampling of the lumbar region of this individual and hence, as was the case with the London County Gaol individual, bone-citrate tests on this individual are not as comprehensive as desired.

7. St. Thomas Church Cemetery

The St. Thomas Church cemetery, located in Belleville, Ontario, was excavated in the summer of 1989 by Dr. Heather McKillop, from Northeastern Archaeological Associates Inc. (Dr. S. Saunders, personal communication). This cemetery dates from ca. AD 1821 to ca. 1874 (Ibid). Sixteen individuals of known age and sex, based upon coffin plaques were selected for bone-citrate analysis and are presented in Table 3:2:7.

Osteological sex was assessed by Ms. Tracy Rogers (personal communication) from the Department of Anthropology, at McMaster University, who employed a variety

Table 3:2:6- Sex, Age and Health-related Status of Wise Cemetery Individual.

Burial No.	Osteological Sex	Documented Sex	Estimated Age	Documented Age	Health-related Status
4	F	F*	40 - 60	?	

* Probable identity

Table 3:2:7- Sex, Age and Health-related Status of St. Thomas Church Individuals.

Burial No.	Osteological Sex	Documented Sex	Estimated Age	Documented Age	Health-related status
	P* / S		1 / 2		
433a	M / M	M	18.5 / 19.8	19	
435	F/F / F	F	+ / +	62	
436	M / M	M	35.2 / 35.8	66(or 56)	
437	F/F / F	F	+ / +	29	
472	M/M / F	M	+ / +	20.06.21	
516	F/F / F	F	60.0 / 47.8	69.11.06	
94		M		13.01.09	
451	M / M	M	28.7 / 24.1	28.06.08	
108	F/F / F	F	48.1 / 55.7	60.11.10	
115	M/M / M	M	35.2 / 29.2	27.09.	
188	F/F / F	F	+ / +	67	
339	M/M / M	M	+ / +	55.11.	
351a	F/M / F	F	19.4 / 20.2	22.03	
406	M/M / M	M	35.2 / 35.9	58	
400	F / F	F	60.0 / 55.7	29.06	
361	F/F / F	F	+ / +	19	

P= Pelvis, S= Skull

* trial 1/ trial 2: some were retested for intraobserver error study (T. Rogers, pers. comm.)

Estimated Age (T. Rogers, pers. comm.):

1. Males (Katz & Suchey 1986)
   Females (Suchey-Brooks 1988)
2. Males (McKern & Stewart 1957)
   Females (Gilbert & McKern 1973)

of visual observations of both the skull and pelvic girdle of each individual tested. Estimated age determinations of the St. Thomas sample were also assessed by Ms. Tracy Rogers by a variety of methods including Katz & Suchey's (1986) and McKern & Stewart's (1957) skeletal age changes for males and Gilbert & McKern's (1973) and Suchey-Brooks' (1988) skeletal age changes in females. Documented ages are based on coffin plaques. Health status was not available at the time of writing but is expected as research on the cemetery proceeds.

3.3- Modern Cadaver Series

The modern sample tested for bone-citrate content in this study are summarized in Table 3:3:1. Of the twenty-five adult individuals of known age and sex sampled, most are of West or East European origin (Wayne Davis, personal communication). As well, the majority of the individuals selected died as a result of heart-related problems (Ibid). Also, most individuals had been infused with embalming fluid consisting of mostly methyl hydrate, glycerine, formalin and phenol for less than one year, following their deaths (Ibid). Two individuals, however, were embalmed for more than two years (Ibid). Steps were taken to eliminate embalming fluid as a potential source of contamination for bone-citrate analysis and are outlined in Chapter Four.

Eighteen individuals tested are males ranging in age from 54 years to 96 years, and seven individuals are females

Table 3:3:1- Sex, Age, Health-related Status and Relative Date of Embalming of Modern Series.

Cadaver No.	Place of Birth	Age at Death	Sex	Health-related Status	Relative Period of Embalming
108	Quebec	54	M	A	1 yr.
83	Ontario	58	M	B	1 yr.
125	unknown	59	M	C	1 yr.
113	England	65	M	D	1 yr.
100	England	66	M	C	1 yr.
97	Ontario	68	M	C	1 yr.
86	Ontario	68	M	A	3.5 yr.
652	unknown	69	M	E	1 yr.
118	Lithuania	72	M	C	1 yr.
120	Manitoba	72	M	B	1 yr.
124	Ontario	73	M	C	1 yr.
90	Ontario	75	M	C	1 yr.
103	Ontario	77	M	C	1 yr.
141	Poland	78	M	C	1 yr.
133	England	81	M	A	1 yr.
116	England	83	M	D	2 yr.
126	England	91	M	D	1 yr.
89	Greece	96	M	F	1 yr.
148	Ontario	67	F	D	1 yr.
136	Ontario	74	F	G	1 yr.
123	Michegan	79	F	A	1 yr.
140	Ontario	81	F	D	1 yr.
134	Ontario	82	F	D	1 yr.
114	Ontario	87	F	F	1 yr.
147	Ontario	93	F	E	1 yr.

A= Respiratory Failure, B= Cardiac Arrythma, C= Myocardial Infarction, D = Atherosclerosis, E= Cardiac Collapse, F= Pulmonary Dysfunction, G= Cerebral Brain Infarct

ranging in age from 67 years to 93 years. Unfortunately, no individuals of either sex, less than 50 years of age, were available for analysis. Also, the series suffers from being predominately male. Hence, bone-citrate results on this control population are not as comprehensive as could be desired.

3.4- *Research Design*

The purpose of this study is to identify and control for potential sources of error in the bone-citrate technique. The following steps were taken, at the outset, to eliminate as many of the variables as possibly which could affect the results. Primarily, the project focuses on the chemical analysis of adult individuals of known sex, age and population affiliation collected from both modern and historic contexts. Increasing the overall sample size ensured control over possible age-dependent factors involved in the deposition of citrate in bone throughout an individual's adult life.

Limiting cadaver selections to individuals whose deaths resulted from heart-related problems, eliminated the risk of producing potentially false high or low citrate values that could skew final interpretation of the results. At the same time, however, it restricted the number of individuals that could be tested, and hence affected the representativeness of the series as a whole.

All of the historic cemeteries selected for analysis

offered an excellent opportunity to test bone-citrate content, for several reasons. First, as a pooled series, the cemeteries provide a single, relatively uniform sample. All individuals are apparently of West or East European origin, with the exception of the London County Gaol sample. In conjunction with the modern series, this allowed for control of potential problems of differential bone-citrate deposition that might possibly arise associated with major dietary differences. Furthermore, within cemetery differences could be assessed. Also, most age-ranges for both sexes are represented in the archaeological series. This assisted in delineating the effects of age-related differences between the sexes as initially observed by Lengyel (1968).

Second, the archaeological series represent a narrow historic period. The earliest documented cemetery in the series is the St. Thomas Cemetery, dating from ca. AD 1821 to ca. AD 1874 (Dr. S. Saunders, personal communication). The use-dates of four of the cemeteries (ie. Stirrup Court, Breslau, Wise, and Harvie) are contemporaneous with the approximately 50 year time span of the St. Thomas Cemetery. The remaining cemeteries (ie. Waterloo County Gaol and London County Gaol) post-date the rest of the series by only a few decades. Thus, all archaeological individuals sampled for bone-citrate content, in this study, were interred between ca. AD 1820 and ca. AD 1920, well within previous

researchers' 1000 year or more time spans (Dennison 1979; Lengyel 1968; Thunberg 1947; see also Kiszeley 1974).

Since soil samples were not available for all cemeteries tested, it had to be assumed that anatomically identical bones from the same period, buried in the same soil and excavated from the same depth, had been exposed to similar diagenesis factors, and acted approximately with the same intensity (Lengyel 1968). The implications of diagenesis for this study are discussed in Chapter Five.

Coffin plaques and other historical documentation have given secure identifications (name, date of death, age at death) for 33 of the 47 individuals in the archaeological series. In this study, in order to resolve problems commonly associated with conventional aging techniques, the bone-citrate content data of the 33 individuals of known sex and age was statistically analyzed and compared to the modern data separate from the rest of the archaeological sample. The data obtained on the 14 remaining 'unknown' individuals was then compared to these results.

Third, nine of the known individuals in the archaeological series were tested for bone-citrate in a double-blind manner (All 8 Harvie family cemetery individuals, and the one Wise family cemetery individual). That is, bone citrate data was obtained on these individuals without historical information in order to ensure that this prior knowledge could not possibly influence interpretation

of the final results. The balance of the archaeological series was tested in a single blind manner. Although information was readily available, it was not accessed until all laboratory analyses on each cemetery was completed.

As noted in Chapter Two, past researchers have used either trabecular or cortical bone as their medium of study. This study uses trabecular material for a variety of reasons. Current research on cortical bone indicates that no age relationship in citrate levels will be observed in male and female subjects ranging in age from 30 years to 80 years (Knuuttilla et al 1985). In contrast, the distribution of citrate between the sexes in trabecular bone as a physiological product of differential oestrogenic/androgenic activity is well documented, including data specifically relating to age relationships (Shorr et al 1942; Hodgkinson 1962; Welshman & McGoewn 1976; Lengyel 1968). Hence, although there is no simple relationship between dietary intake of calcium, its deposition and/or its relationship to the independent production of citrate, for either bone type, in light of these anticipated difficulties, trabecular bone tissue was selected over cortical bone as the medium of study.

Different bones of the skeleton have been shown to contain dissimilar amounts of chemical constituents and even a single bone (ie. a rib) can show demonstrable differences depending on the segment tested (Forbes et al

1976; Grupe 1988). To control for this possible variation and increase the chances of finding the most suitable bone, two different regions of the skeleton were analyzed for bone-citrate content. These regions are the anterior body of the first lumbar and the iliac crest. Exceptions, however, had to be made within the archaeological series as a consequence of pathology, poor preservation and/or missing skeletal elements. These individuals are listed in Table 3:4:1 by cemetery and reasons for selection exception.

Table 3:4:1- Archaeological Sample Body Part Selection Exceptions and Reasons.

Cemetery	Burial No.	Body Part Substituted	Reasons
Stirrup Court Cemetery	19	L2	Trauma evident in L1
	1/2 A	L4 only	Incomplete Skeleton
	1/2 X	L2 or L3	Incomplete Skeleton
Wise Family Cemetery	B (4)	Icr only	Pathology in lumbar region
Harvie Family Cemetery	6	L2	Pathology in L1
London County Gaol	P.L.B	Icr only	Incomplete Skeleton
St. Thomas Church Cemetery	435	L2 or L3	Incomplete Skeleton
	437	L1 or L2	Poor preservation

L= Lumbar vertebra, Icr= Iliac crest

CHAPTER 4: METHOD OF ANALYSIS AND SAMPLE PREPARATION

4.1- Introduction

As noted in Chapter Two, previous researchers determined citrate by converting it to pentabromine-acetone (Lengyel 1968; Dennison 1979; Gibbs 1985; see also Cook et al 1986). This method is problematic in spite of several modifications made to eliminate both the inconsistency and hence, non-reproducibility of results (Gibbs 1985; see also failed modifications by Beutler & Yeh 1959; Welshman & McGeown 1976).

For this study, several alternative methods were considered. Stern's (1957) technique for the determination of citric acid was considered, but it is a modification of the Hess & White (1955) pentabromine-acetone colorimetric technique. It is cumbersome and also proven to be unreliable and/or inconsistent (Welshman & McGeown 1976). Aue et al (1982) present a method for the gas-chromatographic analysis of citric acid (see also Kiszeley 1974). However, access to equipment necessary for the application of this technique posed a problem.

For both ease and precision, the determination of citric acid in this study was carried out by using pre-measured clinical test-combination kits for the

determination of citric acid using the enzyme citrate lyase (CL) prepared from *Aerobacter aerogenes* and supplied by Boehringer Mannheim Corporation (1983). Preliminary testing by Knuuttilla et al (1985) indicated that the pre-measured test-combination kit would be sensitive enough to determine the expected low values of citrate present in archaeological samples.

Important remarks pertaining to sample collection and preparation for this study are discussed in Section 4.2 because the archaeological and modern specimens were handled differently from samples employed by previous researchers using the pentabromide-acetone technique. Reagents and Standard Citric Acid solutions utilized in the citrate lyase assay including their preparation are described in Section 4.3.1.

Although the laboratory technique is readily available from Boehringer Mannheim Corporation (1983), it is described in Section 4.3.2 as it was employed in this study in its clinical test-combination kit form. Finally, Section 4.4 examines the analytical variables observed when utilizing the citrate lyase technique for the determination of citric acid from archaeological and modern trabecular bone specimens.

4.2 Sample Collection and Preparation

Trabecular bone specimens for this study were sampled from two different anatomical regions of the human skeleton.

These included the anterior body of the first lumbar and the iliac crest. Modern cadaver lumbar samples were collected by Ms. Jennifer Nixon from the Department of Pathology, The University of Western Ontario, using a manual bone trephine. Iliac crest samples were obtained using an automated striker saw. Archaeological lumbar and iliac crest samples were collected by both myself and Mr. Richard Lazenby using either a striker saw and/or a coping saw.

Trabecular bone samples for both the archaeological and modern series was carefully separated from any cortical bone present under a light microscope located in Dr. Dingle's laboratory in the Faculty of Life Sciences at McMaster University. The modern cadaver samples were initially rinsed daily for two weeks in this laboratory with double distilled deionized water in order to remove the embalming fluid. Then, both the archaeological and modern samples were washed and ultrasonicated thoroughly with double distilled deionized water. Following this rinsing, both modern and archaeological samples were prepared in Dr. Schwarcz's laboratory in the Department of Geology according to Knuuttilla et al's (1985) suggestions as follows.

After cleaning, oven-drying to constant weight overnight and pulverization to fine powder, the archaelogical and modern bone samples were weighed on a Mettler balance in duplicate sample preparations representing concentrations of 25.0, 50.0 and 75.0

milligrams of bone powder per one milliliter of 2.0N sulfuric acid. The citrate content results of these separate bone sample preparations and implications to this study are discussed in Section 4.4.

Citrate content of the three bone sample concentrations was released by heating the samples in a waterbath for 1 hour at 60+ degrees Celsius. The samples were allowed to cool and were stored at +4 degrees Celsius in a refrigerator located in the Department of Anthropology for two subsequent weeks. This step allowed for additional release of citrate, not previously observed by Knuuttilla et al (1985). This observation is also discussed further in Section 4.4.

Following the dissolution step, all released citrate samples were neutralized with 40% potassium hydroxide. It was noted that during this stage, as the pH approached 7.0, some bone material precipitated out of all samples tested and at all three bone concentrations tested. It was removed in Dr. Dingle's laboratory by centrifugation in an I.E.C. benchtop centrifuge at 3000 rpms, for 5 minutes. The duplicate sample preparations of both the archaeological and modern series were then stored frozen at -20 degrees Celsius in a refrigerator in the Department of Anthropology until they were analyzed in triplicate for citrate content in Dr. Prevec's laboratory in Life Sciences by the enzymatic method described below. The final bone-citrate results of this study are presented in Chapter Five.

4.3- Method of Analysis

1. Principle of U.-V.- CL enzymatic spectroscopy

The principle of Boehringer Mannheim Corporation's (1983) citrate lyase technique for the determination of citric acid involves five successive steps as summarized in Figure 4:3:1. In the reaction catalyzed by the enzyme, citrate lyase (CL), citric acid (citrate) is converted to oxalacetate and acetate (1). The enzyme preparation also contains oxaloacetate decarboxylase which decarboxylates to pyruvate part of the oxaloacetate formed from citrate with citrate lyase (CL) (3).

In the presence of the enzymes, malate dehydrogenase (MDH) and lactate dehydrogenase (LDH), oxalacetate, and its decarboxylation product, pyruvate, are reduced to L-malate and L-lactate, respectively, by reduced nicotanimide-adenine- dinucleotide (NADH) (2),(4). The amount(s) of NADH oxidized in reactions (2) and (4) are stoichiometrically related to the amount of citrate present.

Absorbance measurements (A) are taken at the absorption maximum. The fraction of the incident light absorbed by a solution at a given wavelength is related to the thickness of the absorbing layer and the concentration of the absorbing species. These two relationships are combined into the Lambert-Beer Law (Lehninger 1982) given in integrated form as:

Figure 4:3:1- Enzymatic Determination of Citric Acid by Citrate Lyase.

(1.) Citrate $\xleftarrow{\text{Citrate Lyase}}$ oxaloacetate + acetate

(2.) Oxaloacetate + NADH + H^+ $\xrightarrow[\text{Dehydrogenase}]{\text{Malate}}$ L-malate $+NAD^+$

(3.) Oxaloacetate $\xrightarrow{\text{oxaloacetate decarboxylase}}$ pyruvate+ CO_2

(4.) Pyruvate + NADH + H^+ $\xrightarrow[\text{Dehydrogenase}]{\text{Lactate}}$ L-lactate + NAD^+

(5.) NADH--->NAD^+ + L-lactate + L-malate = Concentration Citrate Present

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$$\log \frac{I_o}{I} = \epsilon c l$$

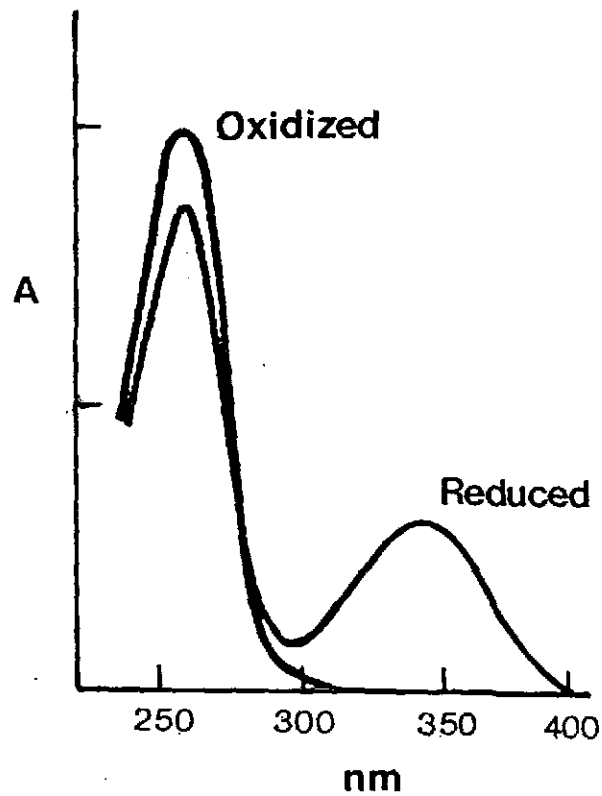
where I_o is the intensity of the incident light, I is the intensity of the transmitted light, ϵ is the molar absorption coefficient (in units of liters per mole-centimeter), c the concentration of the absorbing species in moles per liter, and l the thickness of the light absorbing sample in centimeters. The expression $\log(I_o/I)$ is called the absorbance, designated A.

The molar absorption coefficient (ϵ) varies with the nature of the absorbing compound, the solvent, the wavelength, and also the pH if the light-absorbing species is one that is in equilibrium with another species having a different spectrum through gain or loss of protons. It is the reduction of NADH to NAD+ and L-malate and L-lactate in line 5 which is measured by spectroscopy. The absorption spectra of NAD+ and NADH is presented in Figure 4:3:2. A is the absorbance, $\log I_o/I$. The molar absorbtion coefficient of NADH at neutral pH occurs at 340 nm and is calculated as

$$\approx \quad 6.3 \times 10^{3} \text{ cm}^2 \text{ mol}^{-1}.$$

For this study, a Beckman model DU-7 spectrophotometer located in Dr. Prevec's laboratory was used to measure A at the absorption maximum of NADH. The DU-7 spectrophotometor is a high-speed microprocessor controlled U.-V.-Visible spectrophotometor which operates between wavelengths of 190nm to 800nm. The instrument initiates its own

Figure 4:3:2 Absorption Spectra of NADH to NAD+



calibration routine, after which any number of samples may be measured. The instrument also indicates, immediately upon calibration, lamp failure such as reduced light intensity, which can effect absorbance reading results. The single wavelength (λ) mode was used to obtain absorbance readings at the selected wavelength of 340nm.

The enzyme citrate lyase (CL) is absolutely specific for citrate. Moellering & Gruber (1966) tested isocitrate, cis-aconitate, oxalate, succinate, fumarate, L-glutamate, α-ketoglutarate, tartrate, L-lactate, L-malate, acetate, ascorbate, glucose, fructose and ethanol at concentrations similar to citrate. None of these substances reacted or exhibited any interference with either the speed and/or accuracy of the determination of citrate by the citrate lyase method.

2. Technique of Analysis

Table 4:3:2 lists the reagents included in the kit and description of their preparation. Each kit contains enough reagents for a total of 30 determinations. The kits must be stored in the refrigerator and once opened have a shelf life of approximately 4 weeks. Standard Citric Acid solutions measuring 0.1, 0.2, 0.3, 0.4 and 0.5 millimoles of anhydrous citric acid per liter of deionized double distilled water were also utilized in this study in order to assess both intra- and inter-assay variation between test-combination kits and the optimal performance achieved when utilizing the

Table 4:3:2 Reagents and Their Preparation For 10 Determinations

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Solution 1: 1.4 g lyophilisate consisting of glycylglycine buffer, pH 7.8; malate dehydrogenase, 136 Units; L-lactate dehydrogenase, 280 Units; NADH, 6.0 mg dissolved in 12 ml double distilled water. Stable for 2 weeks at +4 C or 4 weeks at -20 C. Bring Solution 1 to +20-25 C before use.

Solution 2: 50 mg lyophilisate consisting of citrate lyase, 12 Units, oxaloacetate decarboxylase dissolved in 0.3 ml double distilled water. Stable for 1 week at +4 C or 4 weeks at -20 C.

Standards: 0.1, 0.2, 0.3, 0.4 and 0.5 mmole Citric Acid diluted from stock Standard 1.0 mmole Citric Acid solution containing .1921 g anhydrous citric acid dissolved in 1 liter double distilled water. Stable for 1 week at +4 C or 4 weeks at -20 C.

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citrate lyase technique. These solutions were prepared as follows.

Initially, a stock Standard Citric Acid solution representing 1.0 millimole or 0.1921 grams of anhydrous citric acid per liter of deionized double distilled water was weighed and prepared. This solution was then stored frozen until ready for use. Subsequent dilutions of this stock solution of citric acid were prepared weekly representing 0.1 millimoles (0.1ml stock solution-0.9ml deionized water); 0.2 millimoles (0.2ml stock solution-0.9ml deionized water); 0.3 millimoles (0.3ml stock solution-0.9ml water); 0.4 millimoles (0.4ml stock solution-0.9ml deionized water); and 0.5 millimoles (0.5ml stock solution-0.9ml deionized water) of anhydrous citric acid per liter of deionized double distilled water. Theoretically, these dilutions should correspond to 19.2, 38.4, 57.6, 76.8 and 96.0 micrograms of anhydrous citric acid per one milliliter sample solution when assayed by the citrate lyase technique. The results of the assays of the diluted stock standards for this study are presented in Section 4.4.

One milliliter of each of the diluted standards and all samples from both the archaeological and modern series were assayed by the enzymatic analysis described below. Although the citrate lyase reaction is performed at room temperature, archaeological and modern bone sample preparations, diluted standard solutions and reagents were kept on ice during each

assay session.

Figure 4:3:3 summarizes the citrate lyase procedure. As can be seen, the method of determining citrate involves four major steps. The DU-7 spectrophotometor was first zeroed at 340 nm using double distilled deionized water. A blank was prepared containing 1.0 ml of Solution 1 which consists of glyclglycine buffer at pH 7.8, L-malate dehydrogenase, L-lactate dehydrogenase, Nicotanamide-adenine-dinucleotide (NADH) and 2.0 ml double distilled deionized water. This preparation was allowed to equilibrate to room temperature in a +25 degree celcius water bath. Equilibration took approximately 5.0 minutes.

After the 5.0 minutes had lapsed, the absorbance value (A_1) of the Solution 1 was read in the DU-7 spectrophotometor. The enzymatic reaction was then started by adding 0.02 ml of Solution 2 consisting of citrate lyase, oxaloacetate decarboxylase and double distilled deionized water. After a further 5.0 minutes had lapsed, a second absorbance reading (A_2) was taken. Bone preparations and Standard Citric Acid solutions were treated the same as the blank with the exception that the amount of distilled water was reduced to 1.0 ml in order to maintain an overall final volume of 3.02 ml in the cuvette as recommended by Boehringer Mannheim Corporation (1983).

The absorbance differences ($A_1 - A_2$) obtained for both the blank, bone sample(s) and/or standard(s) were then

Figure 4:3:3 - Procedure of Analysis

Wavelength - 340nm
Glass Cuvette - 1cm light path
Temperature - 20-25+ C
Final Volume - 3.02 ml
Read against double distilled water
Sample Solution- 1.0 ml

Pipet into cuvette	Blank	Sample
Solution 1	1.0ml	1.0ml
Double distilled water	2.0ml	1.0ml**
Sample Solution*	-----	1.0ml**

Mix ***, read absorbances of solutions (A_1) after 5.0 minutes. Then start reaction by addition of

Solution 2	0.02ml	0.02ml

Mix ***, on completion of reaction (5.0 minutes) read absorbances of the solutions (A_2)

*Rinse the enzyme pipette or pipette tip of the piston pipette with sample solution before dispensing the sample solution.

**Volume of double distilled water has been adjusted from 1.8ml to 1.0ml to account for sample volume of 1.0ml used in this study and to maintain total volume of 3.02 ml as recommended by B.M.C. (1983)

***For example, with a plastic spatula or by gentle swirling after closing the cuvette with Parafilm.

determined. The absorbance difference of the blank was subtracted from the absorbance difference of each bone sample preparation and/or diluted standard:

$$\Delta A = \Delta A_{(sample)} - \Delta A_{(blank)}.$$

Occasionally, in this study, a negative value with $(A_1 - A_2)$ was obtained. This value was then added to $(A_1 - A_2)$ sample according to the calculation formula. Boehringer Mannheim Corporation (1983) suggested that if the absorbance difference of the sample [$\Delta A_{(sample)}$ measured at 340 nm] was higher than 0.850, the concentration of anyhdrous citric acid in the sample was too high. The sample solution, thus, must be diluted according to their precalculated dilution table presented in Figure 4:3:4.

3. Calculation

According to the general equation for calculating concentration:

$$c = \frac{V \times MW}{x \times x \times v \times 1000} \times A \text{ [g/l]}, \text{ where}$$

V = final volume [ml]
v = sample volume [ml]
MW = molecular weight [g/mol]
d = light path [cm]
ϵ = absorption coefficient of NADH at:
340 nm = 6.3 [1 x mmol $^{-1}$ x cm $^{-1}$]
(Boehringer Mannheim 1983)

It follows for citric acid (calculated as the anhydrous acid):

$$\frac{3.02 \times 192.1}{x \times 1.0 \times 1.0 \times 1000} \times \Delta A \times F = \frac{\text{citrate [g/l]}}{1000 \text{ ml}} = \text{[ug/ml]}$$

$$\frac{580.1}{6300} \times \Delta A \times F = \frac{\text{citrate [g/l]}}{1000 \text{ ml}} = \text{[ug/ml]}$$

Figure 4:3:4- Dilution Factors as Recommended by B.M.C (1983).

Estimated amount of citric acid per liter	dilution with water	dilution factor F
< 0.4 grams	-	1
0.4 - 4.0 grams	1 + 9	10
4.0 - 40.0 grams	1 + 99	100

$$.09207 \times \Delta A \times F = \frac{\text{citrate [g/l]}}{1000\ \text{ml}} = [\text{ug/ml}]$$

If the sample had to be diluted during preparation the result is further multiplied by the dilution factor (F). The citrate content is then converted to micrograms per milliliter of solution.

In this study, final citrate concentration is reported in parts per million (ppm) citrate in whole bone. The concentrations obtained by the citrate lyase method are reported in ug/ml of citrate in the solution prepared for analysis. The concentration of citrate in the bone is thus

$$\text{Citrate (bone)[ppm]} = \frac{\text{C(citrate in solution) [ug/ml]}}{\text{C(bone in solution) [.025g/ml]}}$$

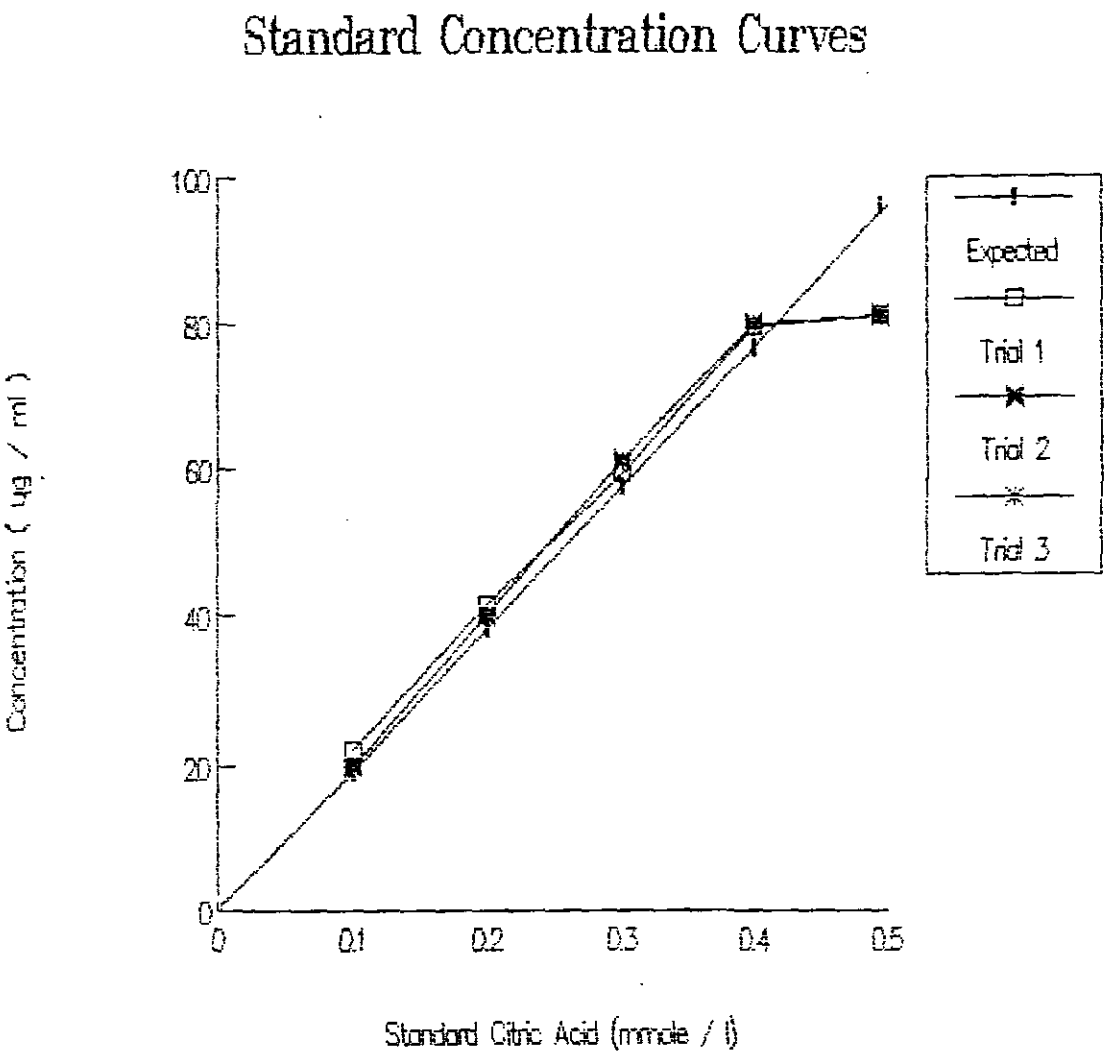
where "C" represents concentration. The concentration of bone in solution is the weight of bone sample used divided by the volume of solution. In this study, although three separate weights of bone were tested, the 25 milligram per milliliter of solution (.025g/ml) placed all of the archaeological samples within the range of the standard concentration curve outlined above. The modern sample at the same concentration, however, had to be diluted. These observations are discussed in Section 4.4.

4.4. Technical Summary of Results

1.Analytical Variables

Figure 4:4:1 illustrates the optimum performance that can be achieved, given the technical design of the assay as

Figure 4:4:1 Standard Citric Acid Curves



described in Section 4.3.1. It was based on the construction of calibration curves by assaying in triplicate, the five Standard Citric Acid dilutions equivalent to concentrations of 0.1 to 0.5 millimoles of anhydrous citric acid per liter of double distilled deionized water discussed in Section 4.3.2

The absorbance data obtained for each of these curves is listed in Table 4:4:1. Part A of the table presents the absorbance differences minus the blank for each standard measured and is based on a 1.0 milliliter sample assay volume (V). Part B of the table lists the citrate concentration results in ug/ml as calculated by the formula for concentration of anhydrous citric acid described in Section 4.3.3.

According to Boehringer Mannheim Corporation (1983), to ensure accuracy of the results, the calibration curve produced by the assay in each test kit should correspond to approximately 8-9 micrograms to 90.0 micrograms per 1.0 milliliter of sample solution per cuvette, where the reduction activity of NADH begins to level off. In this study, however, repeated analysis (n=3) shows that the linear region for the assay fell between 0.1 and 0.4 millimoles or approximately 19.2 to 76.8 micrograms citrate per milliliter sample solution, where NADH is used up in the reaction. In short, all bone samples tested by this assay must fall within the average range of 21.1$\pm$1.0 and 79.8

Table 4:4:1 Data For Calibration Curves Observed at 340 nm

PART A: Abs. Differences - Blank

Sample mmole	Trial 1	Trial 2	Trial 3
0.1	.239	.219	.214
0.2	.450	.446	.437
0.3	.646	.651	.653
0.4	.865	.858	.868
0.5	.882	.892	.882

PART B: $\Delta A \times .09207 \times F$ (1) [g/l]/1000ml

Sample mmole	Trial 1 ug/ml	Trial 2 ug/ml	Trial 3 ug/ml	Trials 1,2 & 3 combined ug/ml	
				Mean	SD
0.1	22.0	21.6	19.7	21.1	1.0
0.2	41.4	41.0	40.2	40.9	0.5
0.3	59.4	59.9	61.3	60.2	0.8
0.4	79.6	79.9	79.9	79.8	0.1
0.5	81.2	82.1	81.2	81.5	0.4

± 0.1 ug/ml per cuvette in order to ensure accuracy of the final concentration results. Whole bone sample preparations containing more citrate than 79.8 ± 0.1 ug/ml per cuvette must be diluted, reassayed and corrected by the dilution factor (F).

In this study, regardless of body part tested, it was found that bone sample preparations of 50 and 75 milligrams per 1.0 milliliter sample volume (V) per cuvette for both the archaeological and modern series were generally too concentrated to ensure their accurate assessment (greater than 79.8± 0.1 ug/ml per cuvette). Correspondingly, although the 25 milligram per milliliter bone sample preparation was sufficient to place all of the archaeological samples tested within the midrange of the calibration curve, the modern cadaver absorbance results at this concentration remained too high to ensure the accuracy of their final concentration assessment. The modern series, at the 25 milligram per milliliter sample preparation was diluted. These samples were then reassayed and their absorbance differences were then multiplied by a 10X dilution factor (F). Both series were then converted to ppms citrate in whole bone.

The calibration curve produced by the method also appears to be relatively stable and precise. To confirm this, inter-assay and intra-assay variation of the three separate trials of the standard curve were compared and are presented in Tables 4:4:2 and 4:4:3, respectively. Trial 1

Table 4:4:2- Inter-assay Variation For Citrate Assay

Part A: Trial # 1*

Sample mmole	Expected ug/ml	Achieved ug/ml	% Difference
0.1	19.2	22.0	12.7
0.2	38.4	41.4	7.2
0.3	57.6	59.4	3.0
0.4	76.8	79.6	3.5
0.5	96.0	81.2	15.4
			$\bar{X}$ = 8.3

Part B: Trial # 2**

Sample mmole	Expected ug/ml	Achieved ug/ml	% Difference
0.1	19.2	21.6	11.1
0.2	38.4	41.0	6.3
0.3	57.6	59.9	3.8
0.4	76.8	79.9	3.8
0.5	96.0	82.1	14.4
			$\bar{X}$ = 7.8

Part C: Trial # 3***

Sample mmole	Expected ug/ml	Achieved ug/ml	% Difference
0.1	19.2	19.7	2.5
0.2	38.4	40.2	4.4
0.3	57.6	61.3	6.0
0.4	76.8	79.9	3.8
0.5	96.0	81.2	15.4
			$\bar{X}$ = 6.4

* onset
**duplicates
***St. Thomas sample

Table 4:4:3 - Intra-assay Variation For Citrate Assay

Part A:

Sample mmole	Achieved Level Trial 1 ug/ml	Achieved Level Trial 2 ug/ml	% Difference
0.1	22.0	21.6	1.8
0.2	41.4	41.0	1.1
0.3	59.4	59.0	0.6
0.4	79.6	79.9	0.3
0.5	81.2	82.1	1.1
		$\bar{X}$=	1.0

Part B:

Sample mmole	Achieved Level Trial 1 ug/ml	Achieved Level Trial 3 ug/ml	% Difference
0.1	22.0	19.7	10.4
0.2	41.4	40.2	2.8
0.3	59.4	61.3	3.0
0.4	79.6	79.9	0.3
0.5	81.2	81.2	0.0
		$\bar{X}$=	3.3

Part C:

Sample mmole	Achieved Level Trial 2 ug/ml	Achieved Level Trial 3 ug/ml	% Difference
0.1	21.6	19.7	8.7
0.2	41.0	40.2	1.9
0.3	59.0	61.3	3.7
0.4	79.9	79.9	0.0
0.5	82.1	81.2	1.0
		$\bar{X}$=	3.0

was a curve produced at the outset of the study. Trial 2 was produced midway through the study, specifically when duplicates of both the modern and archaeological series were assayed. Trial 3 was a curve produced approximately one year later when the St. Thomas Church sample was added to the archaeological series in this study.

Intra-assay and inter-assay variation in citrate content achieved is < 2.4% and < 7.5%, respectively, and is well within previously reported averaged intra- and inter-assay variations of citrate content observed for other biological tissues when employing the test kit (Warty et al 1984: < 3.0% and < 8.0%, respectively). As a final test of precision, a subset of archaeological samples from six of the seven cemeteries used for an initial run were prepared and reanalyzed several months later, when the St. Thomas Church cemetery sample was added to the study.

The results of these assays are presented in Table 4:4:4. The intra-assay variation for the archaeological series per lumbar and iliac crest samples is 2.02% and 1.26%, respectively. The reproducibility of citrate readings in this third run are consistent with the performance trials of the standard curves and do not vary substantially. An overall recovery of greater than 98.0 % and 99.0 % citrate content, respectively, can be expected when analyzing whole trabecular bone using the citrate lyase technique.

Table 4:4:4- Comparison of a Subset of Samples From The Archaeological Series Approximately 1 Year Apart

Sample	Run 1 ($\bar{x}$ Assay 1 & 2) ppm	Run 2 (Assay 3) ppm	Ci % Difference
LVSC21	1222 ± 2.5	1230 ± 3.2	0.65
LVWCG2	1029 ± 3.5	1032 ± 1.4	0.29
LVWCG1	1064 ±48.5	1007 ± 2.1	5.35
LVB2	1168 ±15.5	1197 ± 2.3	2.42
LVB1	976 ±18.5	1010 ± 1.4	3.36
LVH13	1715 ± 0.5	1717 ± 1.6	0.06
		$\bar{x}$ =	2.02
ICrS21	1511 ± 5.0	1501 ± 1.2	0.66
ICrWCG2	1071 ± 1.5	1089 ± 0.8	1.65
ICrWCG1	1026 ± 0.6	1047 ± 3.0	2.00
ICrB2	1191 ± 5.0	1202 ± 3.6	0.92
ICrB1	1023 ± 6.0	1032 ± 1.6	0.87
ICrH13	1769 ±24.0	1795 ± 1.6	1.45
		$\bar{x}$ =	1.26

LV = Lumbar Vertebra; ICr= Iliac Crest; B= Breslau, SC= Stirrup Court, H= Harvie, LCG= London County Gaol, WCG= Waterloo County Gaol

As noted in Section 4.2, additional release of citrate was obtained after allowing preliminary sample preparations to sit refrigerated for an additional two weeks. Table 4:4:5 presents the results of the differences of citrate in ppms of whole bone obtained on a subset of three archaeological samples and three modern cadaver samples, at the 25 milligram per milliliter sample preparation, and for both skeletal regions tested. For both series, a mean percentage difference of 4.5 % and 4.8 % more citrate per body part was released. These results demonstrate that this additional step does more adequately concentrate and release the citrate content of trabecular bone mineral.

A preliminary test of a sample of the embalming fluid used to preserve the modern series yielded no citric acid results by citrate lyase enzymatic spectroscopy. It appeared that constituents of the fluid severely limited the activity of citrate lyase necessary for the reactions to proceed. Successful elimination of the fluid viz. water rinsing could only be based on whether or not absorbance readings within the range of the Standard Citric Acid concentration curves were obtained for a particular sample. Since individual absorbance values for both body parts tested were obtained, it was assumed that all of the embalming fluid was removed by this preliminary washing step.

Tompkins and Toffaletti (1982) have suggested that if an analyte, in this case, citrate, is partly bound to

Table 4:4:5- Comparison Between Sample Preparations

Sample	Skeletal Part	Initial Preparation ppm	Modified Preparation ppm	% Difference
Archaeological Series				
B2	LV	1100±2.8	1152±3.4	4.5
	ICr	1156±3.8	1196±3.4	3.3
SC21	LV	1164±4.5	1220±2.5	4.5
	ICr	1436±2.8	1512±3.3	4.5
WCG2	LV	980±1.9	1033±3.2	5.0
	ICr	1016±2.7	1072±3.3	5.2
			$\bar{X}$ =	4.5
Modern Series				
108	LV	14,976±5.8	15,700±5.4	4.6
	ICr	15,648±4.3	16,420±1.3	4.7
83	LV	15,784±3.5	16,616±2.2	5.0
	ICr	16,036±4.3	16,864±2.1	4.9
125	LV	15,076±3.1	15,856±4.9	4.9
	ICr	15,360±2.9	16,636±2.9	4.8
			$\bar{X}$=	4.8

B= Breslau, SC= Stirrup Court, WC= Waterloo County Gaol
LV= Lumbar vertebra; ICr= Iliac crest

proteins, the absorbance values obtained for citrate by this assay will be proportionately lower than the total concentration present. Although citrate is reported to be bound to protein at pH 5.1 in both blood and urine (Pederson (1971), documentation of similar evidence for citrate in trabecular bone mineral, could not be found. If this is the case for bone material, some loss of citrate content can also be expected due to the neutralization stage of the initial sample preparation. It was assumed, therefore, that since all samples reacted in the same way, any loss of citrate which may have occurred due to protein precipitation was proportionately uniform throughout both the archaeological and the modern series.

The technical results cited above generally demonstrate that the determination of bone citrate content by the citrate lyase technique is more reliable and consistent, and hence, superior, to the Hess & White (1952) pentabromide-acetone technique employed by previous researchers. However, the inability to establish a uniform sample preparation between the two series suggests that separate sample concentration preparations and controls will always be necessary each time different series of human skeletal bone samples are assessed by the assay. This severely limits the assay's ability to adequately determine the citrate content of fragmentary and hence, isolated human skeletal remains.

Nevertheless, the specificity and reproducibility of

U.V- enzymatic spectroscopy by citrate lyase for the determination of trabecular bone citrate content does eliminate problem for previous researchers of having to select high versus low values of Ci-content when attempting to discriminate sex (Dennison 1979; Gibbs 1985, see also Cook et al 1986). The final concentration results obtained in this study are presented in Chapter Five.

CHAPTER 5: RESULTS OF U.V.- ENZYMATIC ANALYSIS OF CITRATE

5.1. Introduction

All samples were analyzed in duplicate by the citrate lyase technique outlined in Chapter Four. The final concentrations observed for duplicate assay runs of citrate in parts per million of trabecular bone tissue for both the archaeological and the modern series per body part are presented in Tables 5:1:1, 5:1:2 and Tables 5:1:3, 5:1:4, respectively.

Sections 5.2 and 5.3 presents the results of trabecular bone citrate analysis for the archaeological and the modern series, respectively. All statistical analyses in Sections 5.2 and 5.3 are based on the means and/or medians of subsets of samples derived from the duplicate runs of Ci-content of individuals in the tables from Section 5:1. Section 5.4 compares the results from both series and summarizes the results of this study in light of previous observations of citrate content as a discriminator of the sex of human skeletal remains.

5.2- Archaeological Series

1. Cemeteries Pooled

As noted in Chapter Three, in order to resolve problems commonly associated with conventional aging

Table 5:1:1- Duplicate Assays of Ci-content of Lumbar Samples Observed for the Archaeological Series

Sample	Citrate Concentration obtained		Assay 1 & 2 compared		
	Assay 1 ppm	Assay 2 ppm	Mean ppm	SD ±	% Difference
B2	1152 ± 3.39	1183 ± 1.48	1168	15.5	2.62
B1	951 ± 1.96	1001 ± 0.12	976	18.5	4.99
H13(B)	1716 ± 3.27	1715 ± 1.89	1715	0.5	0.06
H3(F)	1435 ± 1.89	1365 ± 3.89	1400	35.0	4.88
H11(C)	1743 ± 1.89	1814 ± 4.53	1778	35.5	3.93
H4(H)	1345 ± 3.27	1244 ± 3.68	1294	50.5	7.50
H14(E)	1287 ± 3.77	1296 ± 3.38	1291	4.5	0.69
H8(G)	1241 ± 1.89	1227 ± 3.23	1234	7.0	1.13
H6(A)	1179 ± 1.89	1157 ± 4.29	1168	11.0	1.86
H7(D)	1149 ± 1.89	1138 ± 1.45	1143	16.0	0.95
SC21	1220 ± 3.11	1225 ± 3.18	1222	2.5	0.41
SC10	1631 ± 1.96	1608 ± 4.35	1619	11.5	1.41
SC6	953 ± 3.19	988 ± 1.28	969	18.5	3.54
SCA	863 ± 1.92	886 ± 3.31	874	11.5	2.54
SC4	977 ± 3.39	1010 ± 2.03	993	16.5	3.27
SC18	987 ± 1.98	1015 ± 3.83	1001	14.0	2.75
SC7	990 ± 1.72	1018 ± 1.51	1004	14.0	2.75
SC1/2A	1381 ± 3.14	1383 ± 4.50	1382	1.0	0.14
SC3	1149 ± 2.94	1142 ± 3.40	1145	3.1	0.61
SC5	1551 ± 4.98	1553 ± 5.56	1552	0.8	0.13
SC1/2X	1381 ± 3.14	1383 ± 4.50	1382	1.0	0.14
SC14	1391 ± 3.75	1391 ± 2.05	1391	0.1	0.01
SC19	1391 ± 1.74	1393 ± 3.09	1392	1.1	0.14
SC20	1546 ± 2.18	1541 ± 5.56	1544	2.3	0.32
SC11	1339 ± 1.77	1341 ± 6.48	1340	0.8	0.15
SC17	1550 ± 1.98	1555 ± 3.74	1552	2.5	0.32
SC1/2B	1553 ± 3.35	1554 ± 5.56	1553	0.5	0.06
ST 406	1725 ± 3.35	1795 ± 3.40	1760	35.0	3.90
ST 188	1589 ± 3.31	1602 ± 1.71	1595	6.5	0.81
ST 437	1649 ± 3.43	1637 ± 2.41	1643	6.0	0.73
ST 400	1602 ± 3.61	1630 ± 2.04	1616	14.0	1.71
ST 339	994 ± 1.98	976 ± 2.52	985	9.0	1.81
ST 436	1193 ± 3.39	1209 ± 1.24	1201	8.0	1.32
ST 516	1115 ± 1.97	1147 ± 2.06	1131	16.0	2.80
ST 108	1206 ± 1.84	1226 ± 3.00	1216	10.0	1.63
ST 435	1224 ± 3.31	1242 ± 1.61	1233	9.0	1.45
ST 361	1992 ± 3.31	2025 ± 3.39	2008	16.5	1.63
ST 433a	1995 ± 1.63	2020 ± 2.45	2007	12.5	1.24
ST 94	1947 ± 1.67	1968 ± 0.78	1957	10.5	1.07
ST 472	1710 ± 3.77	1736 ± 3.62	1723	0.5	1.49
ST 451	1868 ± 3.27	1879 ± 2.53	1873	5.5	0.59
ST 115	1691 ± 3.72	1717 ± 1.09	1704	13.0	1.51
ST 351a	1944 ± 3.27	1968 ± 3.97	1956	12.0	1.22
WCG 2	1033 ± 3.24	1026 ± 1.89	1029	3.5	0.68
WCG 1	1016 ± 3.43	1113 ± 7.92	1064	48.5	8.71
				$\bar{X}$ =	1.81

B=Breslau, H= Harvie, SC= Stirrup Court, ST= St. Thomas, WCG= Waterloo County Gaol

Table 5:1:2- Duplicate Assays of Ci-content Observed for Iliac Crest Samples From the Archaeological Series

Sample	Citrate Concentration obtained		Assay 1 & 2 compared		
	Assay 1 ppm	Assay 2 ppm	Mean ppm	SD ±	% Difference
B2	1196 ±3.39	1186 ±1.88	1191	5.0	0.84
B1	1029 ±3.07	1017 ±3.58	1023	6.0	1.17
H13(B)	1745 ±3.23	1793 ±3.18	1769	24.0	2.68
H3(F)	1509 ±3.35	1494 ±2.74	1502	7.5	0.99
H11(C)	1822 ±3.89	1918 ±5.28	1870	48.0	5.00
H8(G)	1389 ±3.31	1428 ±1.20	1409	19.5	2.73
H6(A)	1352 ±3.23	1374 ±2.23	1363	11.0	1.60
H4(H)	1337 ±3.19	1358 ±3.78	1348	10.5	1.55
H14(E)	1272 ±3.23	1297 ±1.93	1285	12.5	1.93
H7(D)	1248 ±3.23	1256 ±1.82	1252	4.0	0.64
LCG1	1323 ±1.89	1321 ±3.02	1322	1.0	0.15
SC21	1512 ±3.27	1510 ±4.62	1511	1.0	0.13
SC10	1672 ±3.27	1694 ±4.90	1683	11.0	1.29
SC6	953 ±3.19	980 ±2.02	967	13.5	2.75
SCA	1028 ±3.18	1046 ±1.47	1037	9.0	1.72
SC4	1070 ±1.93	1088 ±1.74	1079	9.0	1.65
SC18	1034 ±1.93	1047 ±1.62	1041	6.5	1.24
SC7	1114 ±2.15	1129 ±0.41	1122	7.5	1.32
SC3	1058 ±1.80	1061 ±0.92	1059	1.4	0.28
SC5	1612 ±3.27	1622 ±2.78	1617	4.9	0.62
SC14	1591 ±1.91	1594 ±3.33	1593	1.5	0.18
SC19	1552 ±3.27	1556 ±2.60	1554	1.6	0.26
SC20	1636 ±3.27	1651 ±1.72	1644	7.5	0.91
SC11	1431 ±1.89	1440 ±1.42	1436	4.4	0.63
SC17	1592 ±3.27	1599 ±1.04	1596	3.4	0.44
SC1/2X	1463 ±1.98	1467 ±1.73	1465	1.9	0.27
SC1/2B	1592 ±3.27	1592 ±1.36	1592	0.6	0.00
W4	1169 ±0.82	1172 ±2.05	1171	1.7	0.26
ST188	1792 ±3.31	1809 ±1.67	1801	8.5	0.55
ST406	1653 ±3.23	1677 ±2.56	1665	12.0	1.43
ST437	1653 ±3.18	1667 ±1.58	1660	7.0	0.84
ST400	1690 ±1.89	1705 ±4.66	1698	7.5	0.88
ST339	1030 ±3.77	1055 ±2.89	1043	12.5	2.09
ST516	1180 ±3.15	1205 ±2.57	1193	12.5	2.07
ST436	1258 ±4.99	1277 ±2.39	1268	9.5	1.45
ST108	1287 ±1.91	1311 ±1.98	1290	12.0	1.83
ST435	1254 ±1.86	1286 ±2.86	1270	16.0	2.49
ST361	2046 ±1.93	2081 ±0.84	2064	17.5	1.68
ST451	1954 ±2.00	1986 ±2.19	1970	16.0	1.61
ST433a	2089 ±3.64	2104 ±2.21	2097	7.5	0.71
ST94	1992 ±3.31	2025 ±2.55	2009	16.5	1.62
ST115	1780 ±4.29	1798 ±2.31	1789	9.0	1.00
ST472	1785 ±3.47	1805 ±3.31	1795	10.0	1.11
ST351a	2024 ±3.23	2068 ±1.84	2046	22.0	2.13
WCG2	1072 ±3.27	1069 ±3.19	1071	1.5	0.27
WCG1	1028 ±3.27	1023 ±3.63	1026	0.6	0.49
					$\bar{x}$ = 1.25

B= Breslau, H= Harvie, SC= Stirrup Court, ST= St. Thomas, W= Wise, WCG= Waterloo County Gaol, LCG= London County Gaol

Table 5:1:3- Duplicate Assays of Ci-content of Lumbar Samples From the Modern Series

Sample	Citrate Concentration obtained		Assay 1 & 2 compared		
	Assay 1 ppm	Assay 2 ppm	Mean ppm	SD ±	% Difference
86	4,129 ±6.8	4,123 ±3.3	4,126.1	3.2	0.14
116	7,779 ±5.0	7,780 ±6.2	7,779.4	0.6	0.01
126	12,447 ±6.8	12,455 ±3.7	12,450.9	4.2	0.06
652	12,535 ±1.9	12,546 ±4.0	12,540.4	5.7	0.08
100	12,581 ±1.9	12,583 ±3.3	12,582.1	0.9	0.02
140	12,809 ±3.8	12,818 ±2.2	12,813.6	4.4	0.07
97	12,972 ±3.3	12,976 ±8.3	12,974.0	2.0	0.03
124	13,179 ±1.9	13,183 ±2.5	13,180.9	2.1	0.03
141	13,201 ±7.5	13,218 ±1.7	13,209.6	8.4	0.13
134	13,239 ±5.0	13,244 ±2.9	13,241.4	2.6	0.04
118	13,384 ±3.3	13,385 ±3.7	13,384.5	0.5	0.01
103	13,971 ±5.2	13,976 ±4.3	13,973.6	2.4	0.04
147	14,335 ±5.0	14,354 ±6.6	14,344.4	9.7	0.13
89	14,371 ±9.4	14,373 ±3.7	14,371.9	1.1	0.04
120	14,388 ±3.3	14,399 ±1.2	14,393.5	5.5	0.08
148	14,616 ±3.3	14,621 ±4.6	14,618.5	2.5	0.03
133	14,790 ±5.9	14,792 ±6.3	14,791.0	1.0	0.01
90	15,147 ±1.9	15,148 ±1.6	15,147.9	0.1	0.01
114	15,177 ±3.8	15,179 ±5.4	15,178.1	0.8	0.01
113	15,451 ±5.0	15,455 ±4.2	15,452.9	2.1	0.02
136	15,603 ±5.0	15,605 ±4.9	15,603.9	1.1	0.01
108	15,695 ±5.0	15,701 ±5.4	15,697.9	3.2	0.04
123	15,833 ±3.8	15,887 ±4.8	15,860.1	6.9	0.33
125	15,851 ±5.0	15,856 ±4.9	15,853.4	2.6	0.03
83	16,615 ±5.0	16,616 ±2.2	16,615.3	0.7	0.01
				$\bar{x}$ =	0.07

Table 5:1:4:-Duplicate Assays of Ci-content of Iliac Crest Samples From the Modern Series

Sample	Citrate Concentration obtained		Assay 1 & 2 compared		
	Assay 1 ppm	Assay 2 ppm	Mean ppm	SD ±	% Difference
86	4,395±1.9	4,398±3.3	4,396.4	1.6	0.07
116	8,056±3.3	8,060±0.8	8,058.0	2.0	0.05
652	12,369±6.8	12,370±1.2	12,369.6	0.4	0.02
126	12,844±3.3	12,848±1.2	12,846.0	2.0	0.03
100	12,981±1.9	12,984±2.9	12,982.5	1.5	0.02
140	13,192±3.3	13,191±2.5	13,191.5	0.5	0.00
141	13,227±6.8	13,227±1.2	13,226.9	0.2	0.00
97	13,373±1.9	13,374±1.2	13,373.6	0.4	0.02
124	13,540±3.3	13,540±0.8	13,540.0	0.0	0.00
134	13,633±1.9	13,634±2.9	13,633.6	0.4	0.02
118	14,151±5.0	14,156±3.1	14,153.4	2.6	0.04
103	14,305±5.0	14,309±1.2	14,307.1	1.8	0.03
120	14,424±3.3	14,425±2.6	14,424.5	0.5	0.00
147	14,808±3.3	14,805±3.3	14,806.5	1.5	0.02
89	14,813±5.0	14,825±2.9	14,819.1	5.9	0.08
133	14,852±3.3	14,851±1.7	14,851.5	0.5	0.00
148	14,943±1.9	14,942±2.5	14,942.4	0.3	0.00
90	15,529±6.8	15,535±3.7	15,532.1	2.8	0.04
114	15,531±5.0	15,531±1.2	15,530.9	0.1	0.00
113	15,819±5.0	15,816±2.9	15,817.4	1.3	0.03
125	16,132±3.3	16,136±2.9	16,134.0	2.0	0.02
136	16,209±3.8	16,208±1.7	16,208.6	0.6	0.00
123	16,256±3.3	16,258±2.6	16,257.0	1.0	0.01
108	16,423±6.8	16,420±1.2	16,421.3	1.4	0.01
83	16,860±3.3	16,864±2.1	16,862.0	2.0	0.02

				$\bar{x}$ =	0.02

techniques, bone Ci-content of archaeological individuals of known age and sex only from Tables 5:1:1 and 5:1:2 are statistically compared in this section in order to assess the feasibility of utilizing Ci-content as a discriminator of the sex of fragmentary human skeletal remains. Five of the seven cemeteries tested for Ci-content within the archaeological series contained 'known' individuals. These cemeteries included Harvie, Stirrup Court, St. Thomas Church, Waterloo County Gaol and London County Gaol.

The pooled means, medians, standard deviations and standard error of the means of Ci-content of whole trabecular bone observed in this study for both skeletal parts of the five cemetery samples are listed in Table 5:2:1. The Ci-content of the lumbars (n=32) within the pooled series ranged from 874 $\pm$ 11.5 to 2008 $\pm$ 16.5 ppms in whole trabecular bone with an overall mean of 1421.4 $\pm$ 372.6 ppms. The Ci-content of the iliac crest samples tested (n=33) was only slightly higher than the lumbars ranging from 967 $\pm$ 13.5 to 2097 $\pm$ 7.5 ppms in whole bone, with an overall mean of 1363.0 $\pm$ 370.4 ppms.

Chemical content of human bone specimens from different anatomical sites within the skeleton can also show considerable variation. In this study, in order to test the null hypothesis that there are no differences between the medians of Ci-content of whole trabecular bone for both of the body parts tested, Mann-Whitney rank sum tests for

Table 5:2:1- Descriptive Statistics of Ci-content Obtained For Both Body Parts of Known Individuals (Cemeteries, Sexes and Ages Pooled)

BODY PART	NUMBER	MEDIAN ppm	MEAN ppm	SD ppm	SE ppm
LV	32	1293.5	1421.4	372.6	65.9
ICr	33	1363.0	1488.3	370.4	64.5

LV= Lumbar; ICr = Iliac crest

Table 5:2:2- Results of Mann-Whitney Rank Sum Tests Obtained Between Body Parts (Cemeteries, Ages Pooled)

Group	All Individuals		Males only		Females only	
	W	p	W	p	W	p
LV:ICr	977.5	.306	321.1	.533	183.0	.370

This table lists the values of W that were obtained (Two-tailed).
LV= Lumbar vertebra; ICr= Iliac crest

Table 5:2:3- Descriptive Statistics of Ci-content Obtained For Both Body Parts of Known Males and Females (Cemeteries, Ages Pooled)

BODY PART	SEX	NUMBER	MEDIAN ppm	MEAN ppm	SD ppm	SE ppm
LV	F	14	1414.0	1423.0	377.0	101.0
LV	M	18	1293.9	1404.5	359.3	84.7
ICr	F	14	1479.5	1508.7	371.8	99.4
ICr	M	19	1363.0	1457.4	357.0	81.9

LV= Lumbar vertebra; ICr= Iliac Crest; F= Female; M= Male

difference between pairs of samples of Ci-content was performed on the data listed in Table 5:2:1. These results are presented in Table 5:2:2.

No significant differences in the values of W were obtained when the medians of the Ci-content of whole trabecular bone from either lumbar or iliac crest samples were compared ($p \leq .306$). No significant differences in the values of W were obtained for trabecular bone Ci-content when either the individual medians of the pooled male or female lumbar and iliac crest samples were compared ($p \leq .533$; $p \leq .370$). The results of these rank sum two-tailed tests suggests that the Ci-content of whole trabecular bone from either anatomical region can be used, in contrast to previous researchers' reports on trabecular chemical bone variations (Grupe 1988).

Table 5:2:3 lists the means, medians, standard deviations and standard error of the means of the Ci-content of whole trabecular bone observed within the pooled series, grouped by body part and sex. The Ci-content of male lumbar (n=18) samples ranged from 985 $\pm$ 9.0 to 2,008 $\pm$ 12.5 ppms in whole trabecular bone with an overall mean of 1,404.5 $\pm$ 359.3 ppms. The Ci-content observed for pooled male iliac crest (n=19) samples was similar to the lumbars and ranged from 967 $\pm$ 11.5 to 2,097 $\pm$ 7.5 ppms in whole bone with an overall mean of 1,457.4 $\pm$ 357.0 ppms, respectively. The female Ci-content of both lumbar (n=14) and iliac crest

(n=14) bone samples was similar to the male lumbar and iliac crest samples in the pooled series ranging from 875 ± 11.5 to 2,009 ± 16.5 ppms in whole bone with a mean of 1,423.0 ± 377.0 ppms and 1,037 ± 9.0 to 2,064 ± 17.5 ppms with a mean of 1,508.7 ± 371.8 ppms, respectively.

Mann-Whitney rank sum tests for difference between pairs of samples were also performed on the data listed in Table 5:2:3 in order to test the null hypothesis that there are no differences in the medians of Ci-content of whole trabecular bone between the males and females tested within the pooled archaeological series. The results of these tests are listed in Table 5:2:4.

No significant differences in the values of W were obtained when the medians of Ci-content of whole trabecular bone between the sexes were compared, regardless of body part ($p \leq 1.00$; $p \leq .812$). This suggests that Ci-content, alone, cannot discriminate between the sexes of archaeological human skeletal remains, in agreement with previous researchers' reports (Lengyel 1968; Kiszeley 1974; Gibbs 1985).

As noted in Chapter Two, when determining sex, several variables can affect bone citrate observed in any study of archaeological human skeletal remains. These variables include physiological age, geographic distribution, archaeological time and/or health-related status. All of the five cemeteries within the pooled sample date to relatively

Table 5:2:4- Results of Mann-Whitney Rank Sum Tests Between Pairs of Known Male and Female Body Parts (*Cemeteries, Ages Pooled*)

Group	W	p
FLV:MLV	231.0	1.000
FICr:MICr	245.0	.812

This table contains the values of W obtained (Two-tailed).
LV= Lumbar vertebra; ICr= Iliac crest; F= Female; M= Male

the same narrow historical time period (ie. approximately $\leq$ 100 years). Health-related status was not available for all of the known individuals within the pooled series. The effects of health-related status on Ci-content, therefore, could not be dealt with at this time.

In order to test the hypothesis that several alternate variables, including age and/or geographic distribution and hence, diagenesis, could possibly affect interpretations of the Ci-content of trabecular bone observed within this study, Kruskal-Wallis tests for variance between medians of Ci-content were performed on the data listed in Table 5:2:1. The variables included in these tests were individual cemeteries specifically ordered by distance across space, sex and age ranges that included 10 year increments ranging from 10-19 years to 90-99 years. These results are presented in Table 5:2:5.

Regardless of body part, highly significant differences in the values of H were obtained when the medians of the individual cemeteries alone, were compared to each other ($L= p\leq .006$; $ICr= p \leq .010$). No significant differences in the values of H were obtained when the medians of Ci-content between the sexes and/or age range increments were compared within the pooled series. Any significant differences observed in the the comparative statistical analyses of Ci-content in the pooled series, thus, may also be due to the effects of regional differential diagenesis.

Table 5:2:5- Results of Kruskal-Wallis Tests For Variance in Ci-content of Body Parts Grouped by Cemeteries, Sex or Age Ranges

Part A:

LUMBARS	H	p
32 samples: Cemeteries (1,4,5,7) Kruskal-Wallis	12.39	.006
32 samples: Sex (1,2) Kruskal-Wallis	0.00	1.000
32 samples: Age Ranges (1-9) Kruskal-Wallis	0.07	.799

Part B:

ILIAC CRESTS	H	p
33 samples: Cemeteries (1,2,4,5,7) Kruskal-Wallis	13.42	.010
33 samples: Sex (1,2) Kruskal-Wallis	0.07	.799
33 samples: Age Ranges (1-9) Kruskal-Wallis	12.81	.320

This table contains the values of H that were obtained.

Cemeteries: 1=Stirrup Court; 2= London County Gaol; 4= Waterloo County Gaol; 5= Harvie; 7= St.Thomas
Sex: 1= Males; 2= Females
Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+

Table 5:2:6 lists the medians, means, standard deviations and ranges of Ci-content observed for each body part in the pooled series grouped by both sex and age ranges. Kruskal-Wallis tests for variance were also performed on the data of this table in order to test the null hypothesis that when sex is controlled, Ci-content does not vary between either age ranges or the cemeteries. These results are presented in Table 5:2:7.

Significant differences in the values of H were obtained between female age categories within the pooled series, regardless of body part ($L=p \leq .050$; $ICr=p \leq .013$). However, no significant differences in the values of H were obtained between age categories of the males within the pooled series ($L= p \leq .472$; $ICr= p \leq .479$). This suggests that the effect of physiological age on the Ci-content of whole trabecular bone may possibly be more marked within the skeletons of females than within the skeletons of males in the pooled series due to hormonal variations that are known to exist between the sexes as described in Chapter Two.

Figure 5:2:1 illustrates the distribution of Ci-content of whole trabecular bone per body parts obtained on all known individuals in the pooled series separated by both age and sex. In general, it can be seen that, although the Ci-content of both males and females in this study does not significantly vary between the sexes, regardless of skeletal region tested, it does generally decrease with age in both

Table 5:2:6- Descriptive Statistics of Known Individuals' Body Parts Grouped by Sex and Age Ranges

BODY PART	NUMBER	SEX	AGE RANGE	SINGLE OBS. *	MEDIAN ppm	MEAN ppm	SD ±	MIN ppm	MAX ppm
LV	3	M	1	n/a	1,958	1,665	550	1,030	2,008
	4	M	2	n/a	1,713	1,592	359	1,065	1,874
	1	M	3	1,400	n/a	n/a	n/a	n/a	n/a
	1	M	4	1,620	n/a	n/a	n/a	n/a	n/a
	2	M	5	n/a	1,372	1,373	548	985	1,760
	2	M	6	n/a	1,076	1,086	163	970	1,201
	5	M	7	n/a	1,234	1,197	125	994	1,292
	1	F	1	2,009	n/a	n/a	n/a	n/a	n/a
	3	F	2	n/a	1,643	1,738	189	1,616	1,956
	2	F	3	n/a	1,747	1,748	45	1,716	1,779
	5	F	6	n/a	1,216	1,210	259	875	1,602
	2	F	8	n/a	1,003	1,003	2	1,001	1,004
	1	F	9	1,144	n/a	n/a	n/a	n/a	n/a
ICr	3	M	1	n/a	2,009	1,726	569	1,071	2,097
	5	M	2	n/a	1,789	1,580	392	1,026	1,970
	1	M	3	1,502	n/a	n/a	n/a	n/a	n/a
	1	M	4	1,683	n/a	n/a	n/a	n/a	n/a
	2	M	5	n/a	1,354	1,354	440	1,043	1,665
	2	M	6	n/a	1,117	1,118	213	967	1,268
	5	M	7	n/a	1,348	1,297	130	1,079	1,409
	1	F	1	2,064	n/a	n/a	n/a	n/a	n/a
	3	F	2	n/a	1,698	1,801	213	1,660	2,046
	2	F	3	n/a	1,819	1,820	71	1,769	1,870
	5	F	6	n/a	1,270	1,320	288	1,037	1,801
	2	F	8	n/a	1,087	1,082	57	1,041	1,122
	1	F	9	1,252	n/a	n/a	n/a	n/a	n/a

LV= Lumbar Vertebra; ICr= Iliac Crest; M= Male; F= Female

* = Observation on one individual only within age category

Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+

Table 5:2:7-Results of Kruskal-Wallis Tests For Variance in Ci-content Grouped by Cemeteries, Sex and Age Ranges

Part A:

LUMBARS		H	p
14 females: Cemeteries (1,5,7)	Kruskal-Wallis	6.63	.037
18 males: Cemeteries (1,2,4,7)	Kruskal-Wallis	6.63	.085
14 females: Age Ranges (1,2,3,6,8,9)	Kruskal-Wallis	10.69	.050
18 males: Age ranges (1,2,3,4,6,7,8)	Kruskal-Wallis	5.59	.472

Part B:

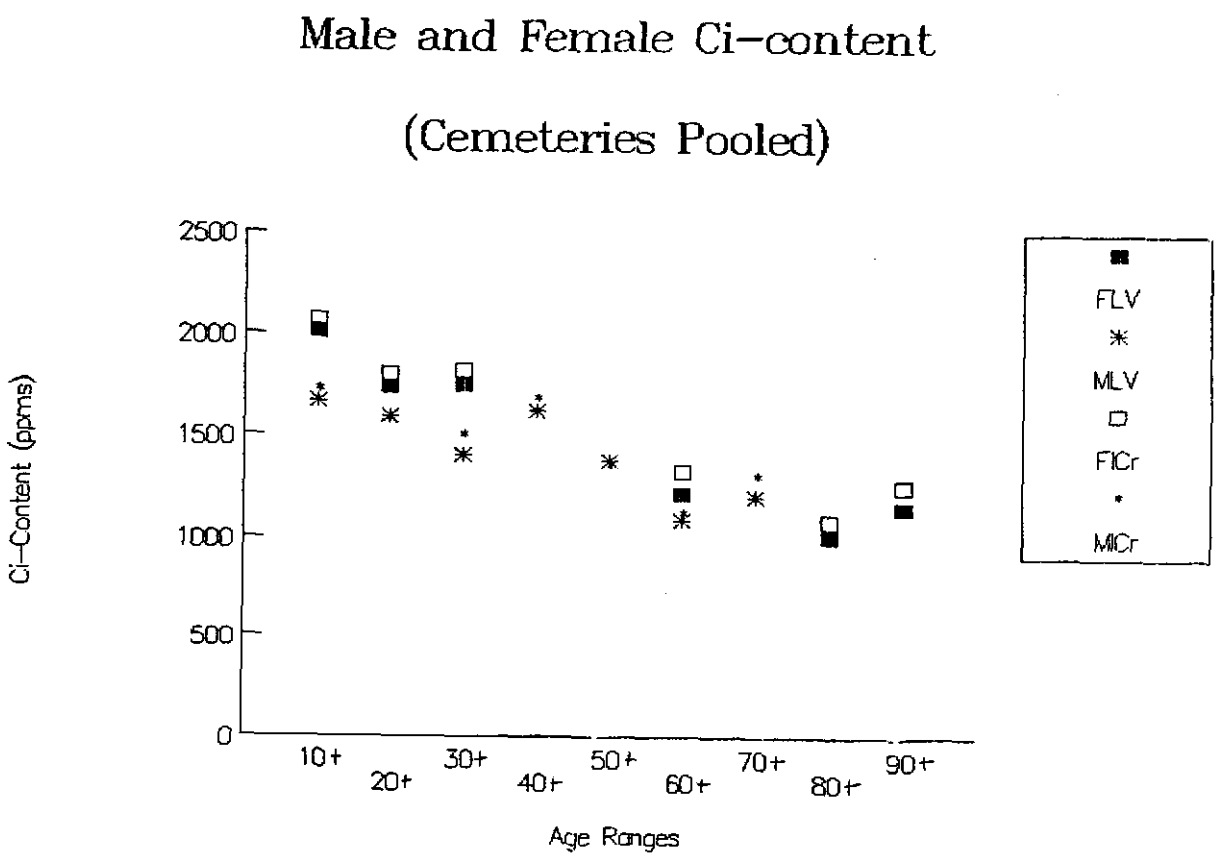
ILIAC CRESTS		H	p
14 females: Cemeteries (1,5,7)	Kruskal-Wallis	6.60	.037
19 males: Cemeteries (1,2,4,5,7)	Kruskal-Wallis	7.16	.129
14 females: Age Ranges (1,2,3,6,8,9)	Kruskal-Wallis	8.93	.013
19 males: Age Ranges (1-8)	Kruskal-Wallis	5.52	.479

This table contains the values of H that were obtained.

Cemeteries: 1=Stirrup Court; 2= London County Gaol; 4= Waterloo County Gaol; 5= Harvie; 7= St. Thomas

Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+

Figure 5:2:1- Distribution of Ci-content of Known Individuals Grouped by Sex, Age and Body Parts (Cemeteries pooled)



sexes, in agreement with previous researchers' observations (Lengyel 1968; Gedalia et al 1969). Ci-content, on its own, therefore, should not be used in order to discriminate the sex of archaeological human skeletal remains.

Furthermore, as noted in Chapter One, the assessment of the age of fragmentary human skeletal remains is virtually impossible. The results of statistical tests on the pooled series, thus far, suggests that Ci-content cannot be used to discriminate the sex of these skeletal remains because it will always be necessary to know the age of the individual at death, prior to accurately interpreting Ci-content results.

In order to further test the effects of geography on the Ci-content observed for the pooled sample, Kruskal-Wallis tests for variance were also performed on the medians of Ci-content from Table 5:2:6. These results are also presented in Table 5:2:7. When the sex of the known individuals from the pooled series is controlled, significant differences in the values of H were still obtained between the medians of Ci-content between cemeteries, regardless of body part (L= $p \leq .037$; ICr= $p \leq .037$). These tests confirm that two of the most significant variables affecting adequate interpretation of Ci-content results within the pooled series are the intrinsic age distribution within each cemetery and possibly regional geography.

Figure 5:2:2- Distribution of Ci-content Per Body Part Across Cemeteries

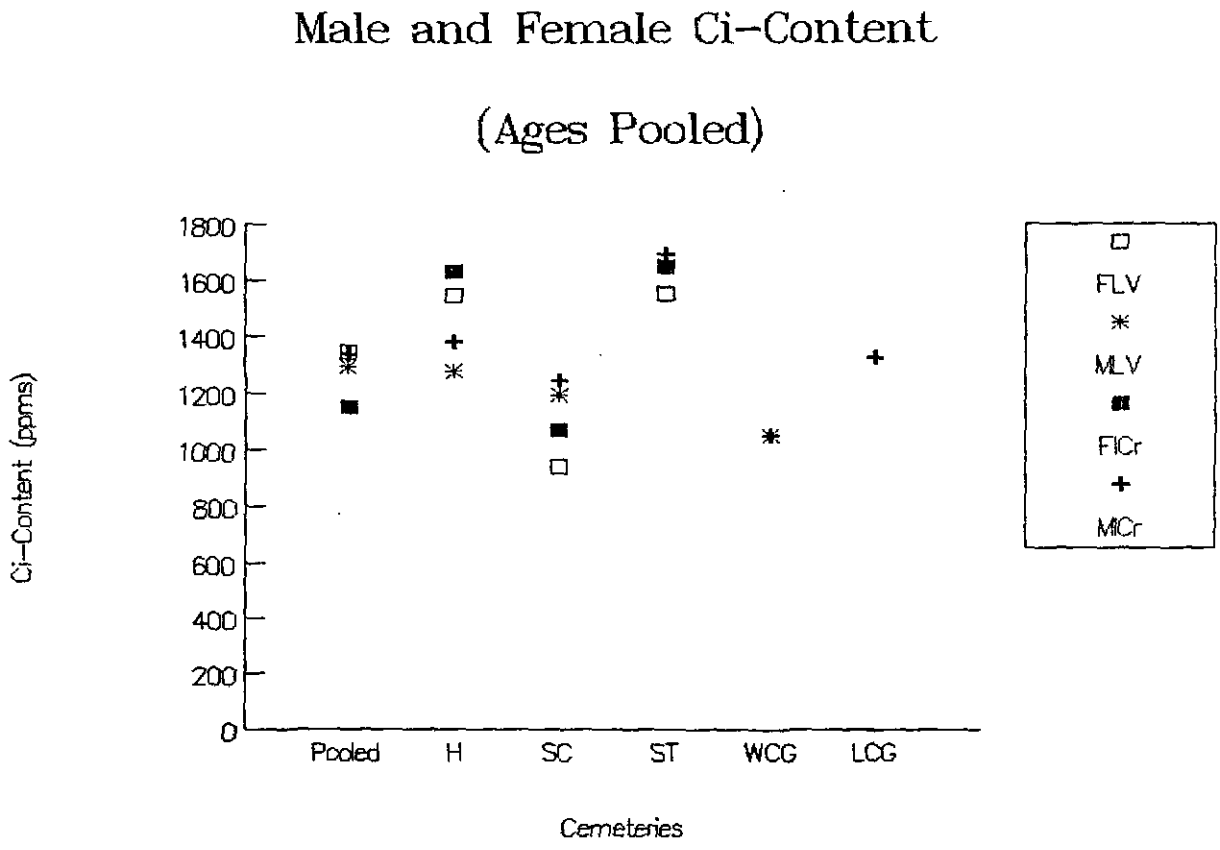


Figure 5:2:2 illustrates the distribution of Ci-content across each of the cemeteries and for both body parts tested. To further test the specific effects of geographic distribution on the Ci-content observed within the pooled sample, Mann-Whitney rank sum tests for difference were performed in order to test the null hypothesis that there is no difference in the medians of Ci-content of trabecular bone between pairs of individual cemetery samples. These results are presented in Table 5:2:8.

The highest significant values of W that were obtained for the pooled series were observed between comparisons of the medians of Ci-content from the Stirrup Court cemetery located in London, Ontario and the St. Thomas Church cemetery located in Belleville, Ontario, regardless of sex distribution or body part ($p \leq .005$). These cemeteries are located the furthest apart geographically (see Figure 3:1:1). They are also, however, the largest individual samples within the series.

The next highest significant value of W obtained occurred between comparisons of pairs of medians of Ci-content from the Stirrup Court and the Harvie family cemetery, the latter located near Kitchener-Waterloo, Ontario ($p \leq .02$). These cemeteries are also situated closer to each other. Nevertheless, these samples are also the next largest in size within the pooled series.

No significant differences in values of W were

Table 5:2:8-Results of Mann-Whitney Rank Sum Tests For Difference Between Pairs of Cemetery Samples (Ages Pooled)

Part A:

LUMBARS			
Group	All Individuals	Males Only	Females Only
SC:WCG	n/a	8.0	n/a
SC:H	27.0**	11.0	6.0***
SC:ST	31.0*	9.0***	6.0
WCG:H	n/a	3.0***	n/a
WCG:ST	n/a	5.0	5.0
H:ST	79.0	19.0	24.0

Part B:

ILIAC CRESTS			
Group	All Individuals	Males Only	Females Only
SC:WCG	n/a	10.0	n/a
SC:H	27.0**	11.0	6.0***
SC:ST	29.0****	10.0***	6.0
WCG:H	n/a	3.0***	n/a
WCG:ST	n/a	4.0	n/a
H:ST	80.0	25.0	18.0

**** $p \leq .004$ *** $p \leq .10$ ** $p \leq .02$ * $p \leq .005$ (Two-tailed)
This table lists the values of W that were obtained.
SC= Stirrup Court; WCG = Waterloo County Gaol; H= Harvie; ST= St. Thomas

obtained, however, when the medians of Ci-content of the Harvie family and St. Thomas Church cemeteries were compared to each other. This might be because these two samples have similar age and sex distribution. Sample size and intrinsic age and sex distribution between the cemeteries, therefore, could also be affecting adequate assessment of the Kruskal-Wallis statistical test results.

When the Ci-content of either sex within the pooled series were compared, less, but still significantly different values of W were obtained between the cemeteries, regardless of body part ($p \leq .1$). This suggests that the significant and non-significant differences in Ci-content of whole trabecular bone observed within this study, thus far, is probably due to variable sample size, physiological age and sex distribution between each of the cemeteries and/or to differential diagenetic factors operating between the cemeteries. Physiological age, sex, geographic distribution, sample size and/or combinations of all of these variables, therefore, are probably the most important factors affecting adequate interpretation of the Ci-content results observed within this study. In short, the results thus far suggest that the samples within the archaeological series should not be pooled.

To summarize, the results of statistical tests on the pooled series suggest that Ci-content of trabecular bone from either skeletal region can be used. Nevertheless,

regardless of body part selected, trabecular bone citrate cannot be used, alone, in order to assess the sex of human skeletal remains. Ci-content also cannot be relied upon as an alternate chemical means for the determination of the sex of fragmentary and hence isolated human skeletal remains since age assessment of these remains is both difficult and usually impossible. Finally, the five cemeteries containing known individuals within this study cannot be pooled as a consequence of the possible effects of geography and intrinsic age and sex distribution. They must be analyzed separately in order to further assess the question whether or not Ci-content can be used as a sex discriminator for 'unknowns' derived from within relatively complete archaeological human skeletal series.

2. Individual Cemeteries

Figure 5:2:3 illustrates the distribution of Ci-content observed for the known individuals grouped by body part and individual cemeteries. Table 5:2:9 lists the medians, means, standard deviations and standard error of the means obtained for the Ci-content of lumbar and iliac crest samples from within each of the five cemeteries.

The Ci-content of the Harvie cemetery lumbar (n=8) and iliac crest (n=8) samples ranged from 1,144 $\pm$ 5.5 to 1,779 $\pm$ 35.5 ppms in whole trabecular bone with an overall mean of 1,378 $\pm$ 241.8 ppms and from 1,252 $\pm$ 4.0 to 1,870 $\pm$ 48.0 ppms in whole bone with an overall mean of 1,474.8 $\pm$ 227.4

Figure 5:2:3- Distribution of Ci-content of Known Individuals For Each Cemetery

Waterloo County Gaol Cemetery

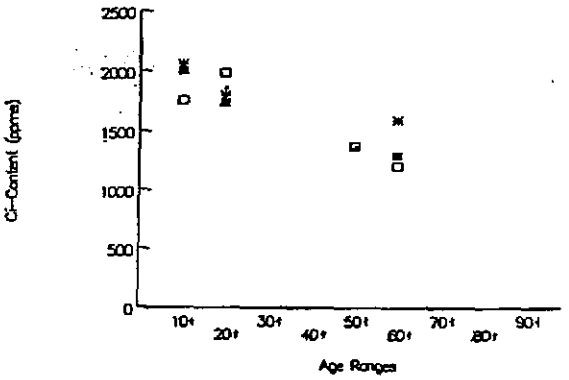
Ci-Content

London County Gaol Cemetery

Ci-Content

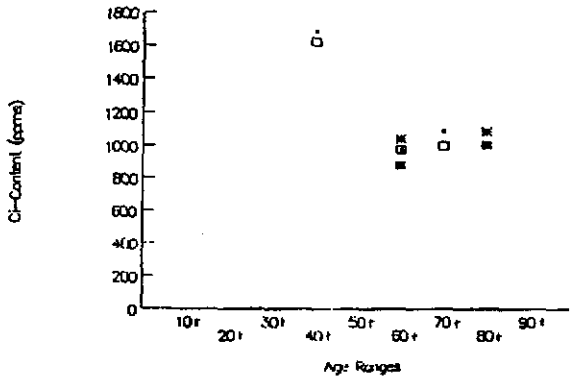
St. Thomas Cemetery

Ci- Content



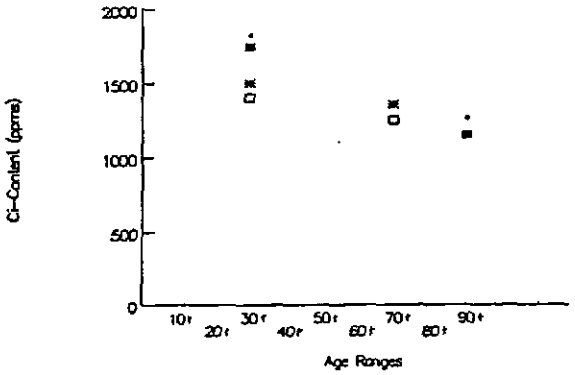
Stirrup Court Cemetery

Ci-Content



Harvie Cemetery

Ci-Content



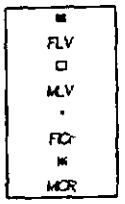


Table 5:2:9- Descriptive Statistics of Individual Cemeteries (Sexes and Ages Pooled)

CEMETERY	BODY PART	NUMBER	MEDIAN ppm	MEAN ppm	SD ppm	SE ppm
H	LV	8	1293.5	1378.5	241.8	85.5
	ICr	8	1386.0	1474.8	227.4	80.4
SC	LV	6	998.0	1077.0	270.0	110.0
	ICr	6	1060.0	1155.0	264.0	108.0
ST	LV	16	1673.5	1618.6	356.2	89.0
	ICr	16	1743.5	1685.6	362.8	90.7
WCG	LV	2	n/a	1047.5	24.7	17.5
	ICr	2	n/a	1048.5	31.8	22.5

LV= Lumbar Vertebra; ICr= Iliac Crest; H= Harvie; SC= Stirrup Court; ST= St. Thomas; WCG= Waterloo County Gaol

ppms respectively. The Ci-content of Stirrup Court lumbar (n=6) and iliac crest (n=6) samples was lower than the Ci-content obtained for both the Harvie and St. Thomas Church cemetery samples ranging from 875 ± 11.5 to 1,620 ± 11.5 ppms in whole trabecular bone with an overall mean of 1,070.0 ± 270.0 ppms and from 967 ± 13.5 to 1,683 ± 11.0 ppms in whole bone with a mean of 1,060 ± 108.0 ppms, respectively.

The Ci-content of St. Thomas Church lumbar (n= 16) and iliac crest (n=16) samples was the highest observed for all of the cemeteries tested and more variable ranging from 985 ± 9.0 to 2,009 ± 16.5 ppms in whole trabecular bone with an overall mean of 1,618.6 ± 356.2 ppms and from 1,043 ± 12.5 to 2,097 ± 7.5 ppms in whole bone with a mean of 1,685.6 ± 362.8 ppms, respectively.

The Ci-content of the Waterloo County Gaol lumbar (n=2) and iliac crest (n=2) samples observed was similar to the Stirrup Court sample ranging from 1,030 ± 3.5 to 1,065 ± 48.5 ppms with an overall mean of 1,047.5 ± 24.7 ppms in whole trabecular bone and from 1,026 ± 2.5 to 1,071 ± 1.5 ppms in whole bone with a mean of 1,048.5 ± 31.8 ppms, respectively.

As noted in Chapter Three, both anatomical regions of the skeleton of the London County Gaol individual selected for analysis in this study were not recovered. Consequently, the London County Gaol sample was not included in many of

the following statistical comparative analyses.

Mann-Whitney rank sum tests for differences between pairs of samples were performed on the data listed in Table 5:2:9 to further confirm/disconfirm the observation above that there is no difference in the Ci-content observed for trabecular bone between either body part tested within each of the cemeteries. These results are listed in Table 5:2:10. No significant differences in the values of W were observed thus confirming that the Ci-content of trabecular bone from either region of the skeletons of known individuals within each of the cemeteries statistically tested can be used.

Table 5:2:11 lists the medians, means, standard deviations and standard error of the means of the Ci-content obtained for lumbar and iliac crest samples of all of the known males and females from within each of the cemeteries. The Ci-content of Harvie male lumbar (n=5) and iliac crest (n=5) samples ranged from 1,168 $\pm$ 11.0 to 1,400 $\pm$ 35.0 ppms in whole trabecular bone with an overall mean of 1,277.8 $\pm$ 85.7 ppms and from 1,285 $\pm$ 12.5 to 1,502 $\pm$ 7.5 ppms in whole bone with an overall mean of 1,381.4 $\pm$ 80.7 ppms, respectively. Harvie female lumbar (n=3) and iliac crest (n=3) Ci-content was slightly higher and more variable than Harvie male Ci-content ranging from 1,779 $\pm$ 35.5 to 1,144 $\pm$ 5.5 ppms in whole trabecular bone with an overall mean of 1,546.0 $\pm$ 350.0 ppms and from 1,252 $\pm$ 4.0 to 1,879 $\pm$ 48.0 ppms in whole bone with a mean of 1,630.0 $\pm$ 332.0 ppms,

Table 5:2:10-Results of Mann-Whitney Rank Sum Tests For Difference in Ci-content Between Pairs of Cemetery Samples (Ages Pooled)

Group Only	INDIVIDUAL CEMETERIES					
	All Individuals		Males Only		Females	
	W /	p	W /	p	W /	p
HLV:HICr	57.0	.270	20.0	.143	9.0	.662
SCLV:SCICr	30.0	.173	10.0	1.00	6.0	.180
STLV:STICr	156.6	.342	62.0	.285	59.0	.372

This table lists the values of W that were obtained.(Two-tailed)

LV= Lumbar vertebra; ICr= Iliac crest; H= Harvie; SC= Stirrup Court; ST= St. Thomas

Table 5:2:11- Descriptive Statistics of Ci-content of Males and Females Within Individual Cemeteries (Ages Pooled)

CEMETERY	BODY PART	SEX	NUMBER	MEDIAN *ppm*	MEAN *ppm*	SD *ppm*	SE *ppm*
H	LV	F	3	1716.0	1546.0	350.0	220.0
	LV	M	5	1292.0	1277.8	85.7	38.3
	ICr	F	3	1769.0	1630.0	332.0	191.0
	ICr	M	5	1363.0	1381.4	80.7	36.1
SC	LV	F	3	1001.0	960.0	73.6	42.5
	LV	M	3	994.0	1195.0	369.0	213.0
	ICr	F	3	1041.0	1066.7	48.0	27.7
	ICr	M	3	1079.0	1243.0	385.0	222.0
ST	LV	F	8	1606.0	1550.0	333.0	118.0
	LV	M	8	1742.0	1652.0	366.0	129.0
	ICr	F	8	1679.0	1629.0	344.0	121.0
	ICr	M	8	1792.0	1704.0	371.0	131.0
WCG	LV	M	2	n/a	1048.5	31.8	22.5
	ICr	M	2	n/a	1047.5	24.7	17.5

LV= Lumbar Vertebra; ICr= Iliac Crest; M= Males; F= Females; H= Harvie; SC= Stirrup Court; St= St. Thomas; WCG= Waterloo County Gaol

respectively.

Male Ci-content of Stirrup Court lumbars (n=3) and iliac crest (n=3) samples was lower than the Harvie or St. Thomas Church males ranging from 970 ± 18.5 to 1,620 ± 11.5 ppms in whole trabecular bone with an overall mean of 1,195.0 ± 369.0 ppms and from 967 ± 13.5 to 1,683 ± 11.0 ppms in whole bone with a mean of 994.0 ± 213.0 ppms, respectively. The Ci-content of Stirrup Court female lumbars (n=3) and iliac crests (n=3) was lower but less variable than the Harvie and St. Thomas Church females ranging from 875 ± 11.5 to 1,004 ± 14.0 ppms in whole trabecular bone with a mean of 960.0 ± 73.5 ppms and 1,037 ± 9.0 to 1,122 ± 7.5 ppms in whole bone with an overall mean of 1,066.7 ± 48.0 ppms, respectively.

The Ci-content of St. Thomas Church male lumbar (n=8) and iliac crest (n=8) samples was the highest of all of the males observed in the study ranging from 985 ± 9.0 to 2,008 ± 12.5 ppms in whole trabecular bone with an overall mean of 1,652.0 ± 366.0 ppms and from 1,043 ± 12.5 to 2,097 ± 7.5 ppms in whole bone with an overall mean of 1,704.0 ± 371.0 ppms, respectively. Female Ci-content of St. Thomas Church lumbars (n=8) and iliac crests (n=8) also was higher than the Harvie and Stirrup Court females ranging from 1,131 ± 16.0 to 2,009 ± 16.5 ppms in whole trabecular bone with an overall mean of 1,550.5 ± 12.5 ppms and from 1,193 ± 12.5 to 2,064 ± ppms in whole bone with a mean of 1,629.0 ± 344.0

ppms, respectively.

As noted in Chapter Three, testing for Ci-content of the Waterloo County Gaol sample was limited to males only. The Ci-content of these lumbar and iliac crest samples (n=2) was similar to the Ci-content observed for Stirrup Court males but also was the least variable of all the males in the study ranging from 1,030 ± 3.5 ppms to 1,065 ± 48.5 ppms in whole trabecular bone with an overall mean of 1,048.5 ± 31.8 ppms and 1,026 ± 2.5 to 1071 ± 1.5 ppms in whole bone with an overall mean of 1,047.5 ± 24.7 ppms, respectively. Furthermore, this was the only case where lumbar Ci-content was higher than iliac crest Ci-content within either the archaeological series tested within this study.

To further test the null hypothesis cited above that there is no difference in the Ci-content observed between the sexes within each of the cemeteries, Mann-Whitney rank sum tests for difference between pairs of male and female body parts was performed on the data listed in Table 5:2:11. These results are presented in Table 5:2:12. No significant differences in the values of W between the sexes was observed within either the Harvie, Stirrup Court or St. Thomas Church cemetery samples. This result thus confirms that Ci-content, alone, cannot be used in order to delineate the sex of 'unknowns' derived from within relatively complete human skeletal series.

Tables 5:2:13 and 5:2:14 lists the means, medians,

Table 5:2:12-Results of Mann-Whitney Tests For Difference in Ci-content Between Pairs of Male and Female Lumbar and Iliac Crest Samples (Ages Pooled)

INDIVIDUAL CEMETERIES		
Group	W	p
HFLV:HMLV	16.0	.551
HFICr:HMICr	16.0	.551
SCFLV:SCMLV	91.9	1.000
SCFICr:SCMICr	10.0	1.000
STFLV:STMLV	61.0	.494
STFICr:STMICr	65.0	.792

This table contains the values of W obtained. (Two-tailed) LV= Lumbar vertebra; ICr= Iliac crest; H= Harvie; SC= Stirrup Court; ST= St. Thomas

Table 5:2:13- Descriptive Statistics of Ci-content Observed For Lumbars Between Individual Cemeteries Grouped by Sex and Age Ranges

CEMETERY	NUMBER	SEX	AGE RANGE	SINGLE OBS. *	MEDIAN ppm	MEAN ppm	SD ±	MIN ppm	MAX ppm
H	1	M	3	1,400	n/a	n/a	n/a	n/a	n/a
	4	M	7	n/a	1,263	1,247	51.6	1,168	1,295
	2	F	3	n/a	1,747	1748	44.5	1,716	1,779
	1	F	9	1,144	n/a	n/a	n/a	n/a	n/a
SC	1	M	4	1,620	n/a	n/a	n/a	n/a	n/a
	1	M	6	970	n/a	n/a	n/a	n/a	n/a
	1	M	7	994	n/a	n/a	n/a	n/a	n/a
	1	F	6	875	n/a	n/a	n/a	n/a	n/a
	2	F	8	n/a	1,003	1,002	1.5	1,001	1,004
ST	2	M	1	n/a	1,983	1,983	25.0	1,995	2,008
	3	M	2	n/a	1,723	1,767	75.8	1,717	1,736
	2	M	5	n/a	1,372	1,373	387.5	985	1,760
	1	M	6	1,201	n/a	n/a	n/a	n/a	n/a
	1	F	1	2,009	n/a	n/a	n/a	n/a	n/a
	3	F	2	n/a	1,643	1,738	154.3	1,616	1,956
	4	F	6	n/a	1,225	1,294	178.4	1,131	1,596
WCG	1	M	1	1,030	n/a	n/a	n/a	n/a	n/a
	1	M	2	1,065	n/a	n/a	n/a	n/a	n/a

H= Harvie; SC= Stirrup Court; ST= St. Thomas; WCG= Waterloo County Gaol; M= Males; F= Females
*= observation on one individual only within age category
Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+.

Table 5:2:14- Descriptive Statistics of Ci-content Observed For Iliac Crests Between Individual Cemeteries Grouped by Sex and Age Ranges

CEMETERY	NUMBER	SEX	AGE RANGE	SINGLE OBS.	MEDIAN ppm	MEAN ppm	SD ±	MIN ppm	MAX ppm
H	1	M	3	1,502	n/a	n/a	n/a	n/a	n/a
	4	M	7	n/a	1,348	1,351	44.4	1,285	1,409
	2	F	3	n/a	1,820	1,820	50.5	1,769	1,870
	1	F	9	1,252	n/a	n/a	n/a	n/a	n/a
SC	1	M	4	1,683	n/a	n/a	n/a	n/a	n/a
	1	M	6	967	n/a	n/a	n/a	n/a	n/a
	1	M	7	1,079	n/a	n/a	n/a	n/a	n/a
	1	F	6	1,037	n/a	n/a	n/a	n/a	n/a
	2	F	8	n/a	1,082	1,081	40.5	1,041	1,141
ST	2	M	1	n/a	2,053	2,053	62.2	2,009	2,097
	3	M	2	n/a	1,795	1,851	102.8	1,789	1,970
	2	M	5	n/a	1,354	1,354	440.0	1,043	1,665
	1	M	6	1,268	n/a	n/a	n/a	n/a	n/a
	1	F	1	2,064	n/a	n/a	n/a	n/a	n/a
	3	F	2	n/a	1,679	1,679	26.9	1,660	1,698
	4	F	6	n/a	1,285	1,393	281.0	1,193	1,809
WCG	1	M	1	1,071	n/a	n/a	n/a	n/a	n/a
	1	M	1	1,026	n/a	n/a	n/a	n/a	n/a
LCG	1	M	1	1,322	n/a	n/a	n/a	n/a	n/a

H= Harvie; SC= Stirrup Court; ST= St. Thomas; WCG= Waterloo County Gaol; LCG= London County Gaol; M= Males; F= Females
*= observation on one individual only within age category
Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+.

standard deviations and ranges of Ci-content obtained for male and female lumbar and iliac crest samples, respectively, grouped by individual cemeteries and separated by age range increments listed above. Kruskal-Wallis tests for variance were performed on the data from these tables in order to further test the null hypothesis that physiological age has no effect on the Ci-content of males and/or females within each cemetery. The results of these tests on both body parts for each cemetery is presented in Tables 5:2:15 and 5:2:16, respectively.

No significant differences in the values of H were obtained when the male medians of Ci-content between age categories within the Harvie and Stirrup Court cemeteries were compared, regardless of body part. This would seem to confirm the observation above that male Ci-content is less affected than female Ci-content by age dependent hormonal relationships within each of the cemeteries tested. However, significant differences in the values of H were obtained when the medians of the male age ranges from the St. Thomas Church cemetery were compared to each other, regardless of body part.

Similar conflicting results for values of H were also obtained between female age range categories within each of the cemeteries. On the one hand, significant differences in the values of H were obtained for the age ranges listed in the table for females of both the Harvie and St. Thomas

Table 5:2:15-Results of Kruskal-Wallis Tests For Variance in Ci-content of Male and Female ICr Samples Within Cemeteries Grouped by Age Ranges.

CEMETERY	H	p
Harvie		
3 Females: Age Ranges (3,9) Kruskal-Wallis	1.50	.021
5 Males: Age Ranges (3,7) Kruskal-Wallis	2.00	.158
Stirrup Court		
3 Females: Age Ranges (6,8) Kruskal-Wallis	1.50	.221
3 Males: Age Ranges (4,6,7) Kruskal-Wallis	2.00	.368
St.Thomas		
8 Females: Age Ranges (1,2,6) Kruskal-Wallis	5.83	.050
8 Males: Age Ranges (1,2,5,6) Kruskal-Wallis	4.81	.087

This table contains the values of H obtained.
Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+

Table 5:2:16-Results of Kruskal-Wallis Tests For Variance in Ci-content of Male and Female Lumbar Samples Within Cemeteries Grouped by Age Ranges.

CEMETERY		H	p
Harvie			
3 Females: Age Ranges (3,9)	Kruskal-Wallis	1.50	.021
5 Males: Age Ranges (3,7)	Kruskal-Wallis	2.00	.158
Stirrup Court			
3 Females: Age Ranges (6,8)	Kruskal-Wallis	1.50	.221
3 Males: Age Ranges (4,6,7)	Kruskal-Wallis	2.00	.368
St.Thomas			
8 Females: Age Ranges (1,2,6)	Kruskal-Wallis	3.89	.044
8 Males: Age Ranges (1,2,5,6)	Kruskal-Wallis	6.25	.090

This table contains the values of H obtained.
Age Ranges: 1= 10-19; 2= 20-29; 3= 30-39; 4= 40-49; 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+

Church cemeteries, regardless of body part. On the other hand, no significant differences in the values of H were obtained when the age ranges listed in the table for females within the Stirrup Court cemetery were compared. Two possible interpretations come to mind which may explain these contradictory observations of Ci-content between age categories for both of the sexes within each of the cemeteries.

First, the results appear to suggest either that the effects of physiological age on Ci-content of trabecular bone tissue may be masked and/or exaggerated by differential decomposition within each of the cemeteries or that within the Stirrup Court sample, it is actually a true reflection of non-significant difference in Ci-content which might be expected to exist between elderly female age categories as outlined in Chapter Two. Small sample sizes within each of the cemeteries not only prevents further statistical testing of this trend, it also could be affecting adequate assessment of the statistical results.

If the latter interpretation is correct, the results of the statistical analyses in part two of this section confirms that Ci-content cannot be used to discriminate the sex of 'unknowns' within specific archaeological cemetery contexts, unless the ages of the individuals can be accurately assessed.

If the former interpretation is correct, the

conflicting statistical results suggest that differential diagenesis might also possibly be operating both within and between each of the cemeteries. As noted in Chapter Three, the Waterloo County Gaol males were limed, following their interment. The Ci-content of the age ranges listed for these individuals (10-19, and 20-29, respectively) is considerably lower and less variable than the Ci-content observed for all other males of corresponding age within the archaeological series. This suggests that the effect of the practice of liming following interment was uniform within this particular cemetery. Prior knowledge of this activity , however, was essential in order to accurately interpret the lower male Ci-content results.

Comparative chemical analysis might have assisted in delineating possible environmental effects of differential diagenesis on trabecular bone Ci-content across separate samples within all of the cemeteries. Soil samples were not systematically collected during the exhumation of any of the individuals within the archaeological series. The results obtained on the archaeological series of this study suggest that it cannot be assumed diagenetic factors operate with the same intensity on Ci-content, both within and/or between each of the cemeteries, as previously suggested (Lengyel 1968). Ci-content, therefore, should not be relied upon at all as an alternate chemical means to determine the sex of human skeletal remains derived from archaeological contexts,

unless these problems can be resolved.

5:3- Modern Series

Figure 5:3:1 illustrates the distribution of Ci-content of whole trabecular bone observed for both lumbar and iliac crest samples from within the modern series. As noted in Chapter Three, bone citrate content results on this control population were not as comprehensive as those observed for the archaeological series due to limitations which occurred during the initial stages of their collection. The modern series is examined in detail in this section in order to resolve some of the confusion seen within the statistical analyses of the archaeological series above.

The means, medians, standard deviations and standard error of the means of Ci-content observed for both skeletal regions of the modern series are listed in Table 5:3:1. The Ci-content of lumbar samples (n=25) ranged from 4,129 $\pm$ 6.8 to 16,615 $\pm$ 5.0 ppms in whole trabecular bone with an overall mean of 13,607 $\pm$ 2,654 ppms. The Ci-content of iliac crest samples (n=25) was slightly higher ranging from 4,398 $\pm$ 3.3 to 16,864 $\pm$ 2.1 ppms in whole bone with an overall mean of 13,947 $\pm$ 2,695 ppms.

Mann-Whitney rank sum tests for difference between pairs of body parts were performed on the data listed in Table 5:3:1 in order to test the null hypothesis that the Ci-content of whole trabecular bone does not vary between anatomical regions of the skeleton. These results are

Figure 5:3:1- Distribution of Ci-content Observed For The Modern Series Grouped by Body Part

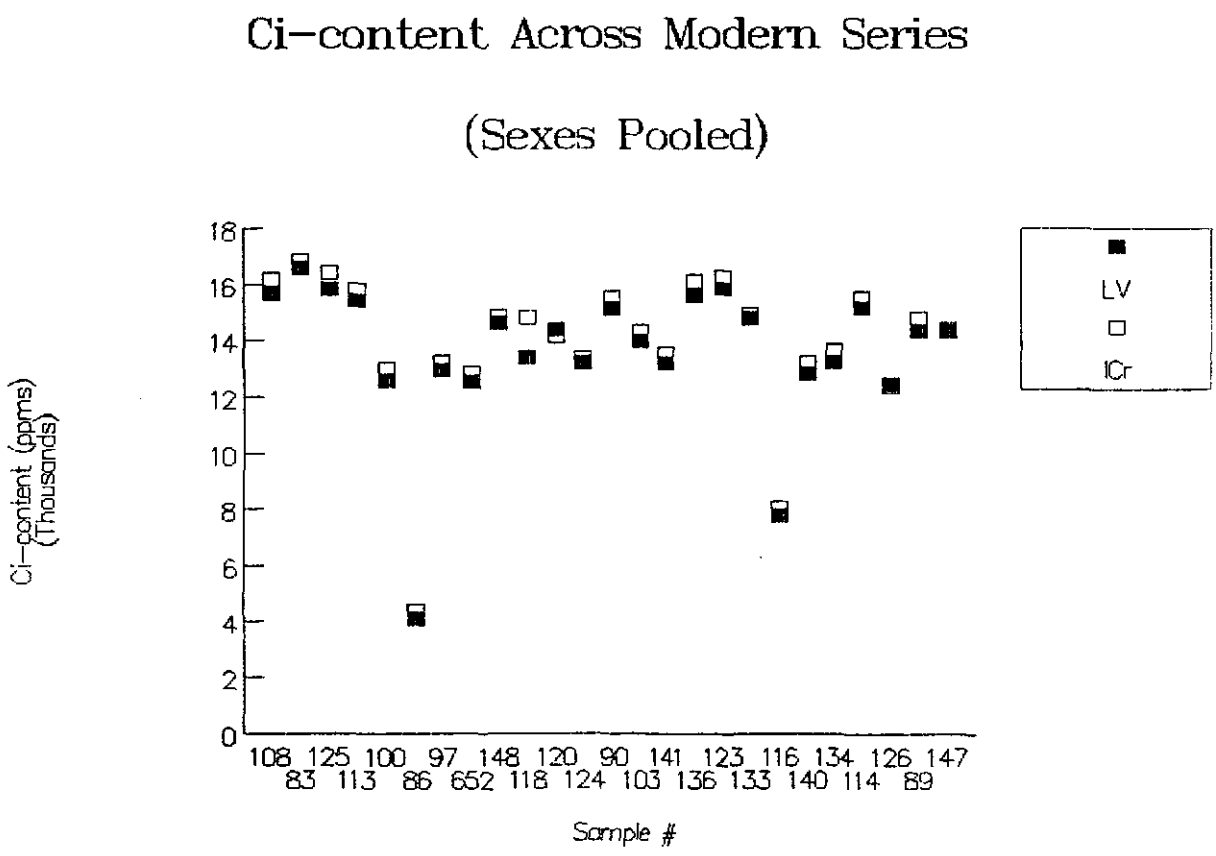


Table 5:3:1 - Descriptive Statistics of Ci-content Obtained For the Modern Series (Sexes and Ages Pooled)

BODY PART	NUMBER	MEDIAN ppm	MEAN ppm	SD ±	SE
LV	25	14,344	13,607	2,654	531
ICr	25	14,424	13,947	2,695	539

LV= Lumbar vertebra; ICr= Iliac crest

Table 5:3:2- Results Mann-Whitney Rank Sum Tests For Difference Between Pairs of Modern Series Body Parts

GROUP	All Individuals		Males Only		Females Only	
	W	p	W	p	W	p
LV:ICr	593.0	.393	333.0	1.00	52.5	1.00

This Table contains the value of W obtained. (Two-tailed)
LV= Lumbar vertebra; ICr= Iliac crest

presented in Table 5:3:2. No significant differences in the values of W were obtained when the medians of Ci-content for each body part of all of the individuals tested in the Modern series were compared ($p \leq .393$).

No significant differences in the values of W were obtained when the body parts of the individual sexes were compared (M= $p \leq 1.00$; F= $p \leq 1.00$). These results substantiate the observations made within the archaeological series that the Ci-content in whole trabecular bone from either skeletal region could be used.

Table 5:3:3 *lists the medians, means,* standard deviations and standard error of the means of Ci-content observed for males and females from the *modern series,* grouped by body part. The Ci-content of male lumbars (n=18) ranged from 4,126 $\pm$ 3.2 to 16,615 $\pm$ 0.7 ppms in whole trabecular bone with an overall mean of 13,251 $\pm$ 2,999 ppms. Female lumbar (n=7) Ci-content was slightly higher and less variable than male lumbar Ci-content ranging from 12,814 $\pm$ 4.4 to 15,860 $\pm$ 26.9 ppms in whole trabecular bone with an overall mean of 14,523 $\pm$ 1,154 ppms.

The Ci-content of male iliac crest samples (n=18) was higher and more variable than corresponding male lumbar samples ranging from 4,396 $\pm$ 1.6 to 16,862 $\pm$ 2.0 ppms in whole trabecular bone with an overall mean of 13,562 $\pm$ 3,032 ppms. Female Ci-content observed for corresponding iliac crest samples (n=7) was also slightly higher than the Ci-

Table 5:3:3- Descriptive Statistics of Ci-content Obtained For Males and Females of the Modern Series (Ages Pooled)

BODY PART	NUMBER	SEX	MEDIAN ppm	MEAN ppm	SD ±	SE
LV	18	M	13,679	13,251	2,999	707
	7	F	14,618	14,523	1,154	436
ICr	18	M	14,230	13,562	3,032	715
	7	F	14,942	14,939	1,188	449

LV= Lumbar Vertebra; ICr= Iliac crest; M= Males; F=Females

Table 5:3:4- Results of Mann-Whitney Rank Sum Tests For Difference in Ci-content Between Pairs Male and Female Body Parts

GROUP	W	p
FLV:MLV	216	0.289
FICR:MICr	110	0.262

This Table contains the values of W that were obtained. (Two-tailed)
LV= Lumbar vertebra; ICr= Iliac crest; M= Males; F= Females

content observed for female lumbar samples and less variable than male iliac crest samples ranging from 13,191 ± 0.5 to 16,257 ± 1.0 ppms in whole trabecular bone with an overall mean of 14,939 ± 1,188 ppms.

Mann-Whitney rank sum tests for difference in Ci-content between pairs of male and female body parts were performed on the data of Table 5:3:3 in order to test the null hypothesis cited above that Ci-content does not vary between the sexes, within the modern series. These results are presented in Table 5:3:4. No significant differences in the values of W were obtained (LV= $p \leq .289$; ICr= $p \leq .262$) thus substantiating the observation made for the archaeological series that Ci-content, alone, cannot be used to determine the sex of human skeletal remains.

Kruskal-Wallis tests for variance in Ci-content were also performed on the data from Table 5:3:3 in order to further test the null hypothesis that physiological age and/or sex has no effect on the Ci-content observed within the modern series. These results are presented in Table 5:3:5.

No significant differences in the values of H were obtained when the medians of Ci-content grouped by sex, were compared, regardless of body part. Significant differences in Ci-content were obtained when the modern series was grouped by age range increments for both body parts tested (L= $p \leq .05$; ICr= $p \leq .05$). This result, therefore, also

Table 5:3:5- Results of Kruskal-Wallis Tests For Variance in Ci-content Between Body Parts From the Modern Series Grouped by Sex and Age Ranges

LUMBAR	H	p
25 Samples:Sex (1,2) Kruskal-Wallis	1.19	0.276
25 Samples:Age Ranges (5,6,7,8,9) Kruskal-Wallis	9.23	0.050
ILIAC CREST	**H**	**p**
25 samples:Sex (1,2) Kruskal-Wallis	1.32	0.250
25 samples:Age Range (5,6,7,8,9) Kruskal-Wallis	8.86	0.050

This table contains the values of H obtained.
Sex: 1= Females; 2= Males
Age Ranges: 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9=90+

substantiates the observation within the archaeological series that physiological age does affect the Ci-content of trabecular bone probably due to differential hormonal fluctuations known to exist between the sexes as discussed in Chapter Two. Small sample sizes within individual age increment categories, however, might also be affecting adequate interpretation of the Kruskal-Wallis test results.

Table 5:3:6 lists the medians, means and ranges of Ci-Ci-content observed for each age range grouped by sex and body part. Krusgal-Wallis tests for variance were performed on the data from this table in order to further test the effects of age on Ci-content deposition within the modern series. These results are presented in Table 5:3:7.

Significant differences in the values of H were obtained for both sexes within the modern series. Physiological age probably does affect interpretation of bone citrate deposition of both sexes, regardless of health-status. Figure 5:3:2 illustrates this distribution of Ci-content between individuals within the modern series separated by skeletal part, sex and age ranges.

In general, Ci-content decreases as individuals' ages increase within the modern series as was the case in the archaeological series. Although Ci-content does vary between the sexes, regardless of skeletal part, the differences, however, are not statistically significant. Small sample sizes within each age increment category of the

Table 5:3:6- Descriptive Statistics of Modern Series Grouped by Body Part, Sex and Age Ranges

BODY PART	NUMBER	SEX	AGE	SINGLE OBS. *	MEDIAN ppm	MEAN ppm	SD ±	MIN ppm	MAX ppm
LV	3	M	5	n/a	15,853	16,056	491	15,698	16,615
	5	M	6	n/a	12,582	11,535	4313	12,540	15,453
	6	M	7	n/a	13,679	13,882	783	13,181	15,148
	2	M	8	n/a	11,285	11,285	4958	7,779	14,791
	2	M	9	n/a	13,411	13,411	1358	12,451	14,372
	1	F	6	14,619	n/a	n/a	n/a	n/a	n/a
	2	F	7	n/a	15,732	15,732	181	15,604	15,860
	3	F	8	n/a	13,241	13,744	1260	12,814	15,178
	1	F	9	14,344	n/a	n/a	n/a	n/a	n/a
ICr	3	M	5	n/a	16,421	16,472	367	16,134	16,862
	5	M	6	n/a	12,983	11,788	4334	12,370	15,817
	6	M	7	n/a	14,230	14,197	803	13,227	15,532
	2	M	8	n/a	11,455	11,455	4804	8,058	14,852
	2	M	9	n/a	13,833	13,833	1395	12,846	14,819
	1	F	6	14,942	n/a	n/a	n/a	n/a	n/a
	2	F	7	n/a	16,233	16,233	34	16,257	16,209
	3	F	8	n/a	13,634	14,119	1243	13,196	15,531
	1	F	9	14,807	n/a	n/a	n/a	n/a	n/a

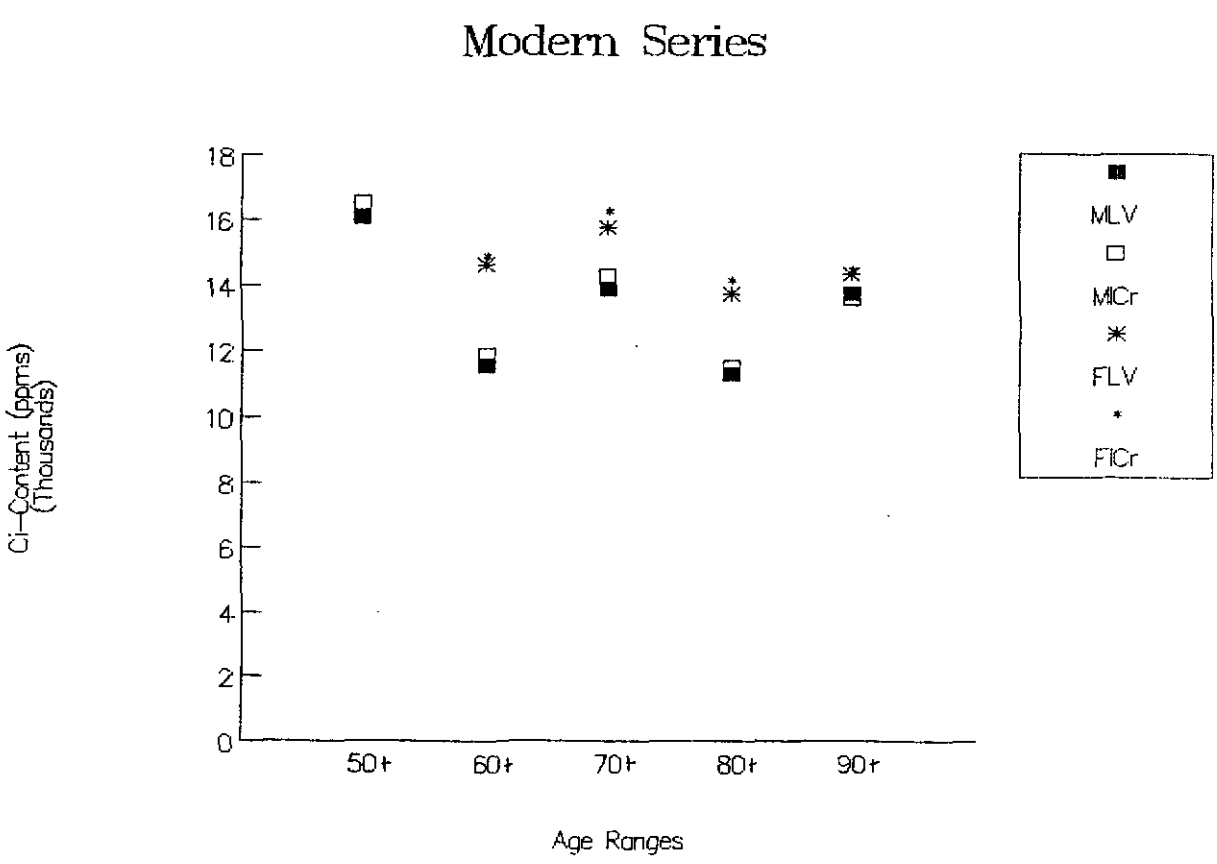
LV= Lumbar vertebra; ICr= Iliac crest; M= Males; F= Females;
Age Ranges: 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9= 90+

Table 5:3:7- Results of Kruskal-Wallis Tests For Variance in Ci-content of Male and Female Lumbars and Iliac Crests From the Modern Series Grouped by Age Ranges.

LUMBAR	H	p
18 Males: Age Range (5,6,7,8,9) Kruskal Wallis	8.60	0.073
7 Females: Age Range (6,7,8,9) Kruskal-Wallis	4.08	0.050
ILIAC CREST	**H**	**p**
18 Males: Age Range (5,6,7,8,9) Kruskal-Wallis	8.28	0.083
7 Females: Age Range (6,7,8,9) Kruskal-Wallis	4.04	0.051

This table contains the values of H obtained.
Age Ranges: 5= 50-59; 6= 60-69; 7= 70-79; 8= 80-89; 9=90+

Figure 5:3:2- Distribution of Ci-content of Modern Series Grouped by Body Part, Sex and Age Ranges



modern series again prevents further testing of this trend.

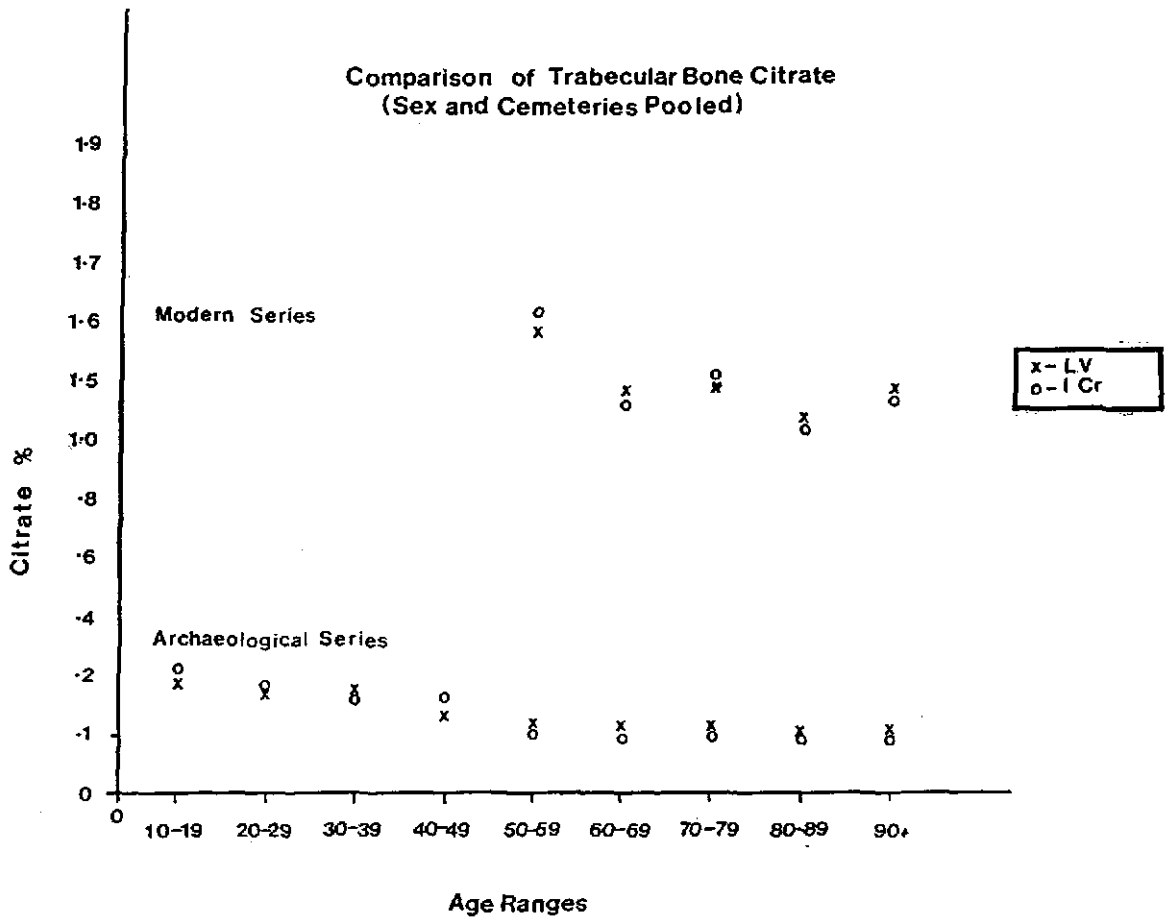
To summarize, despite the limited range of age categories available for comparison and the predominately elderly status of individuals within the modern series, the results of statistical analyses in this section also suggest that Ci-content cannot be used in order to discriminate the sex of modern human skeletal remains. The results further suggest that the conflicting results of the Krusgal-Wallis tests obtained for the archaeological series is probably due to inadequate sample sizes within each of the cemeteries.

5.4- Comparison and Summary Discussion

Figure 5:4:1 compares the results obtained for the archaeological and modern series. Statistical analyses of whole trabecular bone citrate content of individuals from the archaeological and modern series in this study indicates that trabecular bone citrate content from either anatomical region tested can be used. As well, analyses of both series suggests, in general, that as age increases, trabecular bone citrate content decreases, regardless of sex and/or body parts. This is probably related to age dependent hormonal relationships that are known to exist between the sexes as outlined in Chapter Two.

Furthermore, when assessing the deposition of trabecular bone citrate content of individuals from either archaeological or modern contexts, physiological age is an important variable to contend with, in agreement with

Figure 5:4:1 Comparison of Modern and Archaeological Series



previous reports (Lengyel 1968; Kiszeley 1974; Gibbs 1985). Unfortunately, small sample sizes within each age increment in both series of this study prevented further statistical testing of this trend.

Nevertheless, in this study, whole trabecular bone citrate content did not statistically vary between the sexes in either the archaeological or the modern series, in contrast to all previous researchers' reports (Lengyel 1968; Kiszeley 1974; Dennison 1979). Two possible hypotheses come to mind which might explain this difference.

First, the non-significant difference in citrate content between the sexes observed in this study may be due to intrinsic depositional properties of whole trabecular bone citrate, prior to and following the deaths of the individuals tested. For example, Table 5:4:1 compares the results of all previous studies of the determination of bone citrate content within archaeological and modern forensic contexts, to date.

Thunberg's (1947) pioneering analysis of the citrate content of human **cortical** archaeological bone samples utilizing the pentabromide-acetone technique is listed first. His results are compared to a report of citrate content obtained for fresh autopsied human samples (Dixon & Perkins 1952) utilizing the same method of analysis of citrate content. A mean percentage drop of approximately 98.6 % citrate content is observed for his sample, which

Table 5:4:1-Comparison of Citrate Content Results of All Previous Researchers (Cemeteries, Sexes and Ages Pooled)

DATE	BONE TYPE	BODY PART	ARCH. RANGE OBTAINED ppm	MODERN MEAN REPORTED ppm	MODERN RANGE OBTAINED ppm	% Difference I	/ II	/ III
14th-15th Century	C	U	700-1800[1]	95,000[2]	n/a	n/a	n/a	98.6
5th -4th Century	T	LV	4500-7000[3]	67,000[2]	9300-10,200[3]	41.0	85.4	91.4
3000 BC	T	LV	3500-6100[3]	67,000[2]	9300-10,200[3]	50.8	85.4	92.8
1000's of Years	C	U	700-3400[4]	95,000[2]	n/a	0.0	82.6	n/a
'Modern'	C	U	n/a	n/a	32,000-168,000[5]	n/a	n/a	n/a
1820-1920 AD	T	LV	870-2000	n/a	4,126-16,600	88.5	n/a	n/a
	T	ICr	960-2090	n/a	4,390-16,862	85.6	n/a	n/a

C= Cortical; T= Trabecular; LV= Lumbar Vertebra; U= Unspecified
I= Reported loss; II= Attained Loss; III= Modern Fresh Autopsy Loss

1. Thunberg (1947)
2. Dixon & Perkins (1952)
3. Lengyel (1968)
4. Dennison (1979)
5. Knuuttilla et al (1985)

spanned less than 500 years. As noted in Chapter Two, the low recovery and high variability of citrate content between his samples observed utilizing the pentabromide-acetone technique led Thunberg (1947) to conclude that citrate content derived from archaeological contexts could not be used.

The next two lines in the table lists Lengyel's (1968) results obtained for **trabecular** bone citrate content of both modern cadaver samples and archaeological skeletal samples using the same technique. Lengyel (1968) reported a mean percentage drop of citrate content of only 41.0 and 50.8 % per time period when his archaeological series spanning thousands of years was compared to the results obtained for his own modern series.

However, when the citrate content results of Lengyel's (1968) modern series are compared to the results from the modern report cited above, his recovery of citrate content for the archaeological sample was actually 91.4 % and 92.8 % lower than this previously reported norm and is in agreement with Thunberg's (1947) initial analysis. As well, when his own modern series is compared to this norm, a loss of 85.4 % whole trabecular bone citrate following the death and embalming of modern individuals of either sex is also observed when utilizing the pentabromide-acetone technique. The results of his study led Lengyel (1968) to conclude that citrate content derived from the pentabromide-acetone

technique simply could not be compared between time periods. The assessment of sex within each time period was still possible.

The fourth line in the table is Dennison's (1979) pentabromide-acetone citrate content results on cortical bone samples from various archaeological sites which, like Lengyel's (1968) sample, also spanned thousands of years. Dennison (1979) reported a 0 % drop in citrate content through time in contrast to all previous researchers. When his results are compared to Dixon & Perkin's (1952) report on modern cortical bone citrate, a mean percentage drop of 81.6% of citrate content was actually attained between time periods by Dennison (1979) utilizing the pentabromide-acetone technique. This suggests that the analysis of cortical bone from archaeological contexts is also accompanied by much loss in agreement with Thunberg's (1947) pioneering study. Despite technical problems he encountered using the pentabromide-acetone technique, Dennison (1979) suggested that the chemical determination of sex by cortical bone citrate content was reasonable.

The fifth line in the table is Knuuttilla et al's (1985) pioneering study of citrate content in cortical bone fresh biopsies utilizing the citrate lyase technique. The sixth and seventh lines in the table are the ranges of citrate content of trabecular bone samples obtained in this study utilizing the citrate lyase technique for both the

archaeological series which spanned 100 years and the modern series. A mean percentage drop of 88.5 % and 85.6 % citrate content for both lumbar and iliac crest samples between time periods is observed and compares to the results obtained by Lengyel (1968). However, an average of 9.3 % more citrate per sample was recovered between time periods in this study utilizing the citrate lyase technique. This result, in conjunction with the specificity and reproducibility of the technique outlined in Chapter Four suggests that the determination of citrate content by citrate lyase is better than results obtained using the pentabromide-acetone technique.

No alternate comparative studies of citrate content of modern whole trabecualar bone utilizing the citrate lyase technique exist, to date. Given the results of Dixon & Perkins (1952) analysis of citrate content of fresh trabecular human bone utilizing the pentabromide-acetone technique and the results of Knuuttilla et al's (1985) citrate lyase analysis of fresh cortical bone, it would seem reasonable to assume that a similar relatively high loss of trabecular bone citrate would also be observed between fresh autopsied samples and the cadaver samples of the modern series in this study.

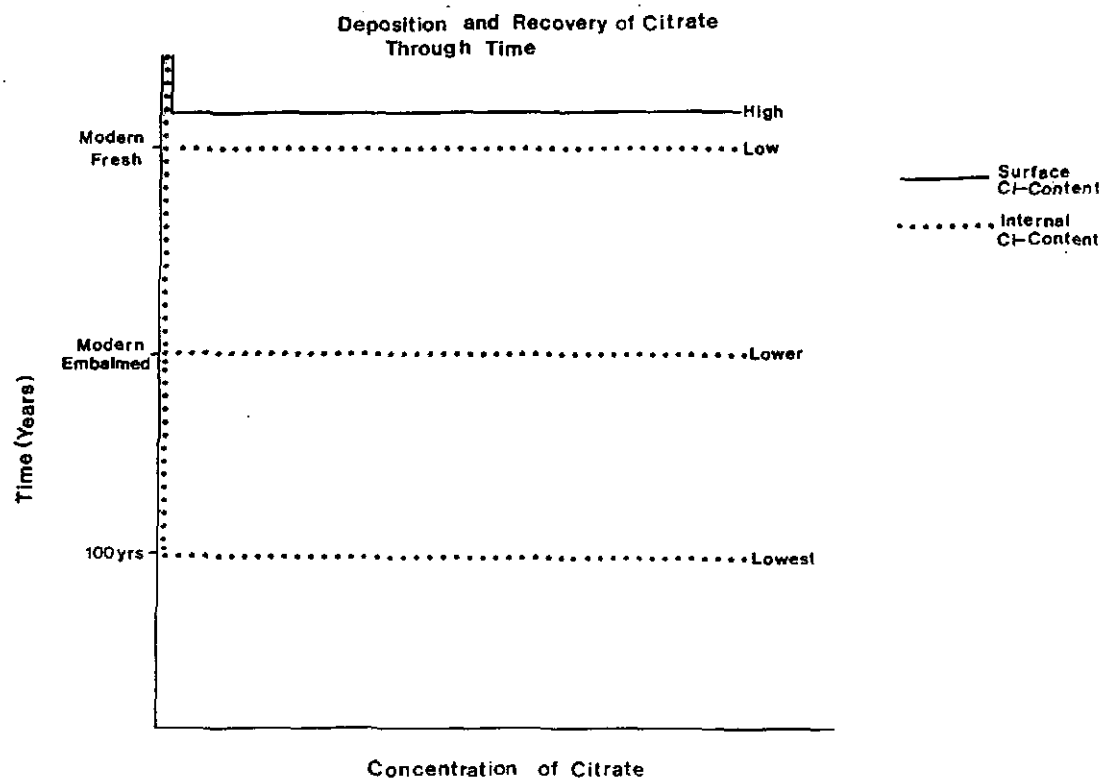
The most striking differences, therefore, observed between the two series in this study and other previous studies cited above, is this high loss of trabecular bone

citrate through time, regardless of sex, age, health-related status and/or skeletal part. This loss may partially be due to methodological problems such as the inability of finding a uniform sample preparation concentration when employing the citrate lyase technique as outlined in Chapter Four or to the difficulties encountered when utilizing the pentabromide-acetone technique as discussed in Chapter Two.

It also reflects intense post-mortem chemical alteration and hence, the high volatility of bone Ci-content, in general, following the deaths of the individuals within both the archaeological and modern series, regardless of bone type. The high loss of citrate content might suggest that both trabecular and cortical bone citrate content observed from either archaeological or modern contexts cannot be compared between time periods, in agreement with previous researchers' reports (Lengeyel 1968; Kiszeley 1974). In this study, it also suggests that the citrate content loss is substantial enough to possibly mask any significant differences in trabecular bone citrate content between the sexes which may actually exist, within the archaeological and the modern series.

As noted in Chapter Two, the location of citrate deposition in either modern trabecular or cortical living bone is still highly debated. Figure 5:4:2 illustrates a theoretically possible model of deposition and the actual recovery of citrate content through time as suggested by the

Figure 5:4:2- Theoretical Model of Citrate Deposition and Recovery



results of this study. It seems reasonable to assume that the concentration of citrate at the surface of the bone, regardless of bone type, is the highest available in living bone. Alternately, that citrate content found internally within the lattice of bone would then represent the lowest in concentration in living human bone. If so, measurement of both concentrations without any chemical alteration such as embalming or diagenesis may possibly reflect the high citrate content results obtained by Dixon & Perkins' (1952) and Knuuttilla et al's (1985) analyses of fresh human bopsied trabecular or cortical bone.

The process of embalming may possibly further exacerbate the depositional and recovery situation by causing the surface citrate to leach from the bone leaving behind only the internal and hence, lower citrate content available for measurement. This might explain the lowered results reported in Lengyel's (1968) study of modern cadaver samples. In short, the lowered values obtained for cadaver samples of the modern series in this study thus may also be what is left of the potential two concentrations of citrate content available for analysis in all living human bone. If so, the loss is substantial enough to mask any differences that may in fact exist between the sexes.

This postulation seems to be substantiated by the observation of citrate content obtained for two of the individuals within the modern series (# 86, #116). When

compared to all other individuals' citrate content within the modern series, these cadaver samples are considerably lower than all other individuals tested in the series. The common variable other than sex which might explain their considerably lowered citrate values is the length of time that had passed since their embalming (3.5 and 2.0 years, respectively) when compared to the rest of the individuals within the series (1.0 year) following all of their deaths. It is also implied in Knuuttilla et al's (1985) suggestion that only a small part of the total citrate available in living human bone is dependent upon the carbonate concentration, and/or vice versa. If this postulation is correct, post-mortem diagenesis within the archaeological series may parallel the actions of embalming within the modern series resulting in even substantially lower concentrations of bone citrate content through time. This would then explain the non-significant results observed between the sexes within the archaeological series.

Second, the non-significant results observed in this study may simply be due to the inadequate size of samples tested for citrate content in both the archaeological and modern series. For example, it did not seem reasonable to assume that the individuals tested for citrate content from either the archaeological or the modern series in this study were from populations exhibiting normal distribution of citrate content. Furthermore, the sample sizes within and

between each cemetery or within the modern series were not large enough so that the distribution of their means of citrate content could be treated as normal. Since parametric procedures require the assumption of normality, and also require large sample sizes, they could not be utilized to analyze the whole trabecular bone citrate data in this study (Sokal & Rohlf 1981). Consequently, non-parametric tests as opposed to parametric tests were used instead.

The advantage of utilizing nonparametric tests in this study is that they required few assumptions about the citrate content data observed (Siegal 1956). Also, the tests could be performed on the small samples of most of the variables known to potentially effect citrate content in both of the series. The disadvantage of using nonparametric procedures in this study is that nonparametric tests are usually not as good at finding differences between groups or variables when the differences may, in fact, exist (Ibid). In short, the non-significant differences of whole trabecular bone citrate observed between the sexes, regardless of skeletal region tested in both the archaeological and modern series, may be due to the fact that the nonparametric procedures used were not powerful enough as their parametric counterparts to distinguish the difference in citrate content between male and females. This suggests that larger, more representative samples in both archaeological and modern contexts are required in order to

adequately assess whole trabecular bone citrate content as an alternate chemical means for the determination the sex of human skeletal remains.

To summarize, the discrepancies in citrate content observed in this study, in conjunction with similar problems raised by all previous researchers' reports, possibly stem from inherent technical difficulties of employing either technique. They also may relate to intrinsic differences between individual archaeological and modern samples tested and/or to combinations of both. The analysis of citrate content of bone derived from archaeological and modern contexts in this study further suggests that citrate content of whole trabecular bone is possibly too volatile and hence, seriously affected through time probably as a consequence of post-mortem alteration and variable decomposing factors. It must be admitted, however, that without precise knowledge of the location of citrate in living human bone, the correlations between total concentrations of trabecular bone citrate postulated above are bound to be somewhat ambiguous.

Because it appears that the extraction and measurement of citrate content is still difficult and accompanied by much loss, it is concluded that the resolution of the effects of differential diagenesis and post-mortem alteration of whole trabecular bone, in particular, prohibits any further consideration of using citrate content. This study also suggests that citrate content,

regardless of bone type, in general, also cannot be used at this time to determine the sex of human skeletal remains. More research is required to obtain precise knowledge of the location of citrate in living human bone, prior to reassessment of the potential of utilizing bone citrate content as an alternate means of determining sex.

CHAPTER 6: CONCLUSIONS

Methodology

1. U.V.-enzymatic spectroscopy utilizing citrate lyase is an accurate and precise technique for measuring the citrate content of human bone samples derived from archaeological and/or modern forensic contexts.
2. The specificity and reproducibility of U.V.-enzymatic spectroscopy by citrate lyase eradicates most of the previous researchers' inherent technical problems when employing the pentabromide-acetone technique.
3. Separate sample concentration preparations and controls are necessary each time different series are assessed by the citrate lyase technique.
4. It is suggested that this restricts the method's assessment of citrate content to 'unknowns' within relatively complete archaeological or modern forensic skeletal series.

Citrate Content Inferences

1. The evidence presented here indicates that observed differences in citrate content of individuals within the seven historic cemeteries does not stem from testing trabecular bone derived from different

anatomical regions of the skeleton.

2. There is also no evidence to show that citrate content varies substantially between known male and female individuals from within five of the seven historic cemeteries. Nor does the citrate content observed differ significantly between the sexes within the modern sample, regardless of health-related status.
3. In contrast to previous research, evidence indicates that citrate content, regardless of age, cannot distinguish the sex of fragmentary or incomplete archaeological or modern human skeletal remains. This is due to the high volatility of citrate content in bone, regardless of bone type.
4. It is proposed that the most important variables affecting citrate content observed within archaeological contexts are small sample size, and geographic distribution, historical time and/or combinations of both. Other studies of bone citrate content derived from archaeological populations show similar results to those reported here.
5. It is suggested that it is not possible to compare bone citrate levels of any individuals in order to determine their sex until more precise information concerning its location in bone is known.

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