DRUG PARTITIONING BETWEEN PLASMA AND ERYTHROCYTES AS A MODEL OF PHARMACOKINETIC VOLUME OF DISTRIBUTION

BY

Joy Wilson

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ABSTRACT

Comparison of plasma: red cell drug concentration ratio (p:rbc) and its predictive factors with known data about V_d was undertaken to investigate the potential of predicting V_d from p:rbc ratio. Blood samples from 75 healthy volunteers, 37 under 60 years old and 39 over 60, were incubated with theophylline, centrifuged and frozen. Hematological and biochemical data were obtained. Plasma theophylline concentrations were analyzed by high-performance liquid chromatography. The p:rbc ratio decreased with age in men but increased in women. Tukey's multiple comparison test found a difference between older men and women; analysis of variance an age-dependent gender effect. **Post hoc** analysis showed factors predictive of the p:rbc ratio: age, cholesterol and creatinine for men, but bilirubin and age for women. An age-dependent gender effect on theophylline V_d has been shown in an animal study, but not in human studies, which were not necessarily optimally designed for the purpose. Cholesterol, creatinine, and bilirubin have not been studied in relation to theophylline V_d. The present study has not supported the similarity of determinants of p:rbc ratio and V_d. Further data could be obtained by direct comparison of partitioning and V_d in one sample, and of V_d with cholesterol, creatinine, and bilirubin. The age-dependent gender effect on the p:rbc ratio was an unexpected finding, of potential relevance to therapy, the significance and mechanisms of which should be clarified.

ABBREVIATIONS

AUC	area under the curve of time and plasma concentration
BBB	blood-brain barrier
Cl	clearance
CSF	cerebrospinal fluid
D	dose
F	free fraction
fl	femtoliter
fmol	femtomole
HPLC	high performance liquid chromatography
i.s.	internal standard
iv	intravenous
L	liter
μg	microgram
p:rbc	plasma: red cell concentration ratio
s:rbc	serum: red cell concentration ratio
$t_{1/2}$	half-life
V_d	apparent volume of distribution

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I. INTRODUCTION

A. Drug Distribution

1. The Apparent Volume of Distribution

The apparent volume of distribution (V_d) has been defined as an arbitrary parameter calculated from drug dosage and plasma concentration, related in an unclear fashion to anatomical or physiological volumes. The body has been described as one compartment or as many, but most often as two: a "central", rapidly equilibrating and a "deep", "peripheral" compartment. In a one-compartment open model with rapid intravenous (iv) injection, V_d was the hypothetical volume relating drug quantity to equilibrium plasma concentration (Koch-Weser et al, 1982). The significance of V_d in regulating drug therapy lay in its ability to predict plasma drug concentrations after a known dose. The V_d has also been related to important pharmacokinetic concepts describing the rate of disappearance of drug from plasma by metabolism, excretion, or tissue uptake. Half-life ($t_{1/2}$) was determined by V_d and clearance. Clearance (Cl) was defined as the ratio of dose, D, to area under the curve of time and plasma concentration (AUC). At best, the models on which the calculations were based, describing the body as one or a few compartments, were oversimplifications of complex equilibria (Wagner, 1971). Slight changes in the semilog plot markedly affected V_d (Berkowitz et al, 1974, Mather et al, 1975). Although useful, V_d must be interpreted cautiously.

2. Tissue Distribution

Tissue distribution has been defined as a physiological parameter indicating drug concentrations in target tissues at a given time. Therapeutic tissue concentrations have not necessarily been related to desirable plasma concentrations. Determinants of tissue distribution have included the drug, the tissue, and the physiologic state. In pregnancy, the antithyroid drugs methimazole and carbimazole, or their metabolites, crossed the human placenta more extensively than the antithyroid drug propylthiouracil or its metabolites (Marchant et al, 1977). The penem antibiotic SUN5555 did not enter rat brain across an intact blood-brain barrier (BBB), although it entered other tissues (Tsuji et al, 1990). Physiologic parameters of importance have included tissue blood flow and capillary permeability. The proportion of total blood flow reaching individual tissues has been difficult to measure and susceptible to rapid change due to varying number and identity of accessible capillary beds. The opening of precapillary sphincters, and hence capillary beds, has been found to be regulated according to the relative importance of the metabolic needs of individual tissues. Capillary permeability has been observed to vary in different organs. The complexity of the physiological variables determining tissue distribution has necessitated direct measurement. Whereas animals have been sacrificed to permit simultaneous measurement of drug concentrations in all tissues

(Benowitz et al, 1974 a, b), man has been studied by measurement of blood drug concentrations, and occasionally by measurement of concentrations in single surgical specimens. Blood, unlike surgical specimens, has been sampled repeatedly without undue trauma (Butler, 1971). A profitable subject of investigation would be a method of determining tissue distribution from the most readily accessible human tissue, blood.

3. Factors Affecting Drug Distribution

a. Tissue Uptake and Binding

The V_d of certain drugs has been found to exceed the body volume; tissue binding has been proposed as an explanation (Smith and Rawlins, 1973). One such drug was amiodarone, with a V_d of 4936 +/- 3920 L (Holt et al, 1983a) and a wet weight in fat of 236 mg/kg (Holt et al, 1983b). For highly tissue-bound drugs, plasma binding affected the free fraction (F) little provided that plasma-bound drug did not displace tissue-bound drug. **In vitro** methods of examining drug interactions by studying competition for plasma protein binding have not always been adequate for highly tissue-bound drugs with a large V_d (Smith and Rawlins, 1973). Investigation of binding to blood cells could be expected to contribute to clarification of such interactions.

Factors influencing drug uptake have been shown to include blood flow, tissue mass, and permeability. Well

perfused tissues have been found to be heart, lungs, kidneys, brain, endocrine and exocrine glands; moderately perfused, muscle, skin, and fat; and negligibly perfused, bone, teeth, tendons and ligaments. Muscle has been described as having a large mass and thus a large drug capacity in spite of being only moderately perfused. Fat was important for lipophilic drugs; perirenal and omental accumulated slightly more than subcutaneous fat (Smith and Rawlins, 1973). Permeability has been considered to affect drug distribution (Sedek et al, 1990): the rapidly equilibrating compartment of simultaneously administered theophylline, urea, and inulin in dogs decreased considerably after resection of the spleen and gastrointestinal tract, tissues with a high prevalence of fenestrated and discontinuous capillaries. The mass, blood supply and permeability of solid tissues have not been adequately simulated by blood samples. Although blood cells could not be used to find actual tissue distribution, they might provide a useful approximation of V_d.

Stereoselective disposition has been poorly understood. Roth et al (1972) found equal affinity of spirosuccinimide enantiomers RAC I and II for red cell ghosts. Takahashi and Ogata (1990) explained rat red cell and tissue stereoselective propranolol uptake by stereoselective plasma protein binding. Gietl et al (1989) found differences in rat tissue R(-) and S(+) prenylamine that might have been, in part, due to stereoselective tissue binding. Evidence for

enantioselectivity of tissue binding has been interesting, though not as yet conclusive. An exciting use of blood might be in the study of stereoselective cell binding.

b. Blood Binding

V_d has been shown to depend on the equilibrium between binding to blood cells or plasma proteins and binding to tissues other than blood (Shand, 1982). Drug binding to plasma proteins has been found to depend on the association constant, number of binding sites, and protein and drug concentrations. The association constant was a measure of affinity, defined as the ratio of association and dissociation rate constants. The number of binding sites was usually one or two per molecule. The bound drug fraction was often approximately constant at therapeutic concentrations, because the saturation curve was usually linear in this drug concentration range (Smith and Rawlins, 1973). Numerous factors have been shown to alter plasma protein binding, including lipophilicity, as for terbinafine binding to lipoproteins (Machard et al, 1989), stereoselectivity, as for methadone (Eap et al, 1990) and propranolol (Lalonde et al, 1990), presence of inherited protein variants (Eap et al, 1990), and alterations in protein concentrations, as in pathological states affecting α_1-acid glycoprotein (David et al, 1983). Albumin, a high-affinity, high-capacity binding protein for acidic drugs, and low-affinity, high-capacity for

basic drugs (Routledge, 1986), decreased in plasma concentration with age (Smith and Rawlins, 1973). Conversely, α_1-acid glycoprotein, the major binding site of basic drugs (Bredesen and Kierulf, 1984), tended to rise in concentration with age (Swift and Triggs, 1987). Basic drugs should be expected to bind more readily to the protein fraction of plasma from the elderly, in the absence of other major changes in plasma protein composition or of a large decrease in low-affinity, high-capacity albumin binding. The V_d of basic drugs has in fact not been observed to change uniformly with age, as exemplified by morphine and diazepam. Factors other than these plasma proteins must be involved.

Red cell drug entry has been described as no less important a determinant of access to other body tissues than plasma protein binding (Perl, 1975; Wilkinson and Shand, 1975; Koch-Weser et al, 1982). Unfortunately, it has been much less well elucidated, although of widespread significance since many drugs have been shown to differ considerably in plasma: red cell (p:rbc) ratios. Red cells contained 20% of blood yohimbine, corresponding to a p:rbc ratio of 5 (Owen et al, 1987). Rat serum to red cell nifedipine, s:rbc ratio, was 3 - 5 (Duhm et al, 1972). Red cells contained 53% of blood detomidine, corresponding to a p:rbc ratio of 0.89 (Singh et al, 1987). Quinacrine and metabolites were more concentrated in red cells than plasma, for blood pooled from several ducks (Marshall and Dearborn, 1946), corresponding to a p:rbc of

less than 0.5, whose physiologic significance might be difficult to interpret. Amopyroquin s:rbc ratio was 0.2 (Pussard et al, 1988). Pipequaline s:rbc ratio was 0.144 (Essassi et al, 1987). Higher red cell than plasma drug concentrations might be explained by active red cell uptake. Other mechanisms of drug association with red cells might have been avid binding to a cell component with passive diffusion of free drug. Pipequaline was associated particularly with plasma lipoproteins but also with red cell membranes, which comprised 12% of blood binding. The free fraction (F) was small, corresponding to an F: red cell ratio of 0.07 (Essassi et al, 1987). The low s:rbc ratio of pipequaline might in part have reflected passive association with the red cell membrane, but the percentage of blood binding accounted for by red cell membranes could not solely have explained the s:rbc ratio. Another mechanism of drug accumulation by red cells might have been rapid clearance from the plasma but not the red cell. The intracellular metabolites of thalidomide left red cells slowly (Keberle et al, 1965; Goldstein et al, 1974). An unusual type of drug uptake, if corroborated by more than one study, would be red cell diazepam accumulation beginning only after 11 weeks of therapy (Zingales, 1973); it has not been clear why only chronic therapy should trigger drug uptake. The mechanisms of drug uptake by red cells should be an intriguing field of study.

Theophylline red cell: plasma ratio in four subjects was

0.58 +/- 0.21, corresponding to a p:rbc of 1.72 +/- 1.14 (Mitenko and Ogilvie, 1973). In five subjects, mean serum: whole blood ratios were 8:8 with a range of +/- 3: +/-3 (Sheehan and Haythorn, 1977), corresponding to a mean p:rbc of 1:0; the results were "inconclusive because of the magnitude of the experimental error" (Sheehan and Haythorn, 1976). Peat et al (1977) obtained comparable results in fifteen subjects. These findings should be corroborated, using larger samples.

The red cell equilibration rate played a role in determining the significance of blood binding with respect to tissue distribution. Interaction of compounds with erythrocyte membranes has been found to be capable of occurring very rapidly (Eskelinen, 1987). Theophylline equilibration with tissues was so rapid that Belknap et al (1987) postulated carrier-mediated transport. Drug binding in blood, when not rate-limiting for tissue extraction, increased drug delivery to tissues (Shand, 1973). The amount of pipequaline supplied to rat brain was greater than that predicted from its F, an observation attributed to rapid dissociation of drug bound both to plasma proteins and erythrocytes (Essassi et al, 1989). Such an effect would not be expected in drugs entering red cells slowly compared to the circulation transit time (Chiou, 1984), for example diodrast (White, 1940; Phillips et al, 1945-6; Chiou, 1984), thiourea (Goresky et al, 1975) and doxorubicin (Lee and Chiou, 1989). Ignoring red cell concentrations of such drugs has led to

overestimation of V_d (Goresky et al, 1975).

Other blood cells have also been observed to contribute to blood binding. Imipramine was found to bind human platelet membranes with a maximum affinity of 667 +/- 100 fmol/mg protein (Langer et al, 1980). The significance of this has been difficult to interpret, as the mass of platelet membrane protein in blood has not been a convenient unit of measure. Amiodarone concentrations in dog red and white cells were highest 5 minutes after dosing, then dropped biexponentially, with $t_{1/2\alpha}$ as in plasma, and $t_{1/2\beta}$ longer. The peak red cell concentration was 2.9 +/- 1.7 μg/ml, similar to the peak plasma concentration; the white cell concentration was 620.5 +/- 277.8 μg/g protein. After 6 hours, the red cell: plasma ratio ranked the lowest among tissue: plasma ratios, and the white cell: plasma ratio second highest. This would have corresponded to, for p:rbc, the highest ranking, 3.6 +/- 1.4 μg/ml:μg/ml, and for plasma: white cell ratio, the second lowest ranking, 0.020 μg/ml:μg/g protein). Unfortunately, all ratios not involving blood cells were measured in μg/g tissue:μg/ml, making interpretation of comparisons difficult. For the metabolite desethylamiodarone, the highest number representing concentration was found in white cells and the lowest in red cells, but the units differed from those for solid tissues (Bandyopadhyay and Somani, 1987). Bergqvist and Domeij-Nyberg (1983) found, 7 hours after oral chloroquine, in one healthy human, 130 nmol/10^9 granulocytes, 3.9 nmol/10^9

thrombocytes, and 0.101 nmol/10^{12} erythrocytes. Serum concentrations of desethylchloroquine were four times greater than plasma concentrations, and of chloroquine twice plasma concentrations. This difference was attributed to drug release from platelets, although a contribution might have been made by binding to proteins involved in coagulation. The affinity of chloroquine for nuclear DNA (Hahn et al, 1966) might have explained its apparent predilection for platelet binding. If the blood distribution of chloroquine and the similar pattern for desethylchloroquine were consistent with the distribution of amiodarone and desethylamiodarone, less chloroquine might have been associated with non-erythrocytic cellular components of blood immediately after dosing than 7 hours after dosing. Immediate determination of plasma concentrations would have minimized the effects of platelet or white cell drug accumulation. A change of double to quadruple the plasma concentration of drug would be expected to be significant to tissue distribution and the use of blood to study V_d. Further study in this area would, however, be necessary before conclusions could be drawn, particularly in view of the sample size of one in Bergqvist and Domej-Nyberg's study (1983).

Ignoring cellular components of blood other than erythrocytes might have led to error in the estimation of V_d. Red cells have been found to comprise approximately 45% of blood volume, white cells 1%, and plasma 64% (Alberts et al,

1989). If drug concentrations were equal in red and in white cells, 2.17% of the drug in blood cells would be lost in the buffy coat. In order for 10% of the drug in the cellular fraction to be lost, white cells must have concentrated drug 4.6 times more than red cells. Data have not become available as to the probability of this occurrence. The error of ignoring blood cells other than erythrocytes might have been minimized by choosing times soon after drug administration when white cell and red cell concentrations might have been more similar, by studying "blood cell" partitioning without assumptions about cell type, or by measuring both red cell and plasma drug. If a marked discrepancy existed between amount of drug added and concentrations in plasma and red cells, the model of V_d might be strengthened by including other formed elements of blood.

c. Membrane Barriers to Drug Entry

i. Determinants of Membrane Permeability

A biologic membrane has been defined as a lipoid molecular sieve, best crossed by small, neutral, lipid-soluble molecules. Most drugs have been shown to be small (Cohn, 1971). Molecules over 0.4 nm did not enter the red cell, whose apparent pore diameter in man was 0.8 nm (Whittam, 1964). Large, especially protein-bound, molecules, have entered cells by endocytosis (Bilej and Vetvicka, 1989) but not red cells. Lipid solubility has depended on ionization,

thus on pH and drug pKa, and on hydrophobicity of structural groups. Drugs have entered cells by carrier binding, or by diffusion across membranes or aqueous channels. The diffusion rate has depended on concentration gradient, area, membrane thickness, and membrane solute partitioning (Cohn, 1971). Lipophilic drugs have partitioned preferentially into membranes. Transmembrane pH gradients have yielded unequal non-ionized F and total equilibrium concentrations on each side (Cohn, 1971). Differences in protein binding on each side of a membrane have also led to unequal F and total equilibrium concentrations (Cohn, 1971), as in red cell drug uptake from buffer or plasma (Kurata and Wilkinson, 1974). A drug capable of ready membrane passage might have a higher V_d than one unable to penetrate the barrier enclosing all cells.

ii. Fat

Lipophilicity has favored fat deposition as well as membrane transit. Lipophilic drugs have often been found to have a high apparent V_d (Butler, 1971). The V_d of amiodarone was 4936 +/- 3290 L with a range of 1375-11810 L (Holt et al, 1983a). In eight patients, its concentration in fat exceeded that in other tissues examined, 236 mg/kg wet weight, as compared to 25 mg/kg in heart (Holt et al, 1983b). The very high concentration of amiodarone in fat, a major body store of the drug, could have explained the large distribution volume and some of the considerable interindividual variability when

V_d was not corrected for body weight. Changes in body fat have been found to influence drug V_d: 0.94 L/kg for desmethyldiazepam in the nonobese, 1.52 L/kg in the obese (Abernethy et al, 1982). Antipyrine was 0.3 times as extensively distributed into excess weight as ideal weight, but diazepam 5 times (Abernethy et al, 1981).

The effect of obesity on theophylline V_d has remained unclear. Ogilvie (1978) cited a study by Danish et al, demonstrating a smaller theophylline V_d in obese than normal subjects, but this study has apparently not been published. Gal et al (1978) found theophylline distribution to rise linearly with total body weight in normal and obese subjects; unfortunately the drug had been given orally, resulting in measurement not of V_d but of the ratio of V_d/bioavailability (Gal et al, 1978). This ratio was assumed to approximate V_d, since theophylline bioavailability has been found to be high and equilibration rapid. Assuming validity of this assumption, the data might have been explained as consistent with the almost equal partitioning of theophylline into water and octanol, with a slight predilection for water (Gal et al, 1978). As no data have become available concerning the effects of body weight on bioavailability and equilibration rate, the validity of the assumption and interpretation of the findings of Gal et al (1978) have remained unclear.

Fat has been shown to range from 10-50% of body weight, rising from 10% at age 20 to 25% at 60 (Rondel, 1982). This

change was insufficient to explain the age variation in diazepam V_d: 1.11 L/kg for 23-37 year-old men, 52.3-79.5 kg, mean 71.7 kg; 1.83 L/kg for 63-76 year-old men, 64.5-118.2 kg, mean 87.9 kg; 1.73 L/kg for 21-32 year-old women, 49.1-70.0 kg, mean 56.8 kg; and 2.64 L/kg for 61-84 year-old women, 45.4-83.6 kg, mean 61.1 kg (Greenblatt et al, 1980). Diazepam tissue binding might have altered with age, a subject which might be examined using the blood model. Alternatively, the difference might have been at least in part related to the distribution of diazepam 5 times more extensively into excess than ideal body weight (Abernethy et al, 1981). Abernethy et al (1981) found diazepam V_d corrected for total body weight to be 1.53 L/kg in 21-61 year-old men and women, weighing 49.1-79.5 kg, with a mean of 60.4 kg; V_d was 2.81 L/kg in 21-64 year-old men and women matched with the previous group for age, sex, and smoking habits, weighing 68.2-197.0 kg, with a mean of 101.1 kg. Abernethy et al (1981) considered both genders and a wide range of ages as a single group, without describing the relative proportions of individuals of each gender or of various ages. Age and gender have confounded the results of this study. The difference in diazepam V_d observed by Greenblatt et al (1980) between the older and younger male groups might have been partly due to weight differences. The older and younger female groups varied significantly in V_d but not in weight. The findings of Greenblatt et al (1980) cannot be solely attributed to weight differences, but further study

would be required to elucidate the effects of age and gender, as distinct from weight, on diazepam V_d.

iii. The Blood-Brain Barrier (BBB)

The BBB has been described as a cellular wrapping around brain capillaries, permeable to lipophilic (Wagner, 1971) or small, <2nm, hydrophilic molecules (Stewart et al, 1987). Characteristics required for BBB entry have been found to resemble those for membrane transit in general, but a few special features have been noted. Two examples have been described: rapid bulk cerebrospinal fluid (CSF) absorption into the arachnoid villi, which washed away slowly-entering hydrophilic drugs, and active transport of certain organic cations out of CSF (Wagner, 1971). For the BBB as for cell membranes, the entry rate has been related to the unionized F at physiologic pH, which in plasma has been 7.4, and in CSF 7.3 (Wagner, 1971). Although F has been a useful first approximation of brain uptake, drug dissociation from erythrocytes (pipequaline, Essassi et al, 1989) and plasma proteins has contributed amounts of drug additional to that comprising the F. In the presence of plasma proteins or erythrocytes, measured rat brain uptake was decreased by less than the expected amount compared to uptake without plasma proteins (Essassi et al, 1989; Machard et al, 1989; Lin and Lin, 1990). The age-related increase in rat brain concentrations of diazepam and its metabolites was not

accounted for by F (Rahman et al, 1986). Postmortem studies suggested an age-related increase in human BBB permeability, but autolytic postmortem changes could not be excluded (Mancardi et al, 1980; Chapel et al, 1984; Alafuzoff et al, 1985; Alafuzoff et al, 1987). Biopsies showed unchanged structures associated with nonspecific permeability, but thinner BBB layers, possibly indicating poorer compensation for transient leaks (Stewart et al, 1987). In this study, conclusions were drawn about function from structural appearance; before acceptance of this interpretation, examination of function would be necessary. Kleine et al (1988) noted protein leakage into CSF in 190 elderly patients with minor neurological symptoms. Although concerns about the invasiveness of lumbar puncture would most likely have precluded its use in the asymptomatic, the presence of symptoms might have indicated neurological differences of the study group from the healthy elderly. In the absence of a relationship between BBB permeability and age, altered drug entry might have been due to altered blood binding, depending on the exchange between bound drug and F. Alternatively, if permeability rose with age, due to slow closure of leaks, drug entry to brain would have increased in spite of constant blood drug concentrations, binding, and receptor sensitivity.

iv. The Erythrocyte

Drug passage into and out of red cells has commonly been believed to be by classical diffusion (Dalmark, 1981; Wilkinson, 1987), although Belknap et al (1987) postulated the existence of a carrier to explain the rapid equilibration of theophylline in body tissues. Factors influencing red cell drug entry have been found to be similar to those influencing membrane crossing in general: lipophilicity, ionization, and plasma protein binding. Drug-drug and drug-membrane interactions have also been of potential significance. Lipophilicity was described by Taylor and Turner (1981) as predictive of the ranking order of red cell partitioning for, from most to least lipophilic, propranolol, atenolol, and pindolol. Red cell: plasma ratios corresponded to a p:rbc ratio of 2.7 +/- 0.6 for propranolol, 3.2 +/- 1.2 for pindolol, and 1.1 +/- 0.5 for atenolol; the reported red cell: plasma ratios were, respectively, 0.39 +/- 0.08, 0.37 +/- 0.14, and 1.15 +/- 0.55. The magnitude of the experimental error in relation to the difference between ratios interfered with the determination of ranking order. Interindividual variability was a confounding factor, as different subjects in the small sample received different drugs (Taylor and Turner, 1981).

Ionization has been significant in erythrocyte membrane crossing: the pH dependence of doxorubicin efflux from red cells corresponded to cell penetration by the unionized form

(Dalmark, 1981). Plasma protein binding of drug determined red cell concentration when red cell uptake was linearly related to F, as for phenytoin (Borondy et al, 1973), diazepam (Kurata and Wilkinson, 1974), and pindolol (Taylor and Turner, 1981). In a series of species, propranolol F rose with $V_{d\beta}$, the apparent volume of distribution of the second, slow phase of disposition, calculated from the total blood drug concentration (Evans et al, 1973).

Drug-drug and drug-membrane interactions were under certain circumstances believed to have been significant with respect to red cell entry. Self-association of planar aromatic drug molecules has been invoked to explain the saturable kinetics of doxorubicin uptake (Dalmark, 1981). Local anesthetics increased doxorubicin membrane passage, possibly by interacting with lipids (Dalmark, 1981). Red cell membrane interactions might sometimes have involved slowly reversible, saturable binding, as for certain carbonic anhydrase inhibitors, rather than partitioning into lipids or through aqueous channels. Drug availability to tissues would have been limited in the presence of slowly reversible membrane binding (Wilkinson, 1987). As characteristics associated with ready drug entry have been similar for the red cell membrane and for cell membranes in general, the red cell would be expected to be a convenient model for other membranes. The effect of drug interactions with tissue membranes on distribution has not been clarified. Such an

effect might be investigated using red cells.

Blood processing has been shown to artifactually affect drug partitioning. Chen et al (1983) reported, in duplicate assays on samples from five volunteers, **in vitro** metabolism of procainamide, and fluctuations in plasma concentrations, during 12 hour room temperature incubation with blood. The application of this information would be questionable, as blood left at room temperature for over six hours would have begun to decompose. Cyclosporine association with red cells differed by about 50% at room temperature and at body temperature (Wilkinson, 1987). Heparin has been found to release lipoprotein lipase, leading to a rise in free fatty acids which competed for drug binding to albumin (Wilkinson, 1987). The stoppers of "Vacutainer" commercial blood collection tubes contained, in 1985, (Wilkinson, 1987, Kremer et al, 1988) plasticizers that leached out when in contact with blood, displacing drug from α_1-acid glycoprotein. The effects of interference by temperature changes and extraneous substances on drug partitioning might lead to consistent results **in vitro**, but misinformation about **in vivo** drug partitioning. Studies of drug partitioning in blood should attempt to avoid the confounding effects of blood processing, within the limits of practicality. It would be difficult to perform all laboratory procedures at body temperature, and impossible to study blood cells without an anticoagulant. Results obtained **in vitro** should be cautiously interpreted

until confirmed by comparison with other studies.

The absence of organelles in red cells has usually been less relevant to drug distribution than the presence of the outer membrane. An exception was drug binding to organelles, such as chloroquine to nuclear DNA (Hahn et al, 1966), or drug transit dependence on metabolism by enzymes less active in the red cell than elsewhere, as possibly for ribavirin (Smith and Wade, 1986). For certain drugs, other formed elements of blood might have been a more useful model than red cells, as for example chloroquine in white cells or platelets.

The red cell has been found to resemble other cells in ways likely to be significant to drug distribution. Red cell membrane phospholipid fatty acid composition correlated with that of brain (DeLucchi et al, 1987) and changed with age (Heckers et al, 1978; Alexander and Justice, 1985). Membrane composition influenced cation exchangers (Holmes et al, 1983) and drug behaviour (Seeman, 1972). Red cell and cardiac membrane digoxin receptors had equal dissociation constants and similar ouabain binding (Erdmann and Hasse, 1975). This finding could explain the correlation between myocardial and red cell digoxin distribution observed by Gorodischer et al (1976). Adsorption isotherms of spirosuccinimide isomers RAC 109 I and II to red cells and synaptosomes were similar (Roth et al, 1972). Partitioning of chlorpromazine, morphine, phenol, and pentobarbital was similar in erythrocyte ghosts, synaptosomes, and sarcoplasmic reticulum: the log of the nerve

or muscle partition coefficient in buffer was directly proportional to the log of the erythrocyte partition coefficient. However, the number of determinations performed was relatively small, and determination of the log of both quantities might have led to oversimplification of the relationship (Roth and Seeman, 1972). Pipequaline binding to red cell membranes and serum lipoproteins was believed to predict distribution to body lipids (Essassi et al, 1987). The importance of lipid solubility in drug distribution might be attributed partly to its reflection of ease of transit through tissue, like red cell, membranes. In the red cell as in the placenta, thalidomide decomposed into polar, teratogenic metabolites trapped by membranes (Keberle et al, 1965; Goldstein et al, 1974). Red cell partitioning has been observed to predict certain effects of drugs on tissues. Lipophilic anesthetics and tranquilizers prevented red cell hemolysis in nerve-blocking molecular forms and amounts (Seeman, 1972). This would be expected if the mechanism of action of these drugs, as has been believed, involved disruption of the cell membrane. Digoxin, which acts on membrane sodium-potassium pumps, interacts similarly with red cells and myocardium. Acetazolamide side effects, which were related to red cell concentration, were attributed to inhibition of the cellular enzyme carbonic anhydrase by the small study of Chapron et al (1985). Red cell butaperazine correlated more closely than plasma butaperazine with dystonic

reactions (Garver et al, 1976) and therapeutic effects (Garver et al, 1977). Garver et al (1976, 1977) concluded that red cells and neurons shared significant characteristics. Gender and age may, however, have been confounding factors. Red cell 6-mercaptopurine correlated with myelosuppression more closely than did plasma 6-mercaptopurine (Chan et al, 1987). The amount of drug penetrating blood cell precursors in the bone marrow would be expected to resemble the amount entering the presumably similar membranes of mature red cells. Even if red cell entry did not predict tissue distribution of drugs in general, it could be useful for specific drugs whose target cells resembled red cells in significant ways.

An association has been demonstrated between red cell and tissue partitioning. Evans et al (1973) reported that the linear relationship of propranolol F to red cell: plasma ratio resembled that of F to peripheral V_d; this would correspond to a nonlinear relationship of F to p:rbc ratio. Digoxin V_d dropped with age in mice and guinea pigs, the inverse of the change in p:rbc and plasma: tissue ratios (Kroening and Weintraub, 1980). The change in plasma: tissue ratios was not explained by altered renal elimination (Kroening and Weintraub, 1980). Similarly, in humans, the age differences in digoxin distribution would correspond to considerably higher s:rbc and serum: myocardium ratios in adults than in infants (Gorodischer et al, 1976). Red cell partitioning ratios might help predict and explain tissue distribution of

other drugs. Kurata and Wilkinson (1974) recommended routine use of such ratios to screen for abnormal plasma binding. Korten and Miller (1978) proposed biomembrane use to quantitatively determine membrane permeability. Taylor and Turner (1981) suggested use of red cell partitioning ratios to combine data on plasma and membranes. Partitioning ratios have been expressed in two ways. For the purposes of the present work, p:rbc ratios have been chosen. This method of expression has had the advantage of comparability with plasma: tissue ratios; overall plasma concentration would have been an approximation of an average of these values, weighted according to blood flow and tissue mass. Conventionally, plasma concentration of drug has been widely used. Disadvantages of using p:rbc ratios have been that red cell: plasma ratios were used in most studies of partitioning, and that red cell: plasma ratios were directly proportional to F and V_d for propranolol. Since V_d has been calculated as the ratio of dose to plasma concentration, it would be expected to be inversely proportional to plasma: tissue concentration.

d. Biological Variability

Individual variation in V_d has sometimes been considerable, depending on genetic, physiological, and environmental factors. Paroxetine V_d was 17.2 +/- 9.9 L/kg, range 8.0-28.0 in four healthy subjects, one given 23 mg and the others 28 mg iv (Kaye et al, 1989). The small subject

number and variable drug dose rendered the results unconvincing. Theophylline V_d was 0.351-0.701 L/kg in 19 healthy subjects (Piafsky et al, 1977b). This more reliable data indicated almost twofold variation. Factors such as circadian and seasonal variation in plasma protein or drug concentrations, dietary composition including quantity of compounds structurally related to the drug of interest, membrane lipid composition, exercise, altitude, pregnancy, or age could be of potential significance to individual variation in V_d. Plasma protein concentrations have been reported, in spite of controversy (Dybkaer et al, 1981), to change seasonally (Chesrow et al, 1958) and diurnally (Yost and DeVane, 1985). Considerable variation in an individual's serum theophylline concentrations has been attributed to a circadian effect (Scott, 1981). Diet pattern varied greatly among 58-89 year-old women (Bourn et al, 1990). Dietary factors have been reported to affect gentamicin pharmacokinetics, but not V_d (Dickson et al, 1986) theophylline metabolism (Monks et al, 1979), and theophylline clearance (Fagan et al, 1987). Dietary differences have been shown to be associated with differing membrane composition, which might alter V_d. Diet was associated with altered brain lipids (DeLucchi et al, 1987; Bourre et al, 1990; Yusuf and Dickerson, 1990), plasma proteins and lipids (Fleck, 1989; Abbey et al, 1990), and red cell membranes (Holmes et al, 1983; Manku et al, 1983; Wahle, 1983; DeLucchi et al, 1987;

Guezennec et al, 1989). Chronic ethanol consumption was associated with altered membrane lipids (Reitz, 1980) although other physiologic factors might have been involved. Exercise and altitude hypoxia apparently affected red cell membranes (Guezennec et al, 1989). Exercise training increased variation in red cell parameters for a given individual (Kaiser et al, 1989). Obesity was associated with reduced theophylline V_d (Ogilvie, 1978). Pregnancy altered salicylate (Krauer et al, 1980) and thiopentone (Morgan et al, 1983) plasma protein binding, as well as anticonvulsant serum concentrations (Kochenour et al, 1980). The significance of these findings has remained unclear. Frederiksen et al (1986) noted in the third trimester of pregnancy a drop in protein binding and rise in theophylline V_d when not corrected for body weight, but no change in V_d corrected for weight. Other pharmacokinetic changes described in pregnancy, such as in anticonvulsant serum concentrations, might also have been apparent changes only. Ritodrine V_d corrected for weight was found to be 2.8 +/- 0.5 L/kg in six pregnant women, and 4.8 +/- 0.7 L/kg in thirteen nonpregnant women (Gross et al, 1988). Flaws in this study were a relatively small sample size, and lack of explanation for the apparent sample size discrepancy of nine pregnant women in determination of V_d when not corrected for body weight, as opposed to six when corrected. Children have been reported to differ from adults in diclofenac V_d (Korpela and Olkkola, 1990). It has been

claimed that biological variability might increase in the elderly, possibly due to individual or organ system differences in rates of aging (Dybkaer et al, 1981; Kenney, 1985), but normal limits have been disputed (Harnes, 1980; Morgan, 1983). Contrary to previous reports, Fraser et al (1989) found biologic variation in hematology of the healthy elderly similar to published data for the young, with large intersubject variation. It has been suggested that variability in the aged might depend on health and remain unchanged for most parameters (Norris and Shock, 1966, Dybkaer et al, 1981). Biologic variation in man has been found to be ubiquitous and its sources very difficult to control. For several biological parameters (Dybkaer et al, 1981, Seiter et al, 1989), interindividual variation has been shown to exceed individual variation. Blood partitioning might allow estimation of V_d for an individual.

e. Drug Interactions

Mechanisms of drug interaction have included alteration of absorption, binding, metabolism, excretion, and receptor response (Rondel, 1982). Drug interactions have been observed to change V_d: probenecid lowered the V_d of nafcillin by a ratio of 2.6 (Klein and Finland, 1963; Gibaldi and Schwartz, 1968). An extensively investigated mechanism of interaction has been alteration of binding to plasma proteins. Numerous drugs have been shown to compete for binding to the major plasma protein

albumin, including phenylbutazone and warfarin (Aggeler et al, 1967) as well as phenylbutazone, propylthiouracil, and acetylsalicylic acid (Kampmann and Molholm Hansen, 1983). Theophylline had only one competitor for plasma protein binding, salicylate, of 19 common drugs and metabolites (Shaw et al, 1982). Competition for theophylline protein binding has been considered relatively unlikely to be important to possible effects of drug interactions on V_d. Drug interactions affecting plasma proteins have rarely been found to involve binding enhancement, such as the effect of increasing chlorothiazide concentrations on pempidine binding to albumin (Dollery et al, 1961). Although very little effect on rat tissue distribution of pempidine was observed, V_d has sometimes been noted to change with altered plasma protein binding. Blood, which has frequently been used to study drug interactions, might be used to estimate the V_d.

Drugs have been found to alter cell characteristics which could affect tissue interactions with other drugs. Arecoline has been shown to alter BBB permeability (Saija et al, 1990). Anesthetics have disrupted membrane cytoskeletal integrity (Nicolson et al, 1986); changes in passive permeability have been described as dependent on cytoskeletal integrity (Kitagawa and Akamatsu, 1983; Bilej and Vetvicka, 1989). Passive permeability to drugs might also be affected. Drugs have been shown to be capable of altering cell permeability: propranolol stimulated rubidium efflux from human peripheral

blood lymphocytes (Murphy and Ryan, 1985). Several drug-induced changes in red cell properties have been demonstrated, but their significance with respect to V_d has not been examined. Drugs have altered red cell membrane fluidity. In glucose-6-phosphate dehydrogenase deficiency, membrane sulfhydril groups have been oxidised by drugs (Hoffbrand and Pettit, 1985). Red cell enzymes and hemoglobin have also been affected by drugs (McGuire et al, 1988). Changes in intracellular structures might have been expected under most circumstances to affect drug access to cells less than changes in membranes. Exceptions might have been changes in enzymes acting on drugs or in intracellular structures to which drugs were extensively bound. A drastic effect of drug binding has been cell destruction, presumably decreasing the number of cells accessible to other drugs. Drugs binding to the red cell surface have promoted antibody-mediated hemolysis (Morse, 1988). Drug interactions involving cells, especially cell membranes, could be conveniently studied using blood cells.

f. Consequences of Disease

Complex combinations of variables likely to affect drug distribution have been encountered in therapy of disease (Crouthamel, 1976). Alterations in protein binding have been attributed to burns, in perfusion to cardiac failure, in hepatic or metabolic function to cirrhosis, in renal function to renal failure, and in receptor number and function to

Parkinson's disease (Jusko, 1976; Rowland et al, 1976; Welling and Craig, 1976; Rinne, 1982). Acidosis and alkalosis have been observed to shift the tissue: plasma ratio of phenobarbital, as expected of pH effects on membrane transit (Butler, 1971). Disease and therapy have been shown to alter V_d. Knoppert et al (1988) noted a greater theophylline V_d in patients with cystic fibrosis than in healthy volunteers. Lidocaine V_d dropped 40% in eight patients soon after cardiac surgery (Holley et al, 1984). One mechanism of alteration in V_d involved altered plasma protein quantity. In severely burned patients, imipramine F decreased and plasma binding increased as α_1-acid glycoprotein rose in response to the injury (Martyn et al, 1984). In inflammatory diseases, altered plasma binding correlated with plasma protein concentrations (Piafsky et al, 1977b). Decreased volume of blood perfusing the tissues has also been shown to affect V_d: loss of 30% of blood volume in the monkey reduced lidocaine V_d. Tissue uptake was slowed and the lidocaine concentration in whole blood was elevated, although the plasma concentration remained constant (Benowitz et al, 1974b). Changes in excretion or metabolism of drug might also have been expected to alter V_d. The V_d of unbound oxazepam rose in renal insufficiency (Murray et al, 1981). Hepatic cirrhosis did not significantly alter theophylline V_d (Piafsky et al, 1977a). The complex, and interacting, effects of multiple physiological alterations due to one or more diseases and their treatment, have not always

been easily predicted. Estimation of V_d in an individual from a blood sample would have simplified therapy.

g. Factors Associated With Aging

Disease and polypharmacy have been common in the aged (Hurwitz et al, 1969 a, b, Aoki et al, 1983, Mitenko et al, 1983). As many as 26.6% of Edinburgh patients 75 years and over took 4-6 drugs when ill; 27% on 6 drugs had adverse reactions, as compared to only 10.8% on one drug (Williamson, 1979). Prediction of V_d might be expected to be more difficult under these conditions. The aged have been found to be more sensitive to a given blood concentration of numerous drugs, including diazepam (Reidenberg et al 1978; Castleden and George, 1979; Wilkinson, 1979). Their greater sensitivity has been attributed to changes in receptor function, as for example ß-adrenoceptor complex affinity (Feldman et al, 1984), or in postreceptor function, as for example ß-adrenoceptor adenyl cyclase coupling (Montamat and Davies, 1989; Swift, 1990). Frequently, alterations in distribution and F have not been excluded before consideration of receptor sensitivity. The V_d of many drugs has been shown to vary with age in man, including diazepam (Klotz et al, 1975), fentanyl (Meuldermans, 1982), morphine (Owen et al, 1984), amantadine (Aoki and Sitar, 1985), cyclosporine (Yee et al, 1986), vinpocetine (Miskolczi et al, 1987), and alfentanil (Sitar et al, 1989). Changes in V_d, although difficult to interpret, might have

been related to changes in the interaction of the entire body with drug. Age-related changes in drug-tissue interactions might have resulted in altered tissue distribution. It has been proposed that organ system aging might have often occurred at different rates (Kenney, 1985); if so, individual tissues should be expected to interact differently with drugs over time. Age-associated changes have been reported which would be likely to alter blood drug concentrations carried to tissues, membrane permeability, and tissue distribution. Concentrations of specific plasma proteins, whose drug binding properties were dissimilar, changed with age (Storiko, 1968, Swift and Triggs, 1987). Membrane composition has been found to change with age (Heckers et al, 1978, Alexander and Justice, 1985), an effect possibly associated with altered permeability. Increased BBB permeability with age has been suggested by two studies (Stewart et al, 1987, Kleine et al, 1988). The evidence for age-related alterations in tissue distribution has not remained solely indirect; brain diazepam concentrations were elevated in older rats as compared to younger rats (Rahman et al, 1986). An elevation of brain diazepam concentrations with age in humans would have explained increased sensitivity of the elderly at plasma drug concentrations similar to those in the young.

Age-related alterations in drug uptake, binding, or effects, dealt with in the above studies, could have resulted from alterations in red cells. Some evidence has suggested

the presence of age-related alterations in red cell properties likely to affect drug distribution, but the data were often unreliable, based on very small sample sizes. Health might also often have been a confounding variable. Diet, which might have been relevant to membrane composition and drug transit, has been reported to change with age and with health. Bourn et al (1990) found rising nutrient intake with age in non-institutionalized women 58-89 years old, whereas Morgan et al (1986) found poorer nutritional indices with illness and dependency in women 61-101 years old. Dietary differences (DeLucchi et al, 1987) and age (Heckers et al, 1978; Alexander and Justice, 1985) were associated with altered red cell membrane composition. Membrane composition influenced cation exchangers (Holmes et al, 1983) and drug behaviour (Seeman, 1972). Aged mice exhibited altered erythrocyte properties: red cell lifespan was reduced, hematopoiesis increased, heterogeneity increased, and function probably impaired (Magnani et al, 1988). Although interesting, this data cannot be extrapolated to man. In elderly humans, red cell lifespan was normal, but cells were more easily disrupted (Garry et al, 1983; Lipschitz, 1990). Changes were observed in several properties likely to affect membranes and drug transit. With age, human erythrocyte osmotic fragility and variability increased (Araki and Rifkind, 1980; Bowdler et al, 1981; Hussain and Voaden, 1984), erythrocyte hemolysis slowed, (Araki and Rifkind, 1980), erythrocyte membrane sodium-

potassium pumps decreased in number in women (Naylor et al, 1977, 1980), mononuclear leucocytes but no other blood cells became less filterable (Ciuffetti et al, 1989), and blood viscosity rose (De Simone et al, 1990). The significance of these findings with respect to drug transit has not been clear. Some of the above findings have been documented by only one investigator in a very small study. Of the above findings, the increase in osmotic fragility and variability has been supported by the largest amount of evidence. Controversial changes in red cells have also been reported, but their significance has been unclear. Bowdler et al (1981) noted a lower mean cell hemoglobin count and a slight drop in the ratio of red cell area to volume (Bowdler et al, 1981). Piomelli et al (1962) and Araki and Rifkind (1980), unlike Hurdle and Rosin (1962) and Bowdler et al (1981), found a raised red cell volume. Garry et al (1983) reported normal red cell count, volume, hematocrit, packed cell volume and iron indices, and criticized previous studies for possible confounding factors. Changes in cell contents have been described: Purcell and Brozovic (1974) reported decreased 2,3-diphosphoglycerate. Evidence, not all of high quality, has supported red cell alteration in older people, most likely in membrane osmotic fragility and possibly in other characteristics. Membrane changes would have been expected to affect drug transit.

According to a controversial theory (Cristofalo, 1990),

aging has been described as secondary to decreased membrane transit due to cross-linking (Bjorksten and Tenhu, 1990) and rigidity from glucose-induced non-enzymatic browning of collagen and elastin: the gas diffusion capacity of human lung dropped 8% per decade (Kohn and Monnier, 1987). Moving red cells, with no fibrous matrix, became more osmotically fragile with age (Bowdler at al, 1981). The use of blood as a model of age changes in distribution would have been expected to depend on the similarity of aging of blood cells and other tissues. Determination of the extent to which age changes of the red cell resemble those of other cells might be expected to clarify aging mechanisms.

Age variation in drug binding to red cells, until recently often neglected (Anonymous, 1978; Rondel, 1982), could have been expected to occur based on the presence of age-related changes in red cell properties. Age variation in red cell drug concentration has been reported, although the sample sizes have usually been inadequate and confounding factors present. Chan et al (1975) noted lower red cell and higher plasma pethidine, but Holmberg et al (1982) and Herman et al (1985) could not replicate this. Nation et al (1977) found that red cell chlormethiazole dropped with age and F rose. Plasma protein binding of acetazolamide dropped with age, and F rose by 4-6%, becoming available to red cells. Raised red cell concentrations correlated with increased side effects (Chapron et al, 1985). Red cell butaperazine

correlated more closely than plasma butaperazine with dystonic reactions (Garver et al, 1976) and therapeutic effects (Garver et al, 1977); red cell 6-mercaptopurine correlated more closely than plasma 6-mercaptopurine with myelosuppression (Chan et al, 1987). The relation of red cell drug concentrations and clinical effects might be significant for other drugs as well. Langer et al (1980) found an age-related decrease in platelet affinity for imipramine, using a considerably larger sample than other studies demonstrating an age effect on drug binding to blood cells. The maximum affinity (B_{max}) was 592 +/- 59 fmol/mg protein for 17 volunteers 50 years and under, but only 386 +/- 41 fmol/mg protein for 18 volunteers 50 years and over. The magnitude of the effect on platelet drug concentrations has not been clear. The evidence of a few small studies for an age-related effect on blood cell drug partitioning, as for chlormethiazole and acetazolamide, has been suggestive, but not incontrovertible. Age effects on drug blood cell concentrations, which could have considerable significance for drug distribution in the elderly, should be more closely investigated.

B. Potential Models of Distribution Volume

Organic/aqueous solvent partitioning, as for hydrochloric acid/trichloromethane (Dayton et al, 1973), has been used to estimate drug entry into tissues (Miller and Yu, 1977). It has not been clear which organic solvent best simulated membranes

(Machleidt et al, 1972). The octanol/water partitioning coefficient did not predict the membrane/buffer coefficient for positively charged drugs (Roth et al, 1972). Taylor and Turner (1981) found it inferior to the coefficient of partitioning in membranes such as red cells. The red cell membrane partitioning of phenobarbital, pentobarbital, and thiopental correlated much better with BBB permeability than did organic solvent partitioning (Korten and Miller, 1978). Red cell membrane partitioning has provided a better estimate of drug entry into tissues than organic solvent partitioning.

Another method of estimating drug distribution has been based on measurement of drug binding to plasma, or more rarely, tissue, proteins. Studies of drug affinity for plasma proteins have been performed by ultrafiltration and equilibrium dialysis. Such studies have made invalid assumptions about plasma, such as the presence of equilibrium and the absence of extraneous molecules, including drug metabolites (Dayton et al, 1973). Drug binding to tissue homogenate **in vitro** was insufficient to explain the very large tissue: plasma ratios, corresponding to very small plasma: tissue ratios, of norchlorcyclizine and chlorcyclizine. Ratio differences for the two drugs or for different tissues were also not explained by **in vitro** studies (Kuntzman et al, 1973). Estimation of tissue distribution based on consideration of protein binding **in vitro** has ignored physiologic conditions such as blood composition, blood flow, and drug interactions

with membranes.

Mice, rats, dogs, and monkeys have often been used to predict tissue distribution in man. Organ concentrations of drug with metabolites have been found by radioactivity counts, and of individual compounds by chromatography (Evans et al, 1973; Russell et al, 1990; Vickers et al, 1990). From the distribution ratio for and anatomic volume of each major drug-binding tissue, V_d has been calculated (Benowitz et al, 1974a). Such studies have been tedious and experimental animals expensive. Extrapolation to man has been unreliable.

A more accurate method of determining distribution in man than studies of organic solvent partitioning, protein binding, or distribution in animals has been direct measurement of concentrations in human tissues or body fluids. Postmortem drug analysis in man (Marcus, 1984) has been confounded by the changes of death, disease and polypharmacy (Dayton et al, 1973). Tissue samples from operation or biopsy (Marcus, 1984) have not necessarily represented nonsurgical patients, and have not examined multiple organs at multiple times. Drug concentrations in saliva have been measured in order to estimate plasma concentrations, as for theophylline (Koysooko et al, 1974), but have been affected by individually variable secretion and by a pH different from that of tissues or blood.

The classical approach to V_d has been blood sampling at frequent intervals after drug administration to animals, healthy volunteers, or patients with single, common diseases.

It could not be routinely performed on all patients to be treated. Infrequent, repeated blood sampling has been used to monitor drug concentrations during therapy (Stewart et al, 1977), but ill effects could have resulted from inappropriate drug concentrations before abnormal results were detected. For large individual variability, small therapeutic indices, rare diseases or complex pathophysiologic and/or pharmacologic variables, safer therapy could have been achieved with prospective approximation of V_d for each patient.

C. Whole Blood as a Model of Distribution Volume

Determination of drug partitioning in whole blood might be expected to be a promising alternative to the previously described models for predicting the V_d of individual human beings with complex pathophysiology. The value of this comparatively convenient method would depend on the properties of blood components and their relevance to tissue distribution in general.

1. Properties of Plasma

Human blood has had the advantage of providing all human plasma components, endowed with the genetic, environmental, or physiological characteristics of the individual of interest, under the conditions prevailing at the time of interest. For compounds rapidly metabolized by red cells, metabolites with similar properties would also have been available to affect

binding, just as **in vivo** immediately after drug dosing. Assumption of the existence of equilibrium would give rise to least error when applied to a minimum period of time, as in this model. The assumption that tissue uptake was determined by F has been found to be approximately valid (Essassi et al, 1989; Machard et al, 1989).

2. Properties of Red Blood Cells

A blood partitioning model would be expected to provide more information than plasma binding or membrane partitioning studies alone. Red cell partitioning ratios were consistent with partitioning ratios of digoxin in myocardium and blood for human infants and adults (Gorodischer et al, 1976). An important implication of this work has been the potential for use of p:rbc ratios in prediction of V_d for other drugs. Tissue characteristics peculiar to the individual to be treated, as for example properties conferred by diet, ethanol consumption, disease, or drug interactions, might be determined by examining drug partitioning into red cells. This might be expected to eliminate the need for invasive sampling of inaccessible tissues, or hazardous therapy based on mean values for a very variable population. Assumptions inherent in the partitioning model have included similarity of red cell-drug interactions to red cell-tissue interactions and similarity of changes in both, such as changes associated with age.

3. Hypothesis

This study has hypothesized that the p:rbc ratio and V_d should be correlated to similar factors.

D. Dissertation Objectives

1. to adapt assay techniques for theophylline in plasma

2. to establish suitable conditions for incubation of whole blood **in vitro** with known amounts of theophylline, in order to determine p:rbc concentration ratios

3. to design and perform an experiment comparing factors correlated to the p:rbc ratio and to V_d

II. MATERIALS AND METHODS

A. Design of Experiment

In this experiment the theophylline p:rbc ratio was determined, an attempt was made to identify parameters correlated to it, and a comparison was performed of p:rbc ratio with reported data regarding theophylline V_d.

1. Inclusion Criteria for Acceptance of Volunteers

Volunteers were healthy adult men and women, under 40 or over 60 years old (Table 1a,b), who had not received systemic medication for at least 7 days, coffee, tea, or chocolate for at least 18 hours, or alcohol for at least 2 days but in most cases 1 week. They gave informed consent to donation of a single morning blood sample after having fasted from 7 to 12 hours. The criteria described were chosen in order to eliminate the possible confounding effects of food absorption or drug interactions, including methylxanthines such as caffeine.

2. Experimental Protocol

a. Sample Collection and Processing

Blood samples were collected between 7:30-12:00 a.m. in heparinized vacuum tubes. Within 2 hours, a 6 ml aliquot of blood was equilibrated with theophylline. Before incubation with drug, the pH was measured to ensure that it had not deviated from the normal range due to anaerobic glycolysis of

Table 1 Demographics of Volunteers.a Younger Volunteers.i Male

no[1]	a[2]	ab[3]	pcv[4]	ch[5]	tg[6]	al[7]	gl[8]	cr[9]	bil[10]	bun[11]	th[12]	p:r[13]
1	18	o	0.445	5.22	2.37	40	29	93	10	5.7	11.7	1.485
2	19	a	0.445	5.42	0.99	50	24	103	14	4.6	12.7	1.915
3	19	b	0.453	3.51	0.26	47	29	93	12	3.2	11.5	1.404
4	20	a	0.416	4.47	1.22	47	25	91	18	7.0	11.0	1.280
5	22	a	0.503	4.27	0.88	47	26	123	17	6.6	12.0	1.496
6	22	o	0.415	3.59	0.51	43	30	95	7	4.3	12.2	1.768
7	24	a	0.434	3.78	0.75	46	32	106	14	4.3	11.0	1.265
8	24	a	0.474	4.39	1.10	47	31	104	23	6.3	12.2	1.614
9	24	o	0.442	6.60	1.36	45	29	108	12	5.3	13.1	2.152
10	25	a	0.450	5.45	3.11	44	29	97	7	5.2	11.8	1.513
11	25	a	0.450	4.29	1.09	42	33	116	12	4.4	12.0	1.588
12	26	b	0.426	5.84	2.05	46	27	92	12	6.8	11.3	1.370
13	28	o	0.463	4.62	0.54	46	29	103	15	7.2	11.1	1.272
14	28	b	0.417	4.08	0.70	44	27	97	7	6.2	10.4	1.102
15	28	b	0.429	4.84	1.24	43	28	89	13	6.7	10.0	1.000
16	29	ab	0.429	5.94	0.93	43	35	101	19	5.4	12.3	1.773
17	30	a	0.440	5.09	0.77	45	31	102	9	6.7	11.8	1.531
18	30	b	0.516	3.74	0.78	49	25	112	17	5.9	10.8	1.168
19	39	a	0.412	4.61	3.12	44	34	97	5	5.1	10.1	1.025

[1]Volunteer number, [2]Age (yr), [3]Blood type, [4]Hematocrit (L/L), [5]Cholesterol (mmol/L), [6]Triglyceride (mmol/L), [7]Albumin (g/L), [8]Globulin (g/L), [9]Creatinine (μmol/L), [10]Bilirubin (μmol/L), [11]Urea (mmol/L), [12]Plasma theophylline (mmol/L), [13]Theophylline p:rbc ratio (mmol/L:mmol/L).

Table 1 (Continued) a Younger Volunteers. ii Female

no[1]	a[2]	ab[3]	pcv[4]	ch[5]	tg[6]	al[7]	gl[8]	cr[9]	bil[10]	bun[11]	th[12]	p:r[13]
20	18	n/a	0.401	4.40	0.91	47	34	81	10	2.9	11.6	1.524
21	18	a	0.374	3.37	0.77	44	26	88	20	7.0	10.6	1.178
22	24	b	0.406	4.97	0.57	38	27	73	14	3.8	11.3	1.395
23	24	a	0.370	4.44	0.59	41	29	74	20	7.3	11.0	1.326
24	24	a	0.395	3.89	0.44	43	27	79	8	5.0	11.0	1.299
25	24	a	0.430	4.86	0.65	42	32	83	21	5.2	9.2	0.832
26	25	a	0.374	4.33	1.22	43	30	76	7	3.0	10.6	1.178
27	26	o	0.389	3.91	0.47	44	33	79	6	2.8	11.0	1.305
28[14]	26	a	0.366	4.16	0.44	47	28	83	10	6.6	10.3	1.087
28[15]	26	a	0.346	3.83	0.44	45	28	86	9	7.3	10.1	1.030
29	27	b	0.350	3.30	0.62	41	30	67	5	4.2	11.8	1.766
30	27	a	0.347	5.68	2.01	41	29	70	5	6.1	11.4	1.548
31	27	ab	0.439	5.15	1.53	40	27	83	15	5.7	11.0	1.261
32	28	a	0.411	4.76	0.85	41	32	84	8	7.1	12.2	1.782
33	29	o	0.349	4.76	1.17	41	29	77	6	4.7	9.5	0.869
34	29	ab	0.425	5.46	1.97	41	33	84	11	5.6	10.9	1.241
35	30	ab	0.380	3.44	0.57	40	26	84	8	3.5	11.0	1.315
36	30	b	0.408	4.09	0.93	44	28	89	9	4.2	12.4	1.903
37	30	o	0.534	4.91	0.92	44	33	79	16	4.9	11.4	1.299
38	36	a	0.375	5.23	0.57	44	31	83	8	4.8	10.4	1.114

[1]Number, [2]Age(yr), [3]Blood type, [4]Hematocrit(L/L), [5]Cholesterol (mmol/L), [6]Triglyceride(mmol/L), [7]Albumin(g/L), [8]Globulin(g/L) [9]Creatinine(μmol/L), [10]Bilirubin(μmol/L), [11]Urea(mmol/L), [12]Theophylline(mmol/L), [13]P:rbc, [14]First sample, [15]Second.

Table 1 Demographics of Volunteers. b Older volunteers. i Male

no[1]	a[2]	ab[3]	pcv[4]	ch[5]	tg[6]	al[7]	gl[8]	cr[9]	bil[10]	bun[11]	th[12]	p:r[13]
39	60	a	0.462	5.86	1.60	38	28	97	6	10.2	9.9	0.979
40	60	a	0.429	5.00	1.56	38	30	101	22	7.8	11.1	1.300
41	61	ab	0.381	4.62	2.86	41	28	94	4	7.3	11.8	1.668
42	62	o	0.461	4.62	0.97	41	25	104	7	5.5	10.1	1.022
43	62	o	0.434	6.15	1.25	44	26	96	10	5.7	11.6	1.466
44	63	o	0.417	6.94	1.38	39	32	116	8	10.7	11.0	1.279
45	65	a	0.401	5.37	1.54	36	29	108	14	7.3	10.9	1.259
46	65	o	0.415	5.80	1.55	44	25	86	13	6.9	11.5	1.458
47	65	o	0.452	5.93	2.39	42	36	112	7	6.6	11.9	1.546
48	67	o	0.395	5.12	0.96	38	30	87	6	6.2	9.5	0.882
49	67	a	0.518	9.01	1.76	43	37	127	25	5.8	11.2	1.261
50	67	a	0.435	4.43	0.92	38	35	100	13	7.1	11.1	1.295
51	69	a	0.424	3.89	0.97	38	31	111	7	8.4	10.5	1.127
52	70	o	0.435	6.05	0.76	40	31	105	8	5.3	11.1	1.295
53	72	a	0.400	4.96	1.36	38	36	88	16	4.6	10.5	1.135
54	73	a	0.455	6.30	2.31	39	29	133	25	12.7	5.5	1.250
55	74	b	0.424	6.27	1.13	39	34	84	7	5.1	10.9	1.242
56	78	o	0.459	5.71	1.33	37	32	107	17	8.6	12.0	1.570
57	84	n/a	0.310	4.14	1.13	28	37	132	9	6.3	10.5	1.182
58	85	a	0.385	4.58	0.90	34	31	89	7	5.8	10.3	1.082

[1]Number, [2]Age (yr), [3]Blood type, [4]Hematocrit(L/L), [5]Cholesterol (mmol/L), [6]Triglyceride(mmol/L), [7]Albumin(g/L), [8]Globulin (g/L) [9]Creatinine(μmol/L), [10]Bilirubin(μmol/L), [11]Urea(mmol/L), [12]Plasma theophylline(mmol/L), [13] P:rbc ratio.

Table 1 (Continued) b Older Volunteers. ii Female

no[1]	a[2]	ab[3]	pcv[4]	ch[5]	tg[6]	al[7]	gl[8]	cr[9]	bil[10]	bun[11]	th[12]	p:r[13]
59	63	o	0.389	5.95	1.50	39	31	97	8	7.3	11.9	1.696
60	66	a	0.391	6.54	1.01	42	25	72	5	3.6	10.7	1.201
61	66	o	0.370	6.10	0.94	37	30	106	13	5.3	10.5	1.148
62	66	a	0.384	5.78	0.57	41	26	76	8	7.3	11.7	1.609
63	67	o	0.422	4.99	1.27	38	36	97	8	6.2	13.3	2.427
64	68	o	0.394	7.12	1.86	43	19	100	1	6.2	12.3	1.903
65	69	o	0.362	5.62	1.16	39	29	86	9	5.5	11.0	1.335
66	70	a	0.415	6.57	1.29	39	32	98	6	7.0	11.3	1.384
67	71	a	0.387	4.36	0.73	36	30	77	7	6.2	10.2	1.053
68	72	a	0.398	4.96	0.66	40	25	95	16	5.9	10.9	1.262
69	73	o	0.345	5.87	1.71	40	31	104	2	6.2	12.2	2.095
70	75	o	0.370	7.34	2.76	35	29	78	6	7.0	11.3	1.451
71	75	b	0.405	5.27	3.07	37	28	75	8	5.5	11.6	1.517
72	76	o	0.352	5.69	1.05	38	31	83	7	6.3	10.6	1.192
73	82	b	0.403	6.80	0.66	35	26	77	7	5.9	12.1	1.756
74	88	n/a	0.367	4.41	1.34	35	29	76	6	5.9	11.4	1.503
75	91	a	0.391	4.30	0.70	39	29	72	10	3.6	12.3	1.917

[1]Volunteer number, [2]Volunteer age (years), [3]ABO blood type, [4]Hematocrit (L/L), [5]Cholesterol (mmol/L), [6]Triglyceride (mmol/L), [7]Albumin (g/L), [8]Globulin (g/L), [9]Creatinine (μmol/L), [10]Bilirubin (μmol/L), [11]Urea (mmol/L), [12]Plasma theophylline (mmol/L), [13]Theophylline p:rbc ratio (mmol/L: mmol/L).

blood cells on standing after collection. Hematological and biochemical analysis were performed on 5 ml and 10 ml aliquots of blood and serum respectively by the Departments of Clinical Chemistry and Hematology at the Health Sciences Centre, as a screening evaluation of the volunteers' health. The following data were obtained: complete blood count including hematocrit (Table 2a,b), and standard biochemical screening, including cholesterol, triglyceride, albumin, globulin, creatinine, bilirubin, and blood urea nitrogen (Table 2a,c). ABO blood type was determined using three drops of blood, antisera from the American Dade Division of the American Hospital Supply Company, and a low power microscope. Any remaining blood, to a maximum of 20 ml, was centrifuged. The buffy coat was discarded. Red cells and plasma were separated and frozen for later use in chromatographic analysis, in comparison with samples from the same individual after addition of drug.

b. Drug Equilibration Procedure

Preliminary quality control studies using outdated blood from the blood bank were performed to determine the incubation time necessary for equilibration between theophylline in plasma and cells, and to ensure that sufficient drug was added to yield plasma theophylline concentrations in the therapeutic range. No difference in plasma theophylline concentrations was found between samples incubated with whole blood at an ambient temperature of approximately 25°C and those incubated

Table 2 Biochemical and Hematological Parameters

a Data Expected to be Relevant to Drug Partitioning

		Younger		Older	
Group[1]		1 (males)	2 (females)	3 (males)	4 (females)
Parameter					
age	mean	25	26	68	73
(years)	S.D.	5	4	7	8
	n	19	20	20	17
			(1,3; 1,4; 2,3; 2,4)[2]		
cholesterol	mean	4.72	4.44	5.54	5.75
(2.97-5.20	S.D.	0.86	0.70	1.16	0.94
mmol/L)[3]	n	19	20	20	17
			(1,3; 1,4; 2,3; 2,4)[2]		
creatinine	mean	102	80	104	86
(70-110	S.D.	9	6	15	12
μmol/l)[3]	n	19	20	20	17
			(1,2; 2,3; 3,4)[2]		
bilirubin	mean	13	11	12	7
(3-18	S.D.	5	5	6	4
μmol/l)[3]	n	19	20	20	17
			(1,4)[2]		

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 a (Continued)		1	2	3	4
hematocrit	mean	0.45	0.39	0.43	0.39
(0.40-0.54,	S.D.	0.03	0.04	0.04	0.02
0.37-0.47	n	19	20	20	17
L/L)[3]			(1,2; 1,4; 2,3; 3,4)[2]		
triglyceride	mean	1.25	0.88	1.43	1.31
(0.30-2.30	S.D.	0.83	0.48	0.55	0.71
mmol/L)[3]	n	19	20	20	17
albumin	mean	45	43	39	39
(35-50g/L)[3]	S.D.	3	2	4	2
	n	19	20	20	17
			(1,2; 1,4; 2,4)[2]		
globulin	mean	29	30	31	29
(10-55g/L)[3]	S.D.	3	3	4	4
	n	19	20	20	17
blood urea	mean	5.6	5.1	7.2	5.9
nitrogen	S.D.	1.1	1.5	2.1	1.1
(2.8-7.1	n	19	20	20	17
mmol/L)[3]			(1,3; 2,3)[2]		

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 (Continued) b Hematological Data Not Expected to be of Major Significance to Drug Partitioning

		Younger		Older	
Group[1]		1 (males)	2 (females)	3 (males)	4 (females)
Parameter					
hemoglobin	mean	151	130	145	130
(140-180,	S.D.	8	10	15	8
120-160 g/L)[3]	n	18	18	20	17
			(1,2; 1,4; 2,3)[2]		
red cell count	mean	5.0	4.3	4.6	4.2
(4.6-6.2,	S.D.	0.3	0.4	0.6	0.3
4.2-5.4	n	18	18	19	15
x 10^{12}/L)[3]			(1,4; 3,4)[2]		
mean cell	mean	90	89	92	91
volume	S.D.	3	3	4	3
(86-100	n	18	18	20	17
fL)[3]					
mean cell	mean	31	31	31	31
hemoglobin	S.D.	1	1	2	1
(27-31 pg)[3]	n	18	18	19	16

[1] Groups classified according to age and gender

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 b (Continued)		1	2	3	4
mean cell hemoglobin concentration (330-370 g/L)[3]	mean	343	340	341	336
	S.D.	4	6	6	4
	n	18	18	20	17
			(1,4; 3,4)[2]		
red cell width distribution 11.5-14.5 no units)[3]	mean	12.9	12.9	13.5	13.7
	S.D.	0.7	0.8	1.0	0.7
	n	18	18	20	17
			(2,4)[2]		
platelet count (140-440 x 10^9/L)[3]	mean	263	280	261	284
	S.D.	70	51	62	59
	n	18	18	20	17
leukocyte count (4.5-11.0 x 10^9/L)[3]	mean	5.5	6.0	6.2	5.6
	S.D.	1.3	1.4	1.5	0.9
	n	18	18	20	17

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 b (Continued)		1	2	3	4
lymphocyte count (1.1-3.6 x 10^9/L)[3]	mean	1.9	1.9	1.7	2.1
	S.D.	0.6	0.5	0.5	0.7
	n	18	18	19	16
granulocyte percentage (55-81%)[3]	mean	57	55	68	62
	S.D.	6	11	7	9
	n	12	10	11	11
			(1,3; 2,3)[2]		
monocyte percentage (3-7%)[3]	mean	6	6	6	5
	S.D.	1	4	3	3
	n	12	10	11	11
mean platelet volume (7.4-10.4 fL)[3]	mean	9.9	9.8	8.9	9.7
	S.D.	1.7	1.1	0.7	1.2
	n	9	7	10	5

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 (Continued) c Biochemical Data, Not Expected to be of Major Significance to Drug Partitioning

		Younger		Older	
Group[1]		1 (males)	2 (females)	3 (males)	4 (females)
Parameter					
sodium	mean	142	137	140	140
(135-147	S.D.	6	3	4	3
mmol/L)[3]	n	19	20	20	17
			(1,2)[2]		
potassium	mean	5.2	6.2	5.4	5.5
(3.5-5.3	S.D.	1.5	2.9	1.9	1.7
mmol/L)[3]	n	19	20	20	17
chloride	mean	107	104	106	107
(95-110	S.D.	7	3	5	5
mmol/L)[3]	n	19	20	20	17
total CO2	mean	26	24	26	25
content	S.D.	2	2	2	2
(22-30	n	19	20	20	17
mmol/L)[3]			(1,2)[2]		

[1] Groups classified according to age and gender

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 c (Continued)		1	2	3	4
calcium	mean	2.31	2.22	2.21	2.2
(2.10-2.60	S.D.	0.07	0.06	0.11	0.09
mmol/L)[3]	n	19	20	20	17
			(1,3)[2]		
phosphate	mean	1.21	1.23	1.02	1.2
(0.81-1.45	S.D.	0.13	0.12	0.19	0.10
mmol/L)[3]	n	18	17	19	16
			(1,3; 2,3; 3,4)[2]		
magnesium	mean	0.76	0.73	0.74	0.77
(0.70-0.96,	S.D.	0.10	0.08	0.08	0.05
0.63-0.94	n	19	19	19	15
mmol/L)[3]					
glucose	mean	4.3	4.1	5.0	4.7
(3.6-6.1	S.D.	0.5	0.6	0.7	0.6
mmol/L)[3]	n	19	20	20	17
			(2,3; 2,4)[2]		

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

Table 2 c (Continued)		1	2	3	4
uric acid	mean	322	217	328	233
(120-420	S.D.	58	46	70	47
μmol/L)[3]	n	19	18	20	17
			(1,2; 2,3)[2]		
alanine	mean	25	16	21	13
transaminase	S.D.	15	14	6	4
(0-30, 0-25	n	18	20	20	17
U/L)[3]			(1,4)[2]		
aspartate	mean	22	17	20	20
transaminase	S.D.	6	6	6	4
(10-32 U/L)[3]	n	19	20	20	17
gamma glutamyl	mean	19	11	30	14
transferase	S.D.	11	4	20	4
(5-38 U/L)[3]	n	19	19	20	17
			(2,3)[2]		
lactate	mean	162	152	166	177
dehydrogenase	S.D.	34	28	20	15
(63-200 U/L)[3]	n	18	19 (2,4)[2]	19	16

[2] Pairs of groups differing by Tukey's multiple comparison test at p=0.05, separated by semicolons.

[3] Normal range; if a gender difference, values for males first

at 37°C. The final incubation conditions involved addition of 60 μl of 1.0 mg/ml theophylline in methanol to 6 ml of blood, yielding a final whole blood concentration of 10 mg/L theophylline. Blood was incubated with shaking at 37°C for 30 minutes. Cells and plasma were centrifuged in a hematocrit tube immediately after incubation, the buffy coat was discarded and red cells and plasma were separated and frozen for later analysis.

c. Analysis by High Performance Liquid Chromatography (HPLC)

i. Theory

HPLC has been described as a separation technique based on equilibration between a liquid mobile phase, the solvent, and a stationary phase, most commonly a steel column filled with particles of silica gel whose bonded coating determines its characteristics. Types of chromatography have included adsorption, characterized by a liquid mobile phase and solid stationary phase, and partition, characterized by a liquid mobile phase and liquid stationary phase. Ion exchange chromatography has separated compounds according to charge, and size exclusion chromatography according to size (Rodgers, 1974). The apparatus used for HPLC has been as follows: solvent has been blended by a stirrer and propelled through the system by a pump; sample containing solutes of interest added to the solvent by an injector; solutes, after separation by the column, measured by a detector; the ensuing data

depicted graphically by a recorder, and the area of any peak on this graph calculated by an integrator (Schram, 1981). The goals of HPLC have been good resolution, determined by peak width, retention time, and separation. Resolution, R, has been defined as the ratio of distance between peaks to their average base widths. Peak width has been measured by efficiency, N. Retention time has been measured by capacity, k', the distance between a peak representing solute and a peak not retained on the column. Separation has been measured by selectivity or α, the ratio of solute retention times. The above has been expressed as follows:

$$R_s = 1/4 \left[(\alpha - 1)/\alpha \right] \left[\sqrt{N}\right] \left[k'/(k' + 1)\right]$$

R_s, the resolution of a solute, has been improved by varying column packing or length, solvent components or strength, flow rate, and pressure. It has been necessary to balance large k' values, yielding good, but slow resolution, against practical limits on available time (Anonymous, unknown publication date). An internal standard (i.s.), similar to the solute of interest, has sometimes been added to the sample to correct for variations in extraction and handling that might alter the quantity of solute of interest.

ii. Sample Analysis

Sample assay was a modification of the technique described by Borga et al (1983). The final assay conditions have been presented in Table 3. Reproducibility had

Table 3 Specifications for HPLC Assay

Internal standard[1]:	3,7-dihydro-1-ethyl-3-(2-hydroxypropyl)-H purine-2,6-dione
Solvent[2]:	20:80 methanol: 0.01 M acetate buffer pH 4.0
Column:	C^{18}, 30 x 0.39 cm internal diameter, 10 μm particles
Temperature:	ambient (25°C)
Flow rate:	1.5 ml per min
Injection volume:	50 μL
Run time:	15 min
Ultraviolet detection:	280 nm

[1] D4126, Astra Pharmaceuticals Limited

[2] HPLC grade methanol, Fisher; reagent grade acetic acid, Fisher

previously been demonstrated for this assay in the present laboratory (Sitar et al, 1987). Outdated blood and plasma from the blood bank were used for calibration curves relating theophylline: i.s. concentration ratios to known amounts of drug added to plasma. Fresh blood samples were taken to characterize the pattern of chromatographic peaks in plasma collected in vacuum tubes. Heparin was preferred to EDTA as an anticoagulant because fewer interfering peaks were present. At the conclusion of the experiment, plasma samples from drug incubations were thawed and analysed in a few days under consistent conditions.

B. Data Analysis

Calibration curves of theophylline added to plasma enabled determination of plasma drug concentrations corresponding to peak height ratios of drug to i.s. The p:rbc concentration ratios were calculated from the plasma concentration (C_p), the total drug concentration in whole blood (T), and the hematocrit (H) of the donor, as follows:

$$p:rbc = C_p \; / \; \{ \; [T - C_p(1-H)] \; /H \; \}$$

This procedure ignored the potential contribution of uptake by other types of blood cells.

C. Statistical Analysis

Calculations were performed with the aid of the SYSTAT and SAS statistics computer programs. A two-way analysis of

variance of p:rbc was carried out with respect to age, gender, and any interaction between these variables, at an appropriate degree of stringency to yield an overall p value of at least 0.05 for a significant result. In order to explain the observations, a similar analysis of variance was performed for each previously selected hematological or biochemical parameter; age and gender categories were compared by Tukey's multiple comparison test at $p = 0.05$. The theophylline p:rbc ratio was tested for adherence to a normal distribution by the X^2 test. Non-normally distributed data was log transformed before further analysis. The data were separated according to gender and a Pearson correlation matrix was generated for the transformed p:rbc ratio with hematological and biochemical parameters, in order to identify variables sharing the greatest amount of variance with the p:rbc ratio. Stepwise regression was performed separately for each gender, using the independent variable p:rbc ratio and the dependent variables age and the following hematological and biochemical parameters: albumin, cholesterol, triglyceride, bilirubin, creatinine, and blood urea nitrogen.

III. RESULTS

A. Characteristics of Volunteers

The demographics of the volunteers, as well as age, gender, plasma theophylline, and selected hematological and biochemical data, have been presented in Tables 1a and b. A summary of the relationship of age and gender category to theophylline p:rbc ratio and hematological and biochemical data has been presented in Table 2a, b, and c and Figure 1a, b, and c. The age distribution was not significantly different for males and females (Table 2a). By far the majority of hematological and biochemical values were within the normal range (Table 2a,b,c). Potassium concentrations were somewhat elevated in all age and gender categories. Blood urea nitrogen was raised modestly in older females. The mean red cell count in older people was at the lower limit of normal, although hemoglobin and hematocrit were within normal limits.

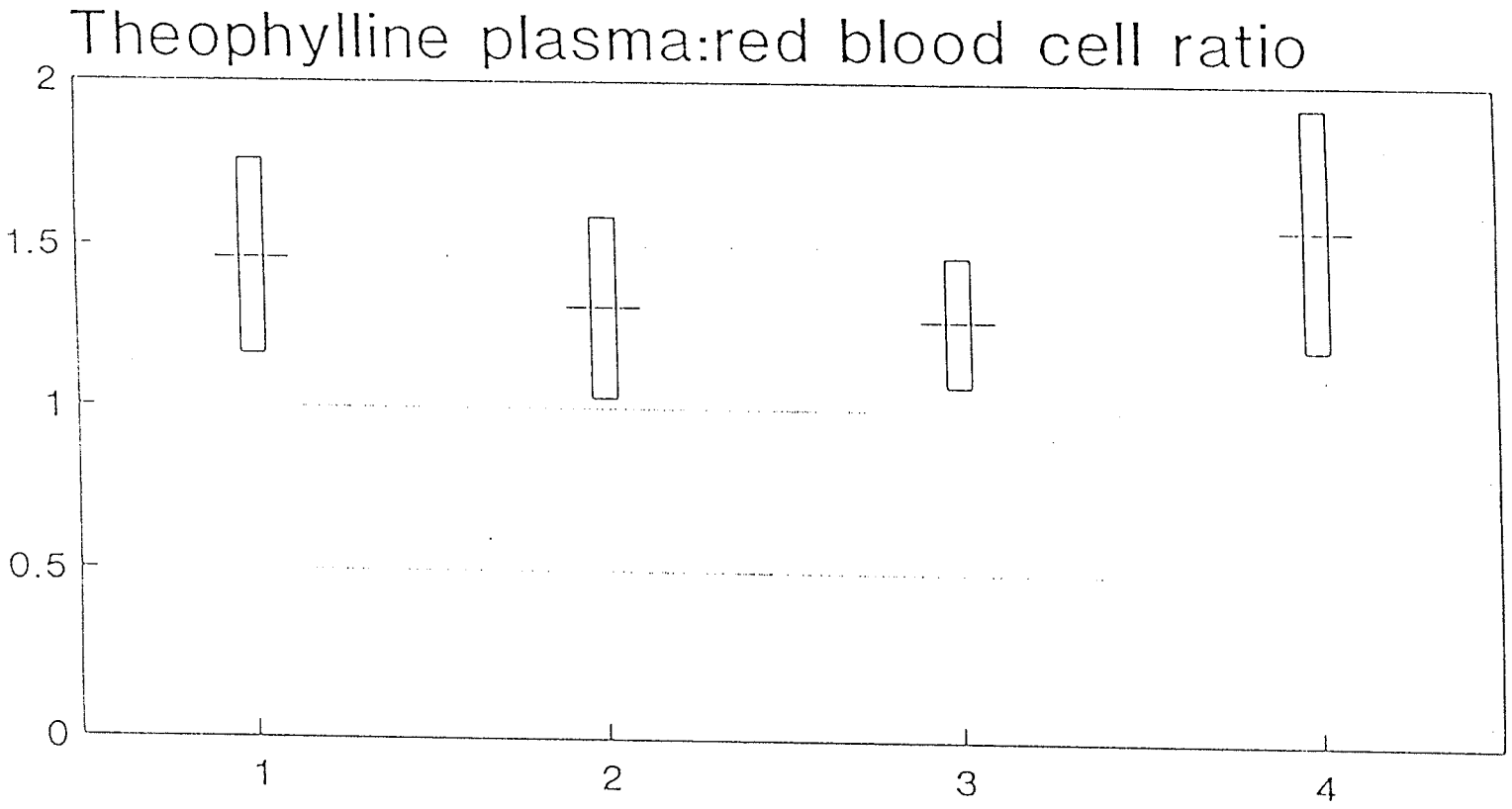
Several age and/or gender differences were observed in the biochemical and hematological parameters selected for potential relevance to drug partitioning, as for hematocrit, cholesterol, albumin, creatinine, bilirubin, and blood urea nitrogen (Table 2a). Hematocrit did not differ significantly for each gender, although all other comparisons were significant. Cholesterol was higher in the elderly but not different by gender for a similar age range. The large individual variation in triglyceride and in globulin precluded

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

a. Theophylline p:rbc Ratio

This figure has demonstrated the relationship between age and gender category and mean theophylline plasma: red blood cell partitioning ratio, a quantity without units, represented as a horizontal line with a bar signifying one standard deviation extending above and below this line. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

The theophylline plasma: red blood cell partitioning ratio exhibited a significant age-related gender effect: it tended to decrease with age for men and increase for women. A significant difference was obtained by Tukey's multiple comparison test, at $p=0.05$, between older males and older females only. This unique finding was examined **post hoc** to determine the presence of any possible association with changes in measured hematological and biochemical parameters.



1 younger males
2 younger females
3 older males
4 older females

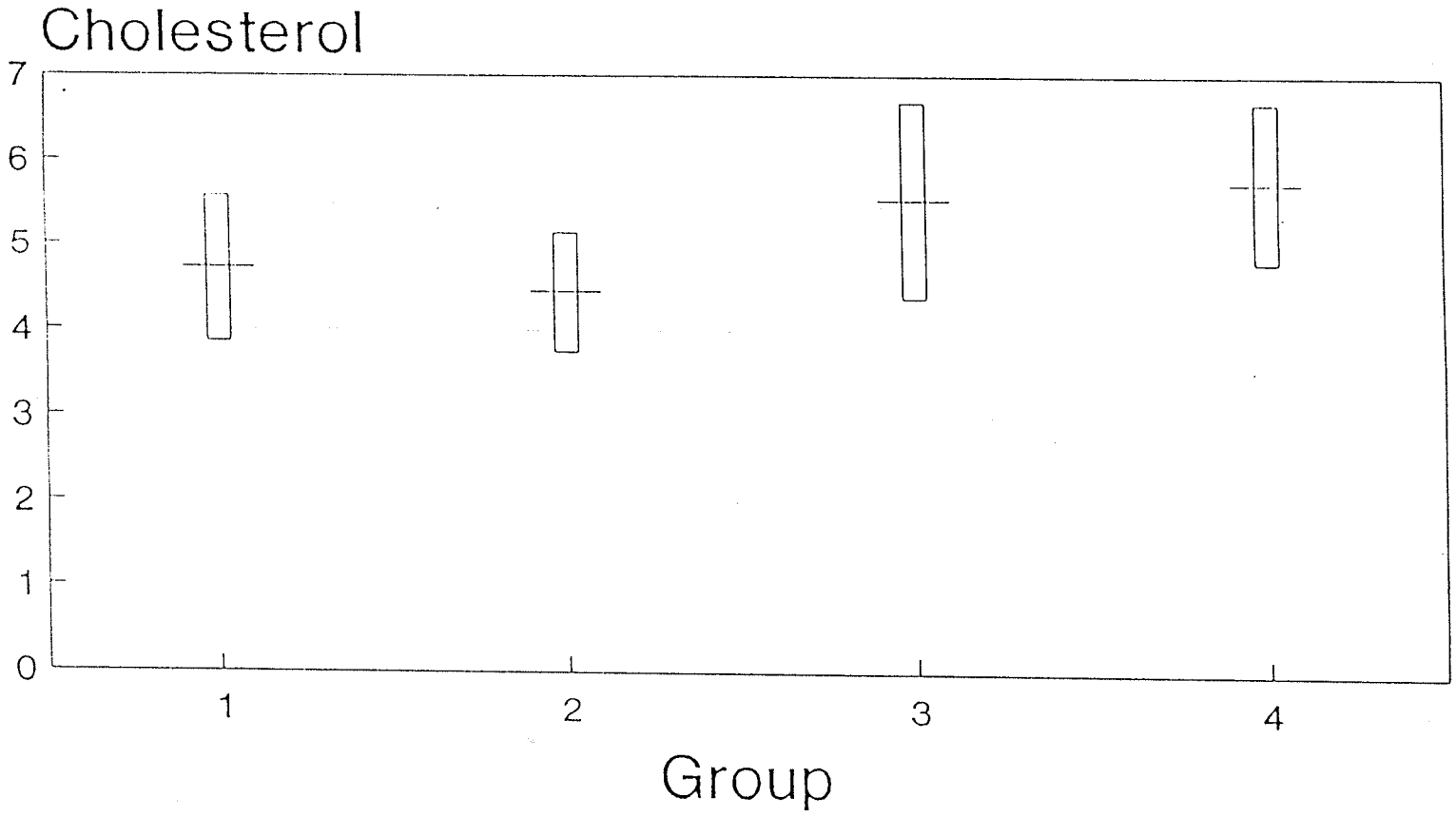
Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

b. Hematological and Biochemical Parameters Correlated with Theophylline p:rbc Ratio

i. Cholesterol

This figure has demonstrated the relationship between age and gender category and mean serum cholesterol concentration, measured in mmol/L and represented as a horizontal line with a bar signifying one standard deviation extending above and below this line. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Cholesterol was the second most significant determinant of the theophylline plasma: red blood cell partitioning ratio in men, next after age. Cholesterol tended to increase with age for both men and women. Significant differences existed between any younger and any older group, according to Tukey's multiple comparison test at $p=0.05$.



1 younger males
2 younger females
3 older males
4 older females

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

b. Hematological and Biochemical Parameters Correlated with Theophylline p:rbc Ratio

ii. Creatinine

This figure has demonstrated the relationship between age and gender category and mean serum creatinine concentration, measured in μmol/L and represented as a horizontal line with a bar signifying one standard deviation extending above and below this line. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

The third most significant determinant of the theophylline plasma: red cell partitioning ratio (p:rbc ratio) in men, creatinine tended to remain approximately constant with age and to be slightly higher in men than women. The significant differences among the four age groups were between younger men and women, older men and women, and younger women and older men, according to Tukey's multiple comparison test at $p=0.05$.

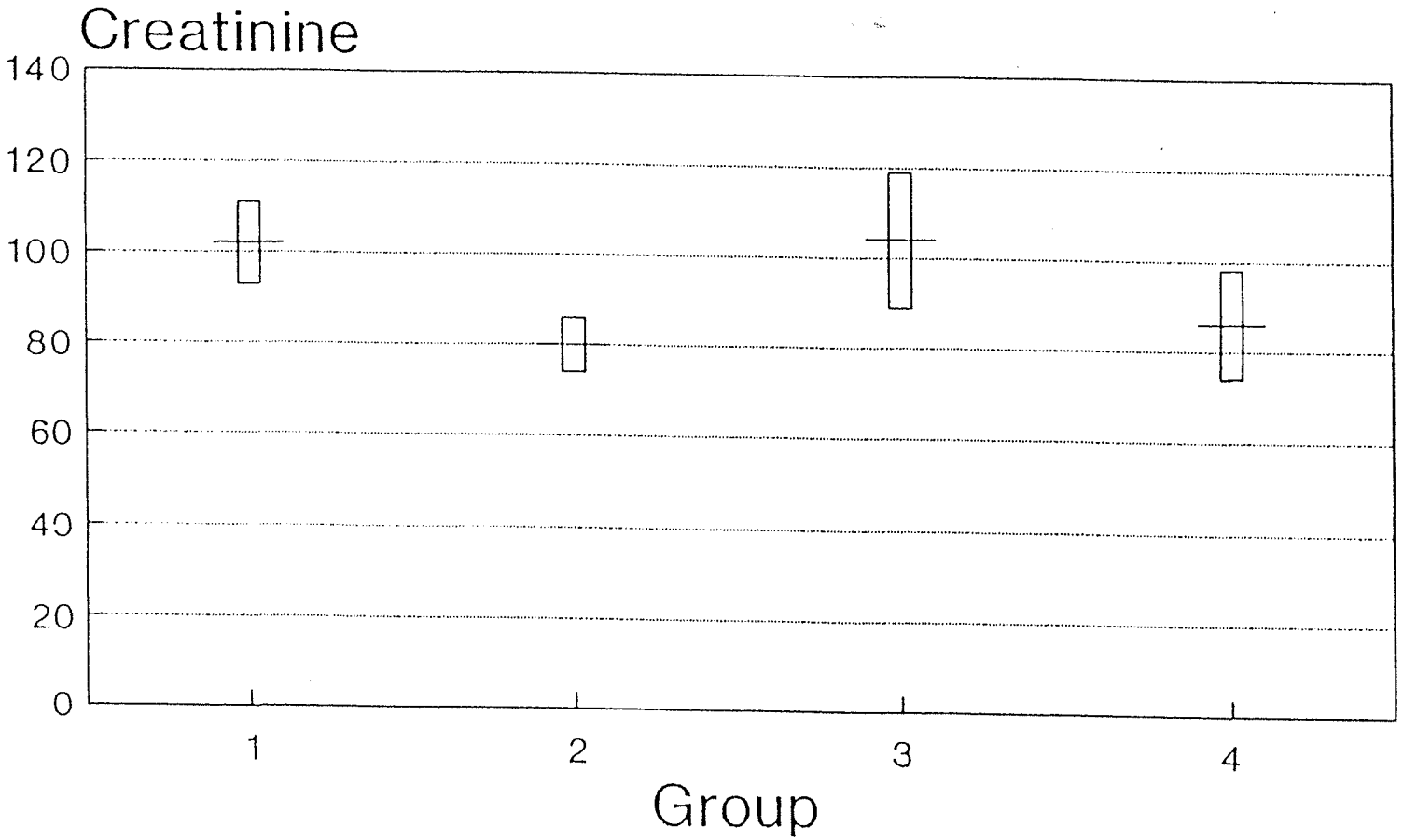

Creatinine
140
120
100
80
60
40
20
0
1
2
3
4
Group
1 younger males
2 younger females
3 older males
4 older females

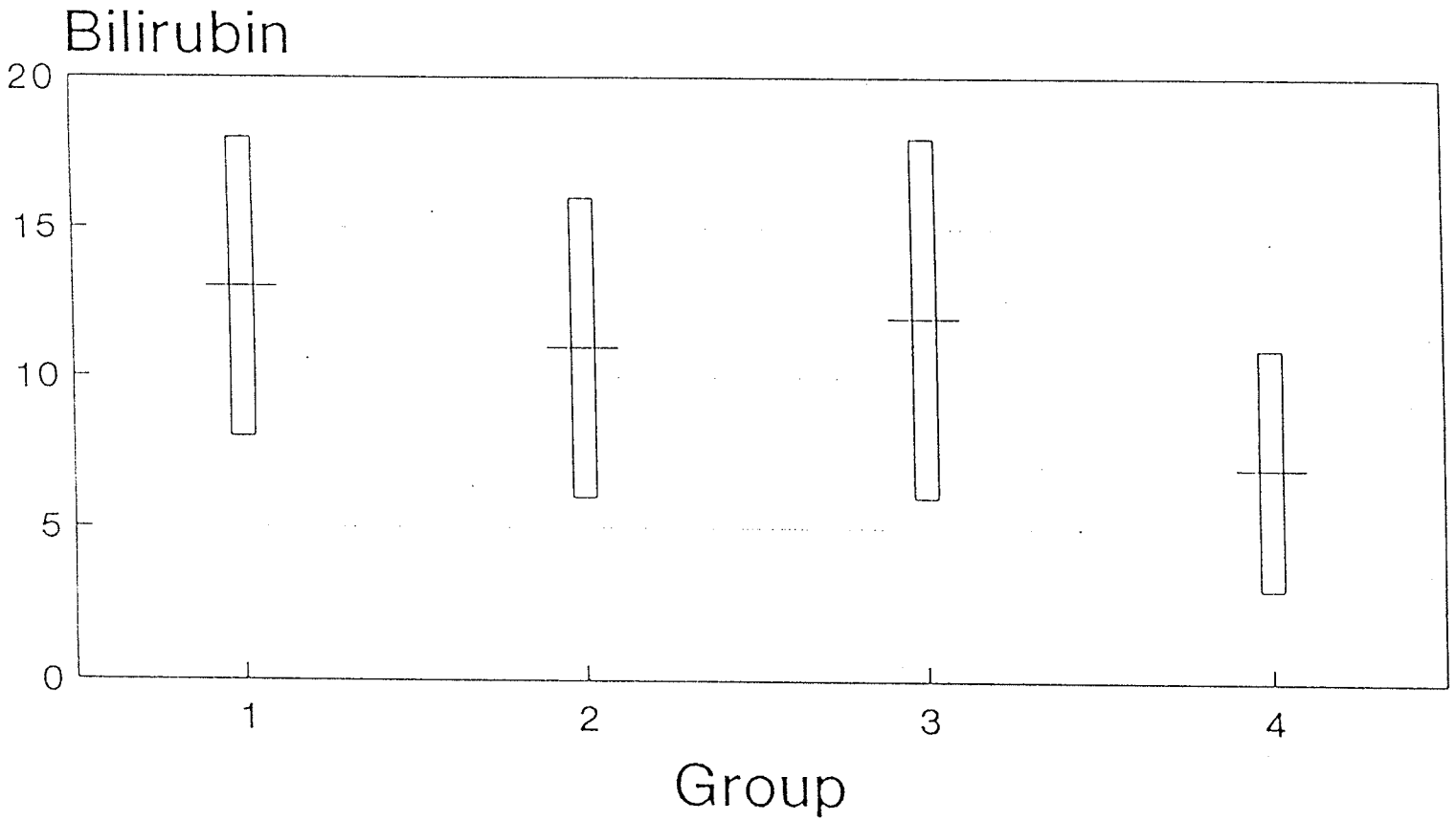
Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

b. Hematological and Biochemical Parameters Correlated with Theophylline p:rbc Ratio

iii. Bilirubin

This figure has demonstrated the relationship between age and gender category and mean serum bilirubin concentration, measured in μmol/L and represented as a horizontal line with a bar signifying one standard deviation extending above and below this line. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Bilirubin, the most significant determinant of the theophylline plasma: red cell partitioning ratio (p:rbc ratio) in women, was less in older women than younger men, significantly according to Tukey's multiple comparison test at $p = 0.05$.



1 younger males
2 younger females
3 older males
4 older females

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

c. Hematological and Biochemical Parameters Found Not to be Correlated with Theophylline p:rbc Ratio

i. Hematocrit

This figure has demonstrated the relationship between age and gender category and mean serum hematocrit, measured in L/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Hematocrit was less in women than men, significantly so according to Tukey's multiple comparison test at $p = 0.05$.

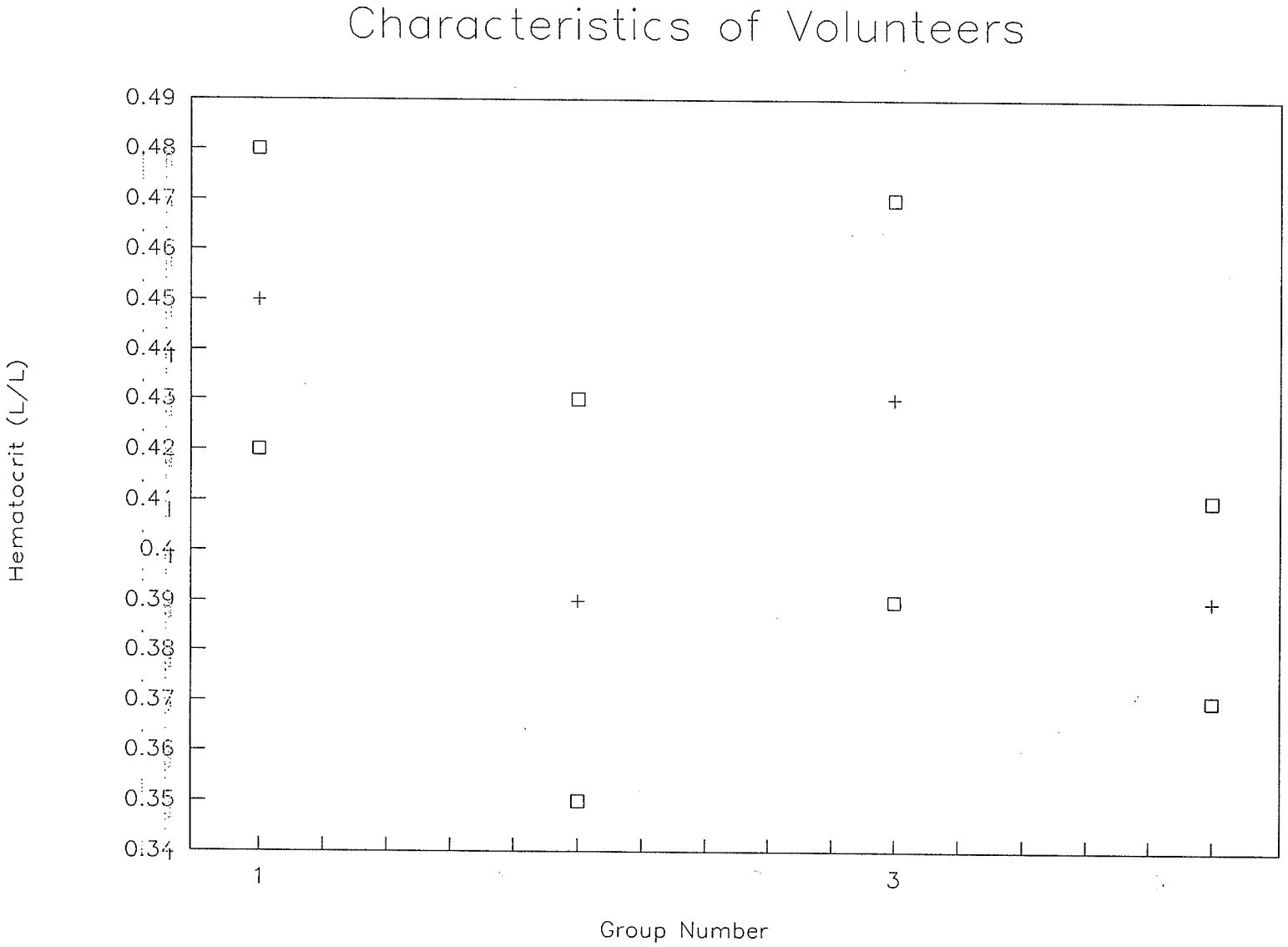

Characteristics of Volunteers
Hematocrit (L/L)
0.49
0.48
0.47
0.46
0.45
0.44
0.43
0.42
0.41
0.4
0.39
0.38
0.37
0.36
0.35
0.34
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

c. Hematological and Biochemical Parameters Found Not to be Correlated with Theophylline p:rbc Ratio

ii. Triglyceride

This figure has demonstrated the relationship between age and gender category and mean serum triglyceride concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Triglyceride did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

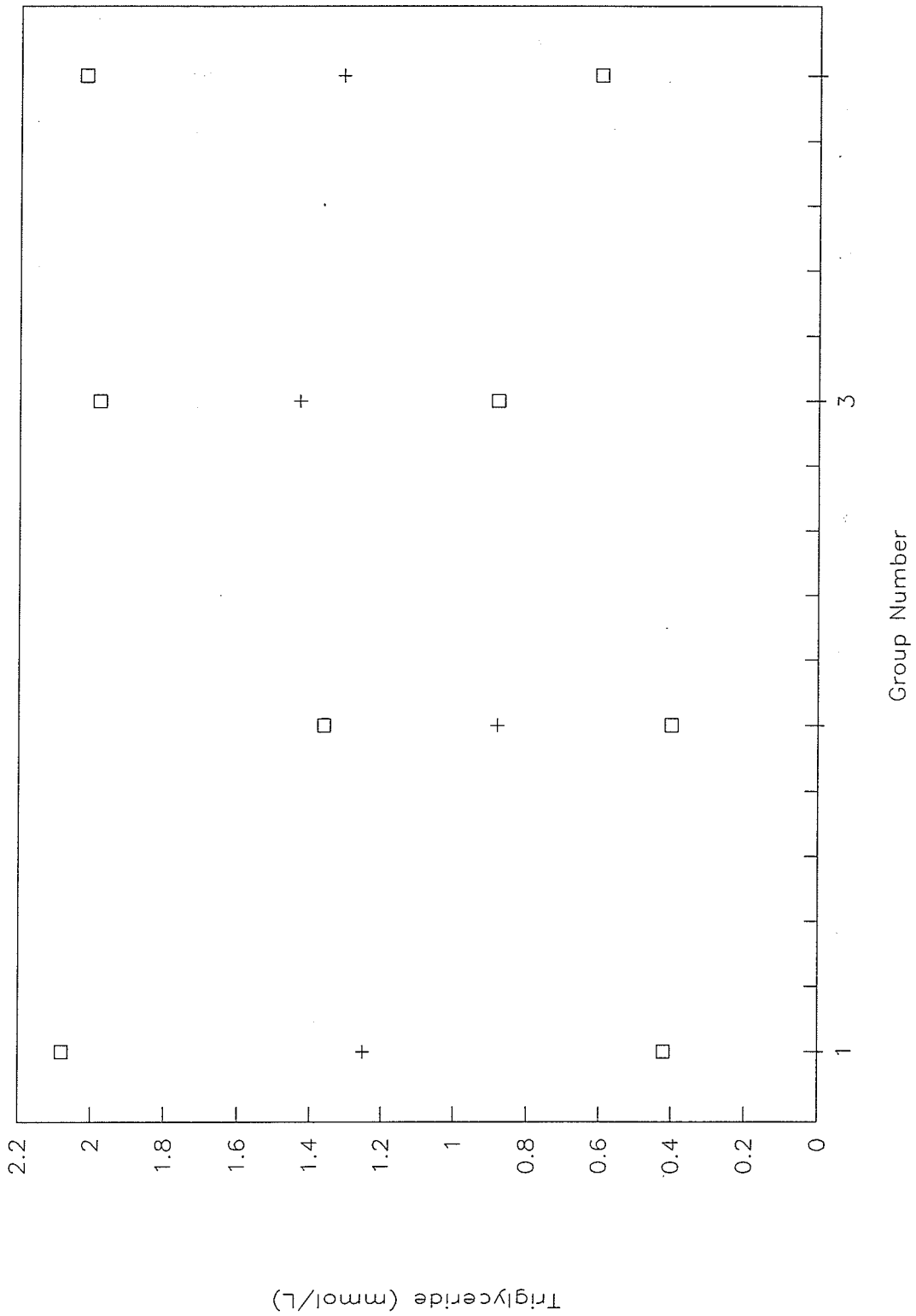

Triglyceride (mmol/L)
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

c. Hematological and Biochemical Parameters Found Not to be Correlated with Theophylline p:rbc Ratio

iii. Albumin

This figure has demonstrated the relationship between age and gender category and mean serum albumin concentration, measured in g/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Albumin was highest in younger men and tended to decrease with age. Significant differences were present among younger men and younger women, younger men and older women, and younger and older women, according to Tukey's multiple comparison test at $p = 0.05$.

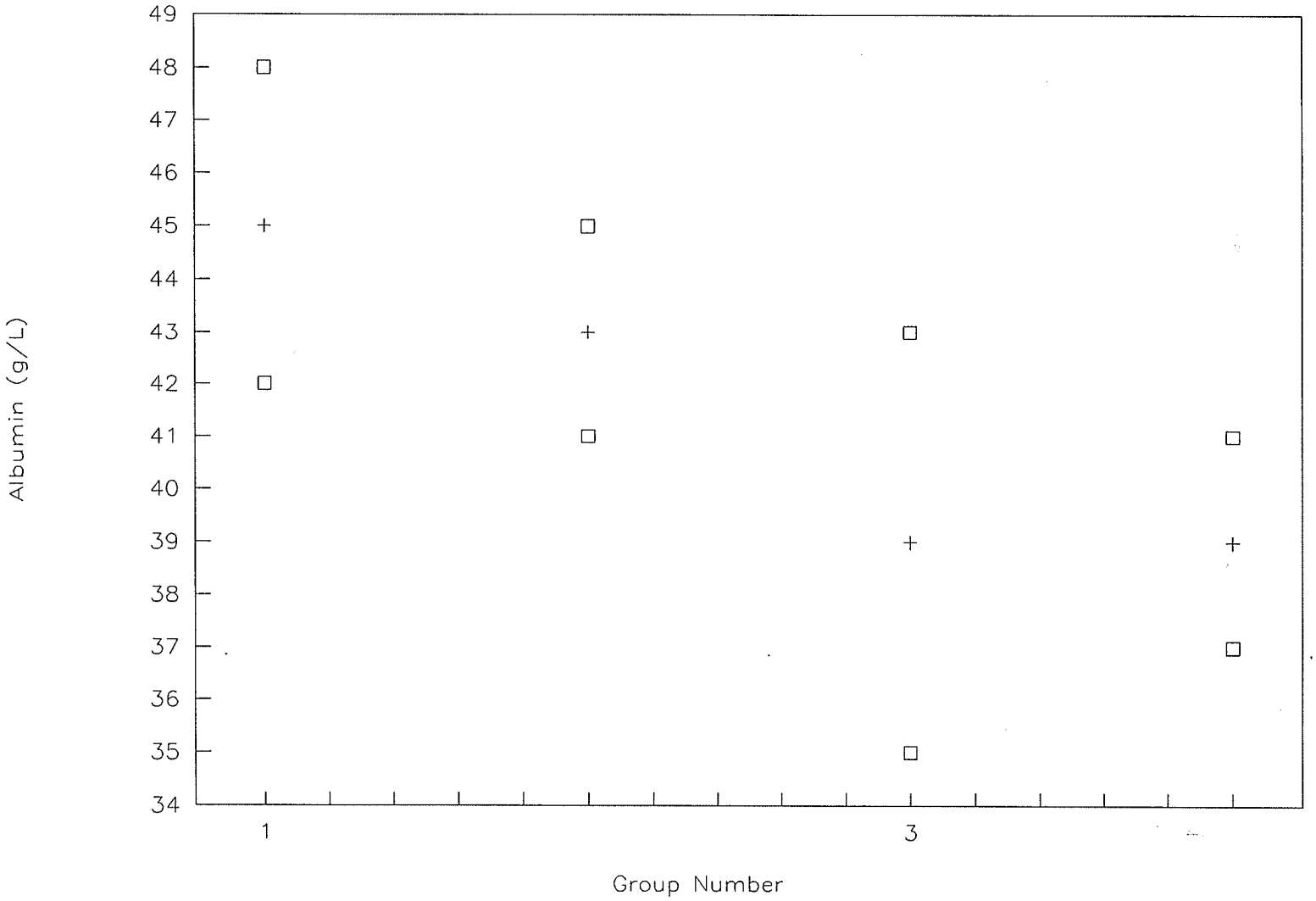

49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
Albumin (g/L)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

c. Hematological and Biochemical Parameters Found Not to be Correlated with Theophylline p:rbc Ratio

iv. Globulin

This figure has demonstrated the relationship between age and gender category and mean serum globulin concentration, measured in g/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Globulin did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

Globulin (g/L)
Group Number
1
3

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

c. Hematological and Biochemical Parameters Found Not to be Correlated with Theophylline p:rbc Ratio

v. Blood Urea Nitrogen

This figure has demonstrated the relationship between age and gender category and mean blood urea nitrogen, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Blood urea nitrogen differed significantly among younger and older men, and between younger women and older men, according to Tukey's multiple comparison test at $p = 0.05$.

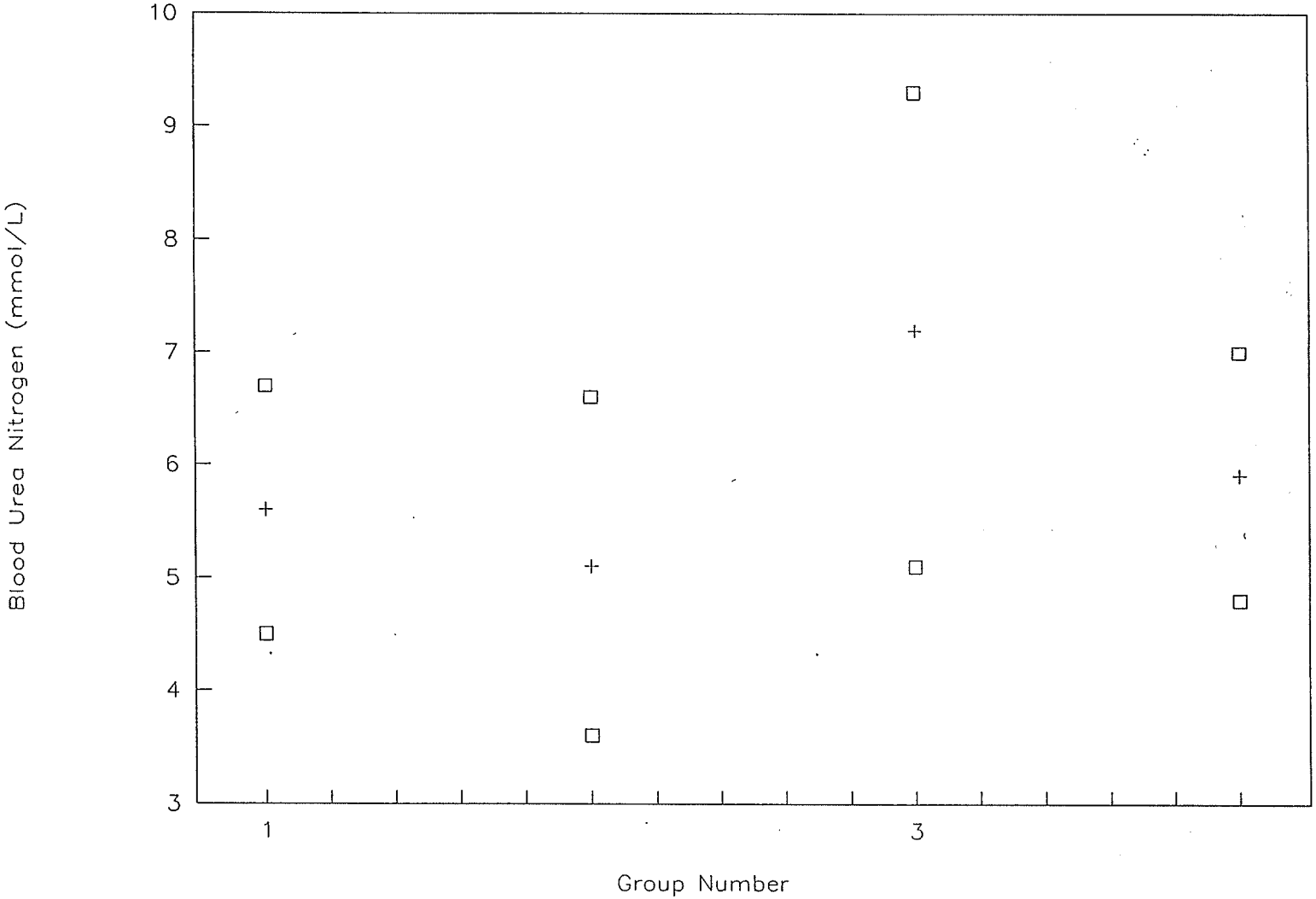

Blood Urea Nitrogen (mmol/L)
10
9
8
7
6
5
4
3
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

i. Hemoglobin

This figure has demonstrated the relationship between age and gender category and mean hemoglobin concentration, measured in g/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Hemoglobin differed significantly among younger males and females, younger males and older females, and younger females and older males according to Tukey's multiple comparison test at $p = 0.05$.

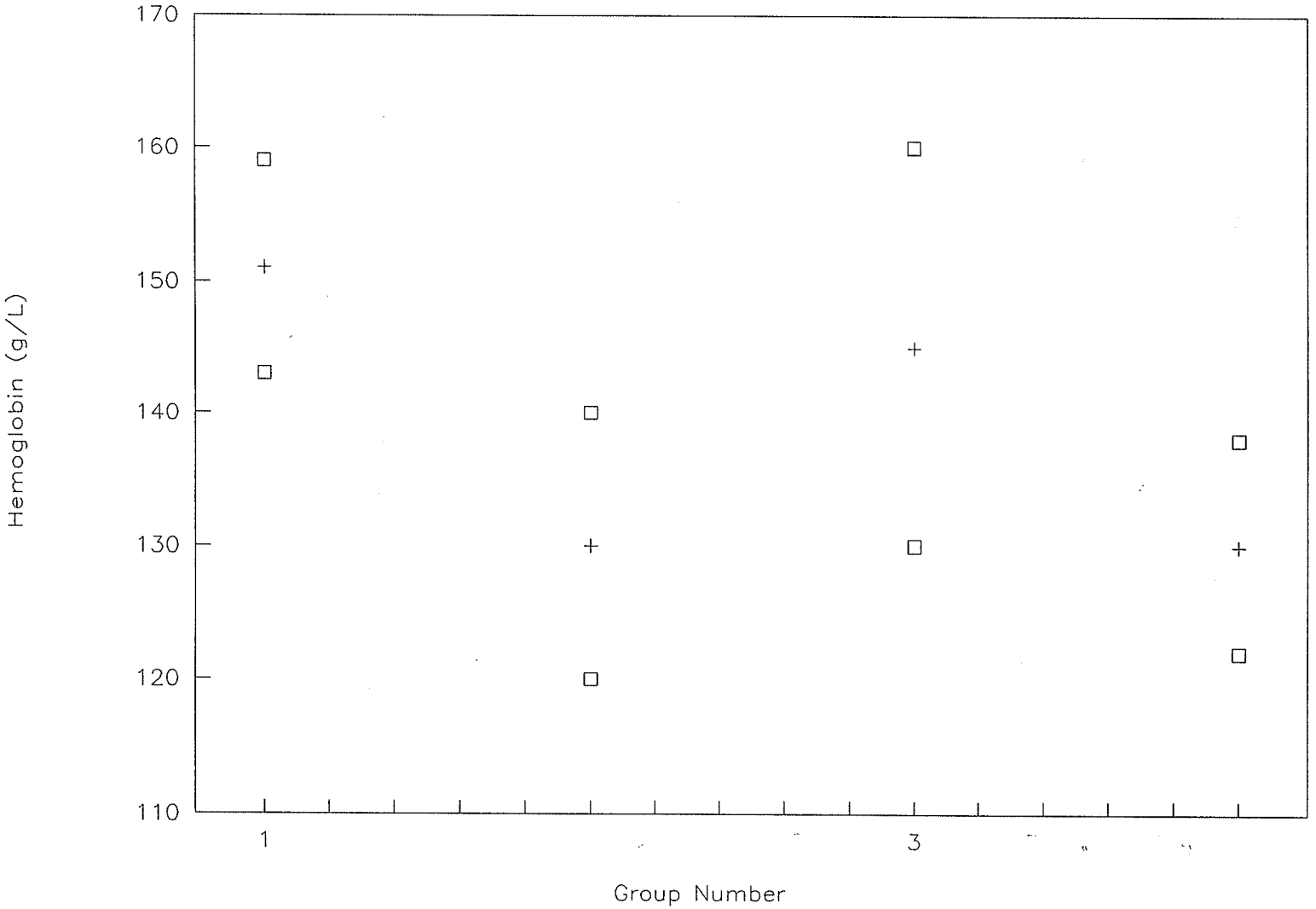

170
160
150
140
130
120
110
Hemoglobin (g/L)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

ii. Red Cell Count

This figure has demonstrated the relationship between age and gender category and mean red cell count, measured in number x 10^{12} /L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Red cell count differed significantly between younger males and older females, and between older males and females according to Tukey's multiple comparison test at $p = 0.05$.

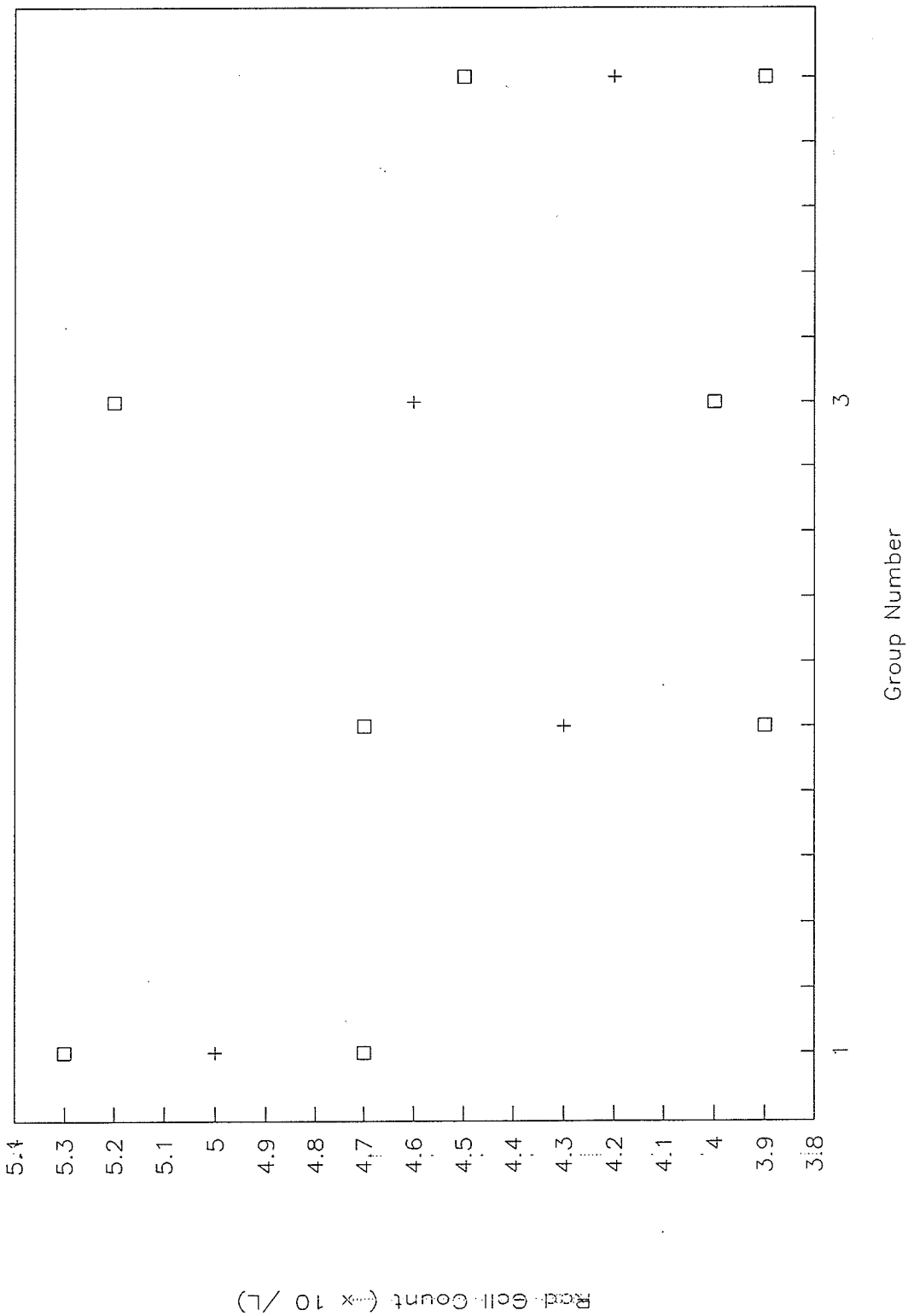

Red Cell Count (x 10 /L)
5.4
5.3
5.2
5.1
5
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4
3.9
3.8
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

iii. Mean Cell Volume

This figure has demonstrated the relationship between age and gender category and average mean cell volume, measured in femtoliters and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Mean cell volume did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

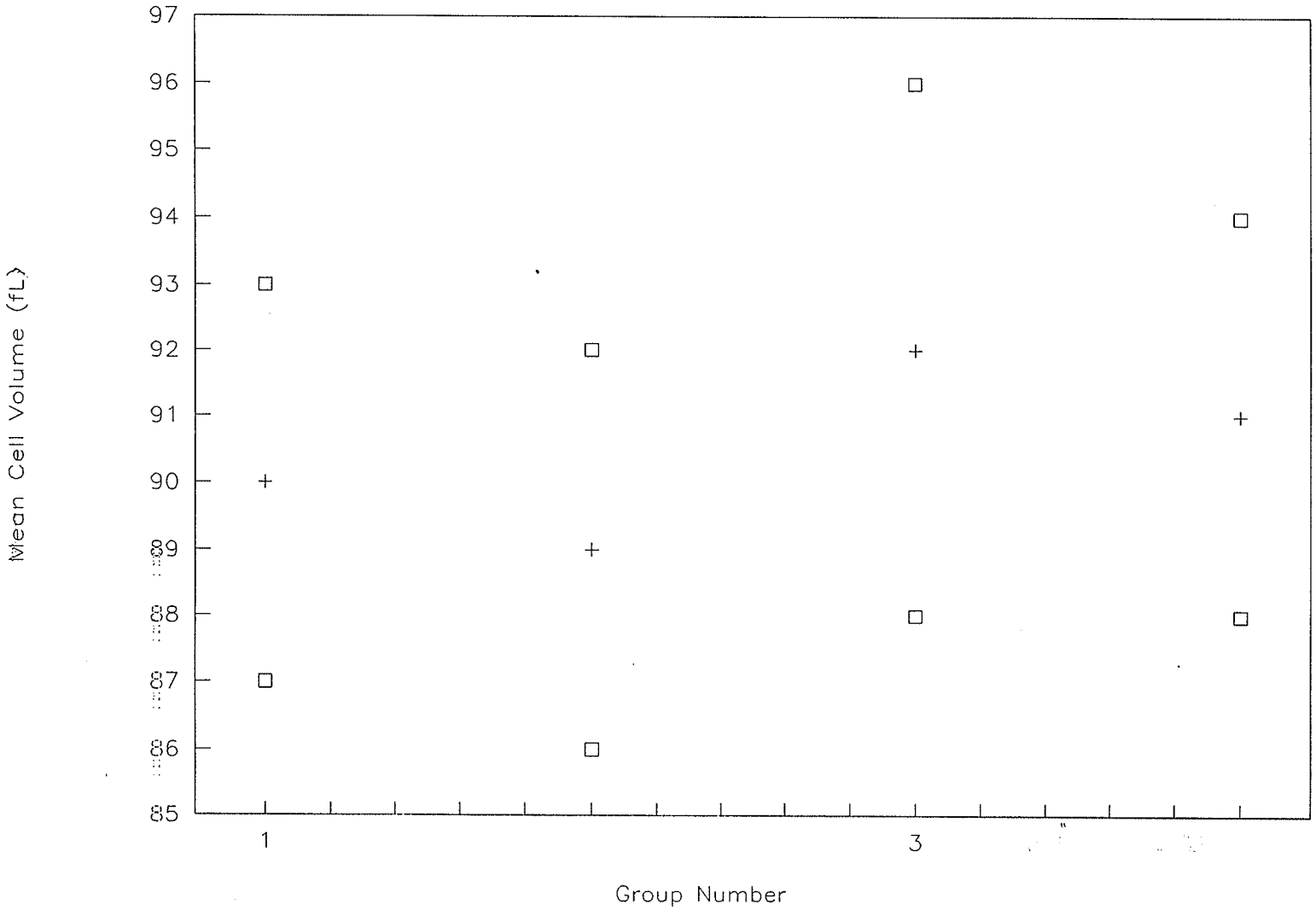

97
96
95
94
93
92
91
90
89
88
87
86
85
Mean Cell Volume (fL)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

iv. Mean Cell Hemoglobin

This figure has demonstrated the relationship between age and gender category and average mean cell hemoglobin, measured in picograms and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Mean cell hemoglobin did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

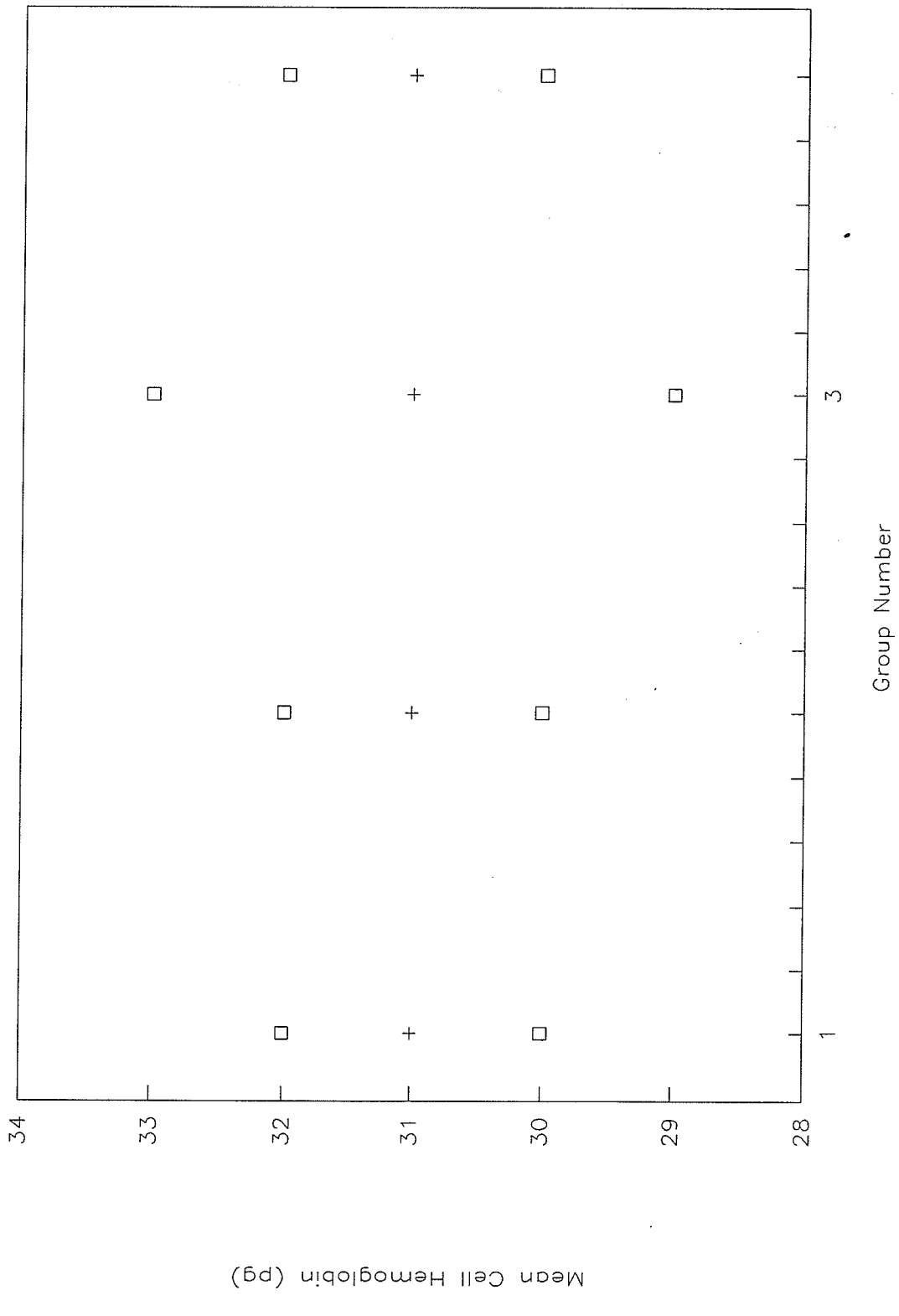

Mean Cell Hemoglobin (pg)
34
33
32
31
30
29
28
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

v. Mean Cell Hemoglobin Concentration

This figure has demonstrated the relationship between age and gender category and average mean cell hemoglobin concentration, measured in g/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Mean cell hemoglobin concentration differed significantly between younger men and older women as well as between older women and men according to Tukey's multiple comparison test at $p = 0.05$.

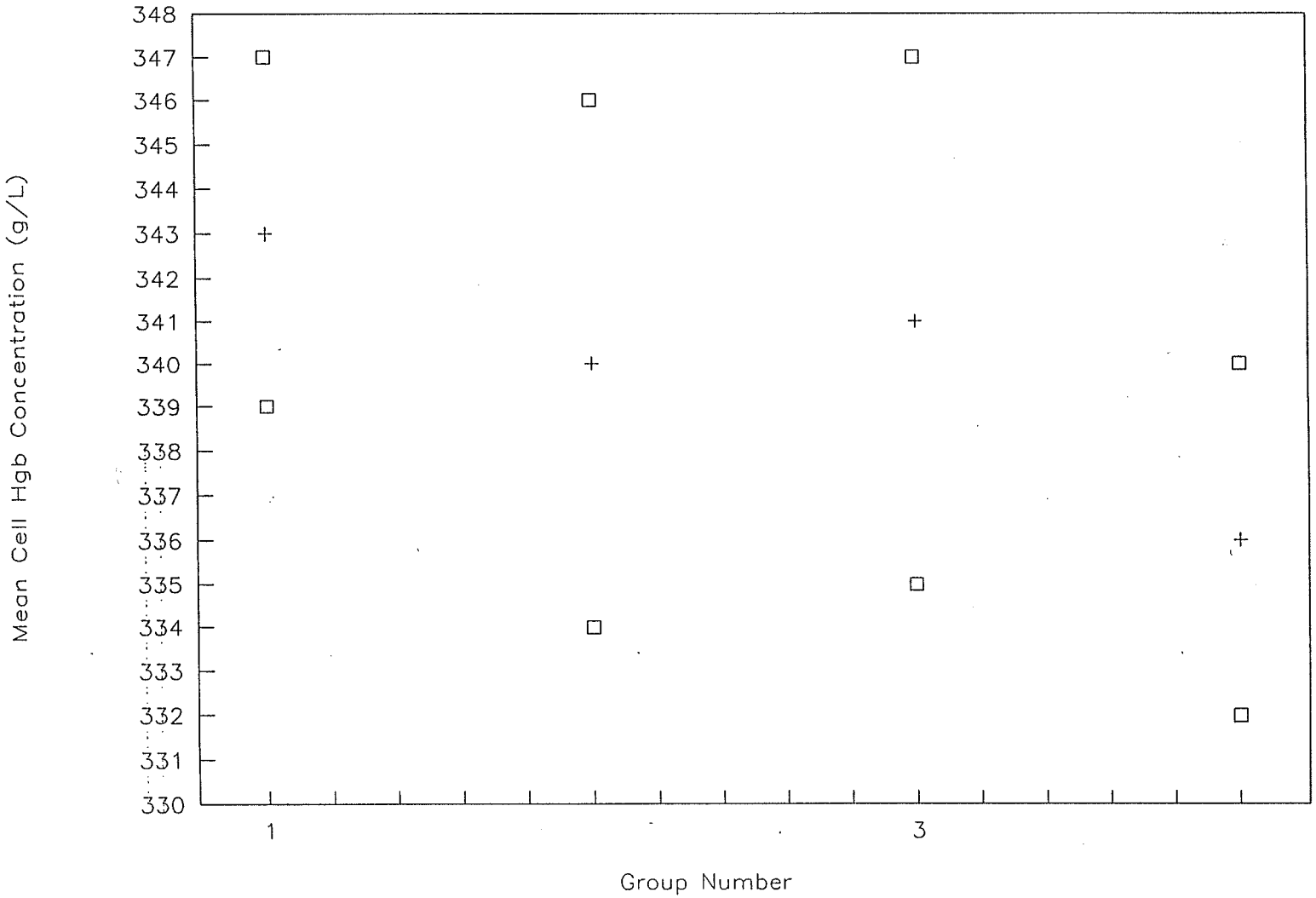

Mean Cell Hgb Concentration (g/L)
348
347
346
345
344
343
342
341
340
339
338
337
336
335
334
333
332
331
330
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

vi. Red Cell Width Distribution

This figure has demonstrated the relationship between age and gender category and mean red cell width distribution, a quantity without units, represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Red cell width distribution differed significantly between younger and older women according to Tukey's multiple comparison test at $p = 0.05$.

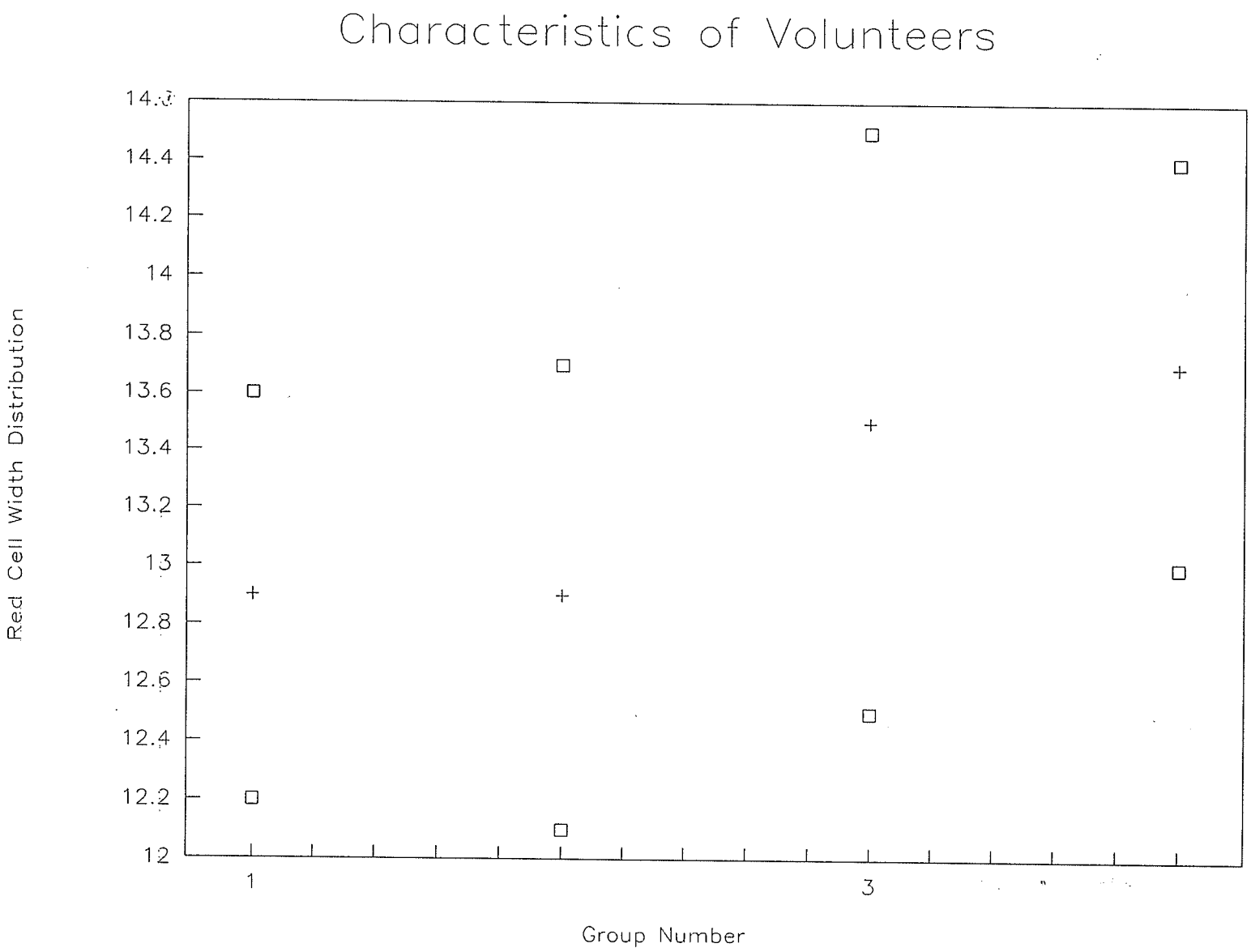

Characteristics of Volunteers
Red Cell Width Distribution
14.6
14.4
14.2
14
13.8
13.6
13.4
13.2
13
12.8
12.6
12.4
12.2
12
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

vii. Platelet Count

This figure has demonstrated the relationship between age and gender category and mean platelet count, measured in number x 10^9/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Platelet count did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

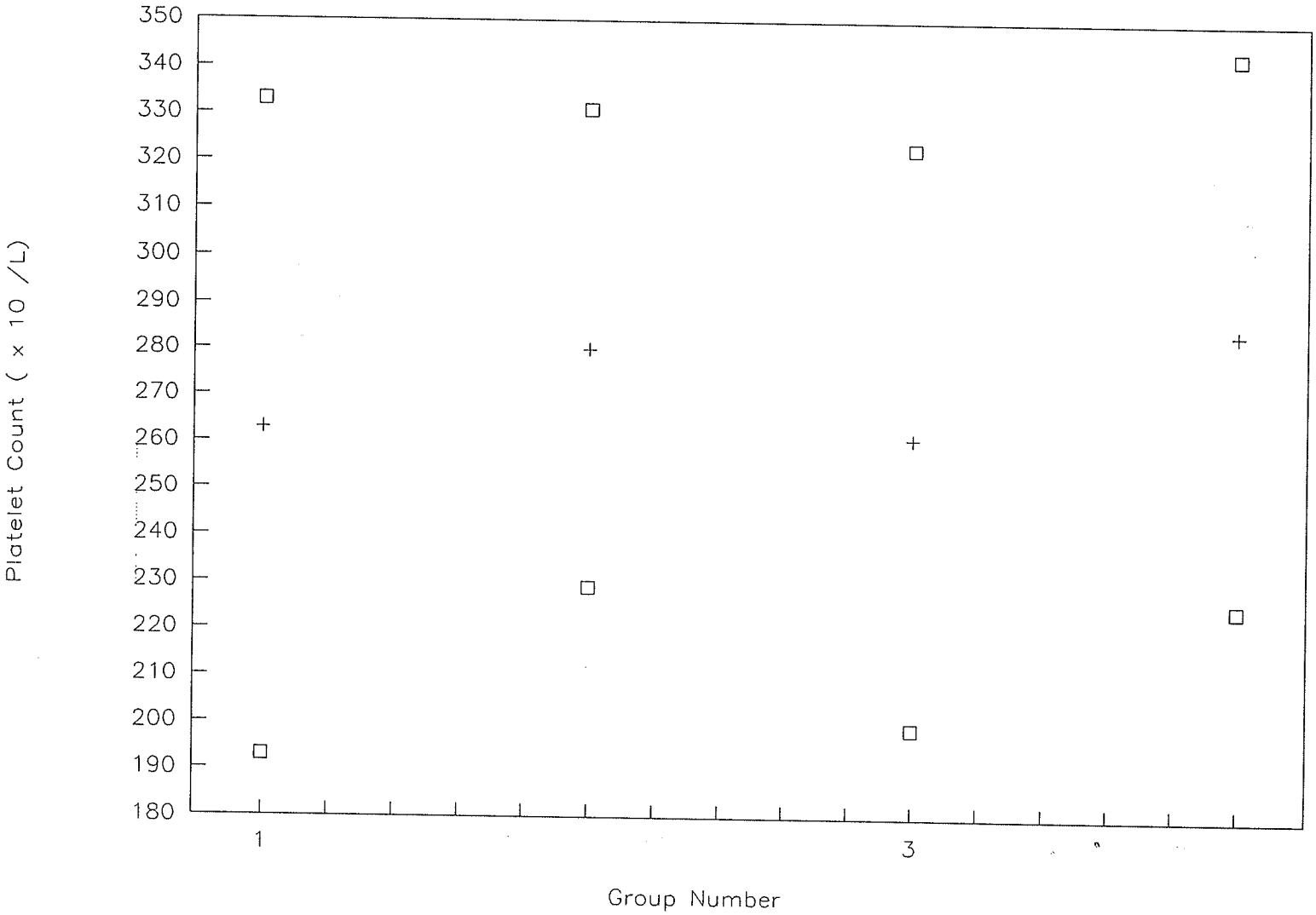

350
340
330
320
310
300
290
280
270
260
250
240
230
220
210
200
190
180
Platelet Count (x 10 /L)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

viii. Leukocyte Count

This figure has demonstrated the relationship between age and gender category and mean leukocyte count, measured in number x 10^9/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Leukocyte count did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

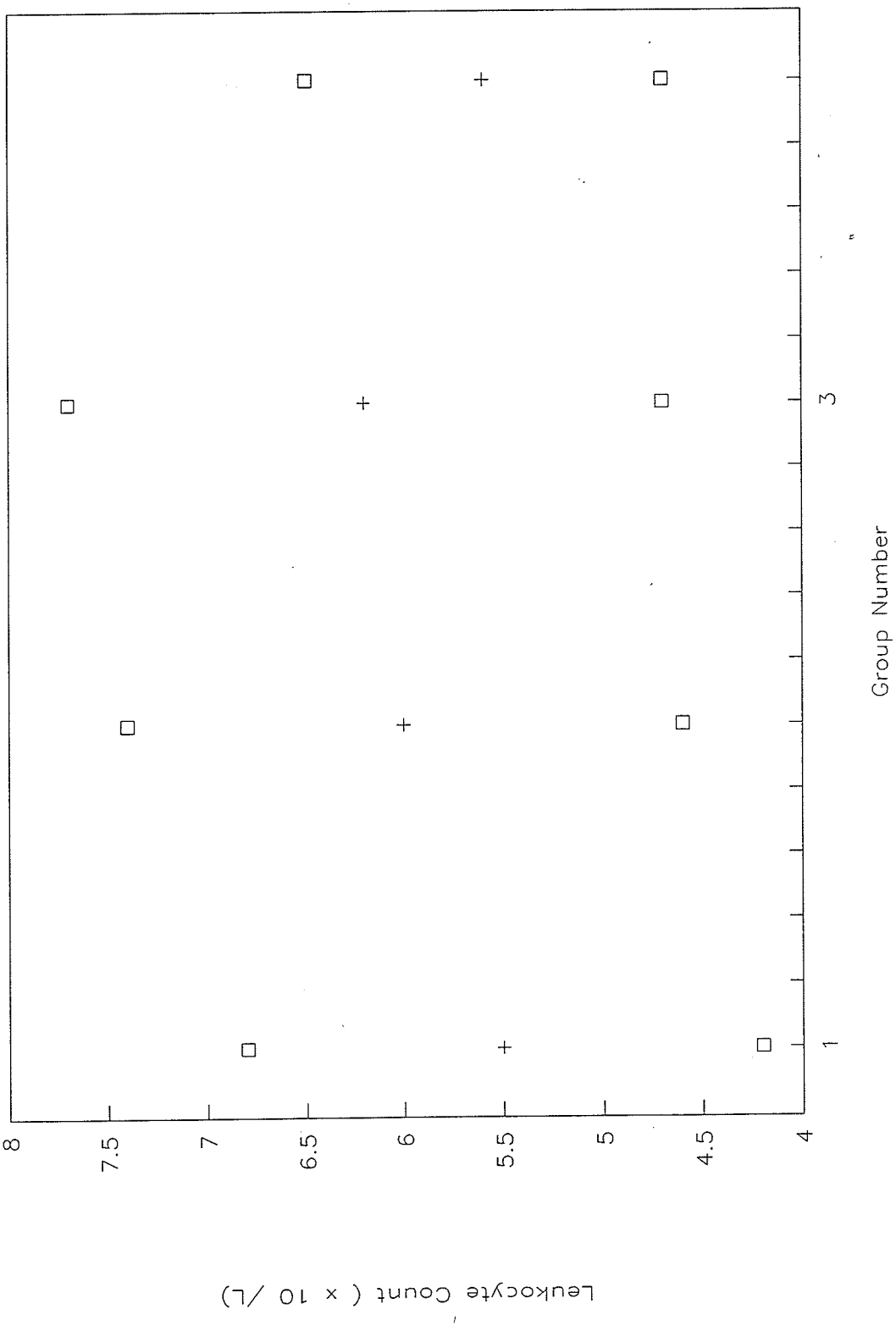

Leukocyte Count (x 10 /L)
8
7.5
7
6.5
6
5.5
5
4.5
4
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

ix. Lymphocyte Count

This figure has demonstrated the relationship between age and gender category and mean lymphocyte count, measured in number x 10^9/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Lymphocyte count did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

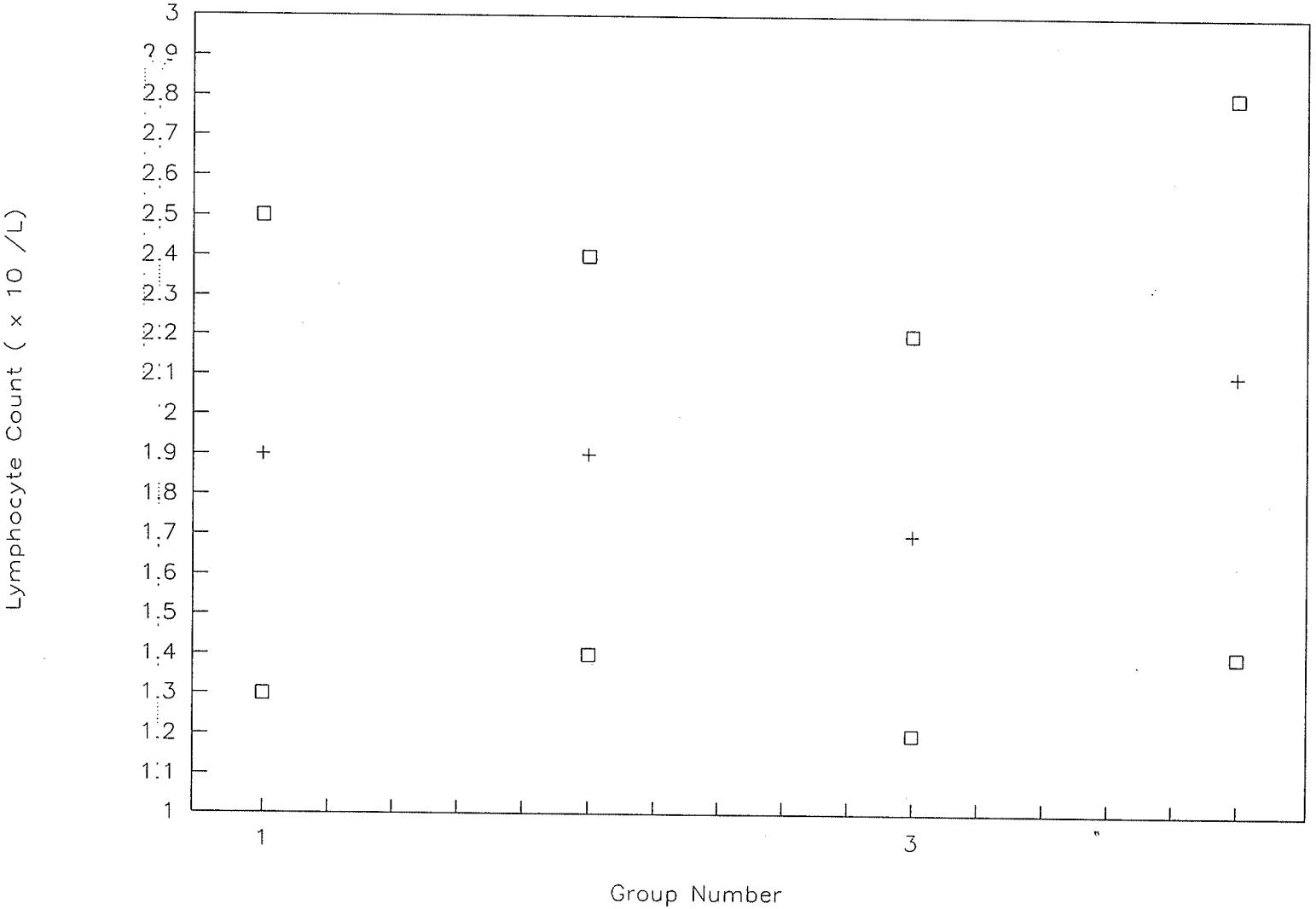

Lymphocyte Count (x 10 /L)
3
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

x. Granulocyte Percentage

This figure has demonstrated the relationship between age and gender category and mean granulocyte percentage, measured in percent and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females. Granulocytes were reported as a percentage whereas lymphocytes were reported as an absolute count due to alteration in the laboratory protocol for selecting and reporting measured values.

Granulocyte percentage differed significantly among younger and older men and among younger women and older men, according to Tukey's multiple comparison test at $p = 0.05$.

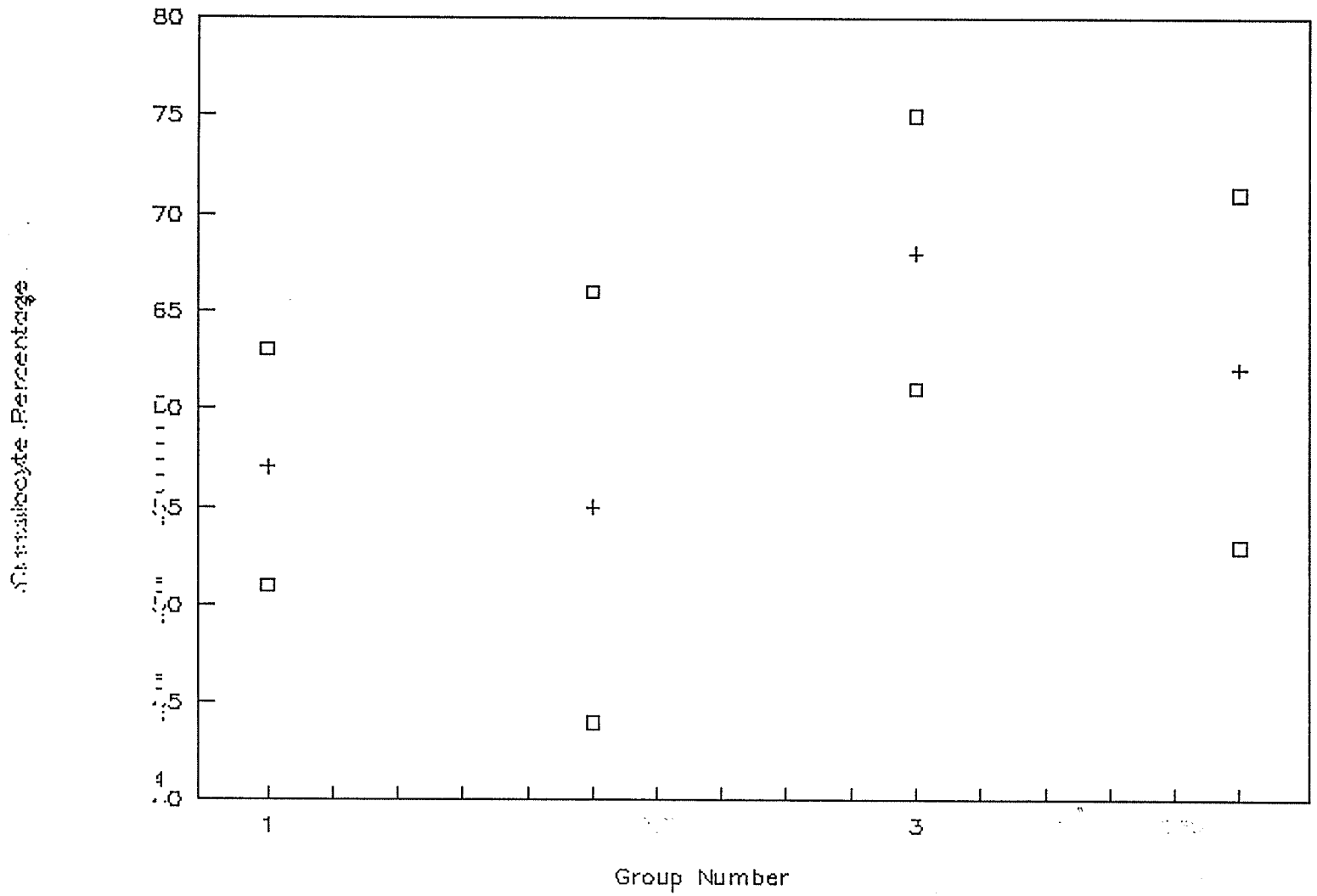

80
75
70
65
60
55
50
45
40
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xi. Monocyte Percentage

This figure has demonstrated the relationship between age and gender category and mean monocyte percentage, measured in percent and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females. Monocytes were reported as a percentage whereas lymphocytes were reported as an absolute count due to alteration in the laboratory protocol for selecting and reporting measured values.

Monocyte percentage did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

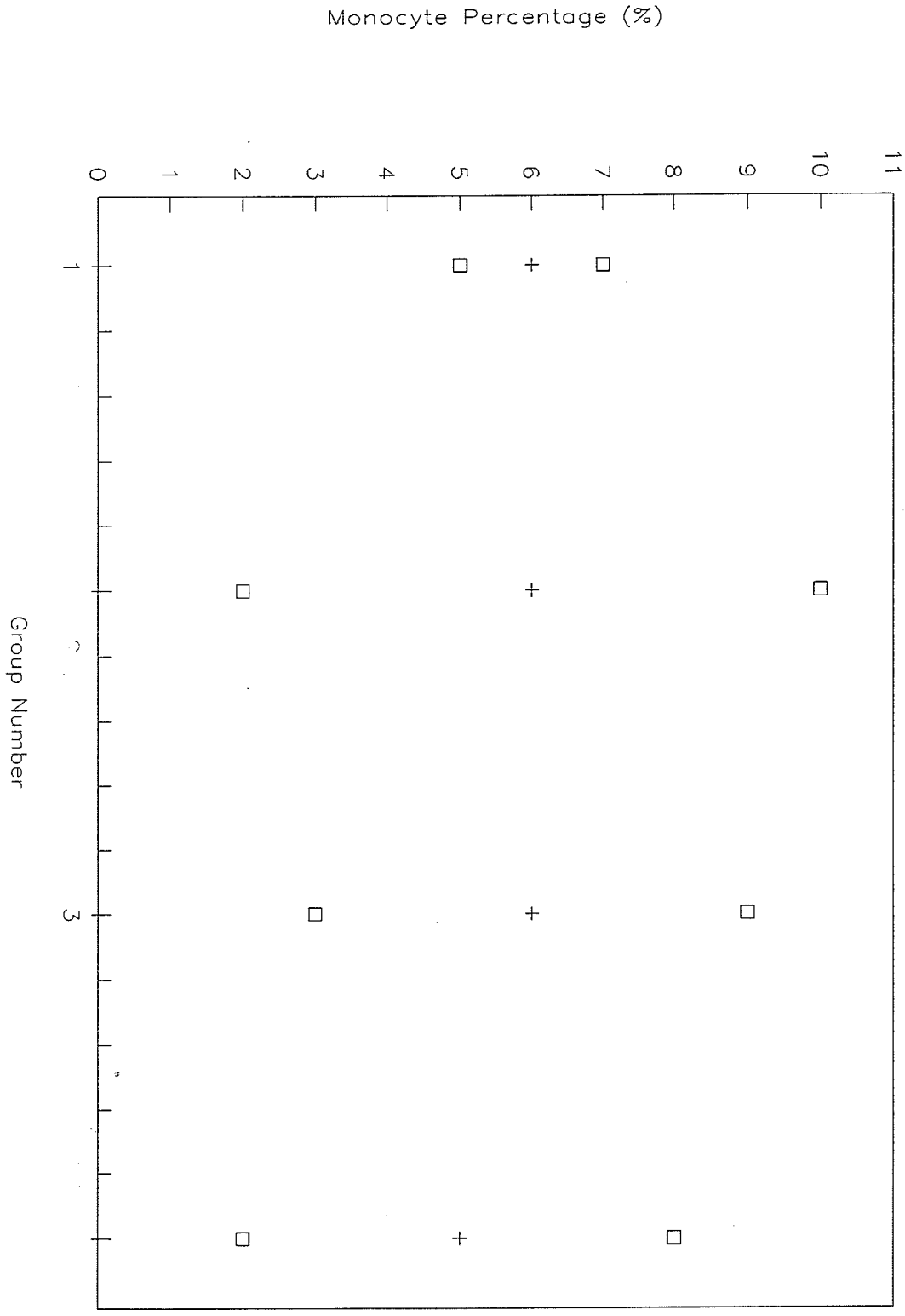

Monocyte Percentage (%)
0
1
2
3
4
5
6
7
8
9
10
11
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xii. Mean Platelet Volume

This figure has demonstrated the relationship between age and gender category and average mean platelet volume, measured in femtoliters and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Mean platelet volume did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

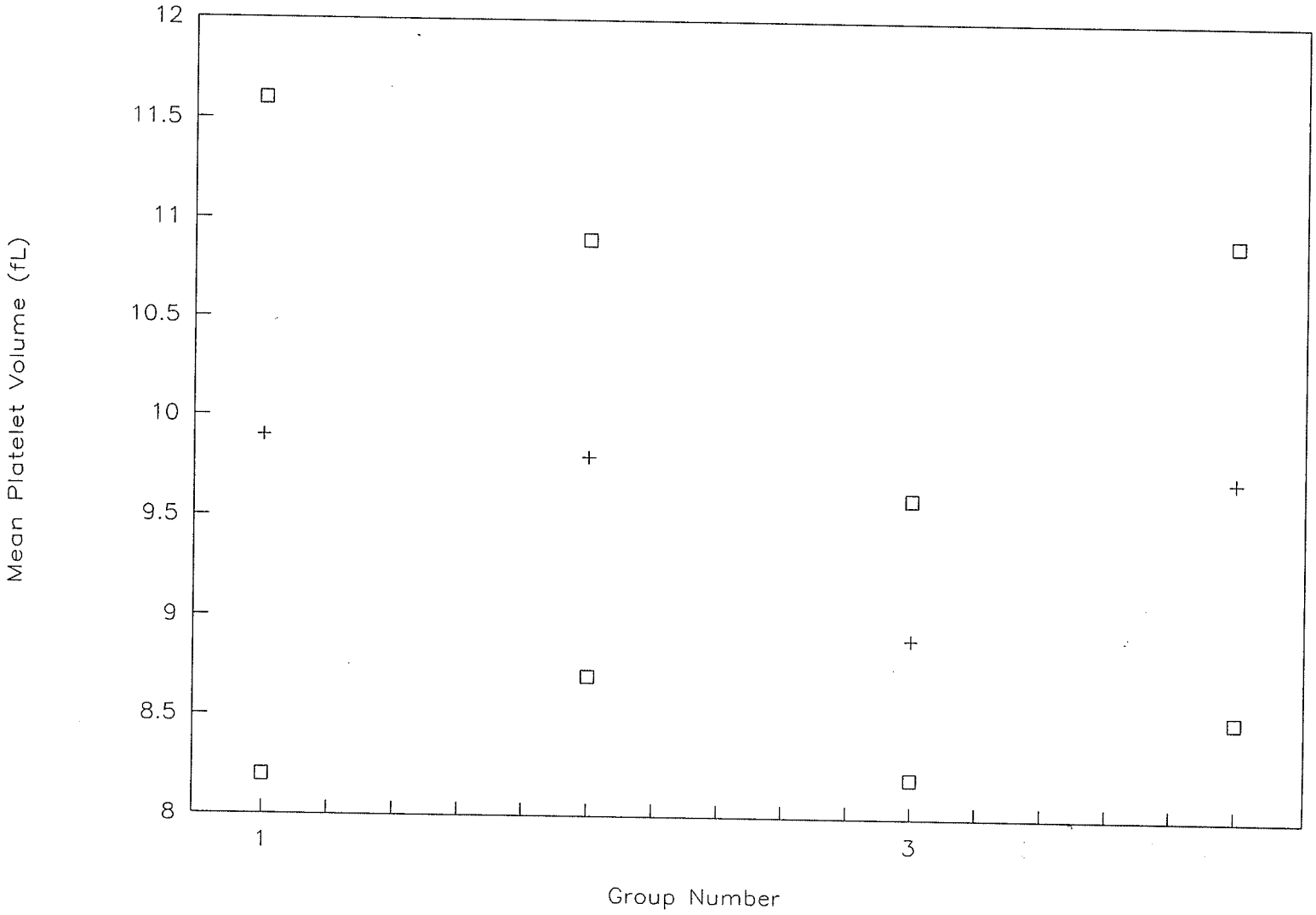

12
11.5
11
10.5
10
9.5
9
8.5
8
1
3
Mean Platelet Volume (fL)
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xiii. Sodium

This figure has demonstrated the relationship between age and gender category and mean serum sodium concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Sodium concentration differed significantly between younger males and younger females according to Tukey's multiple comparison test at $p = 0.05$.

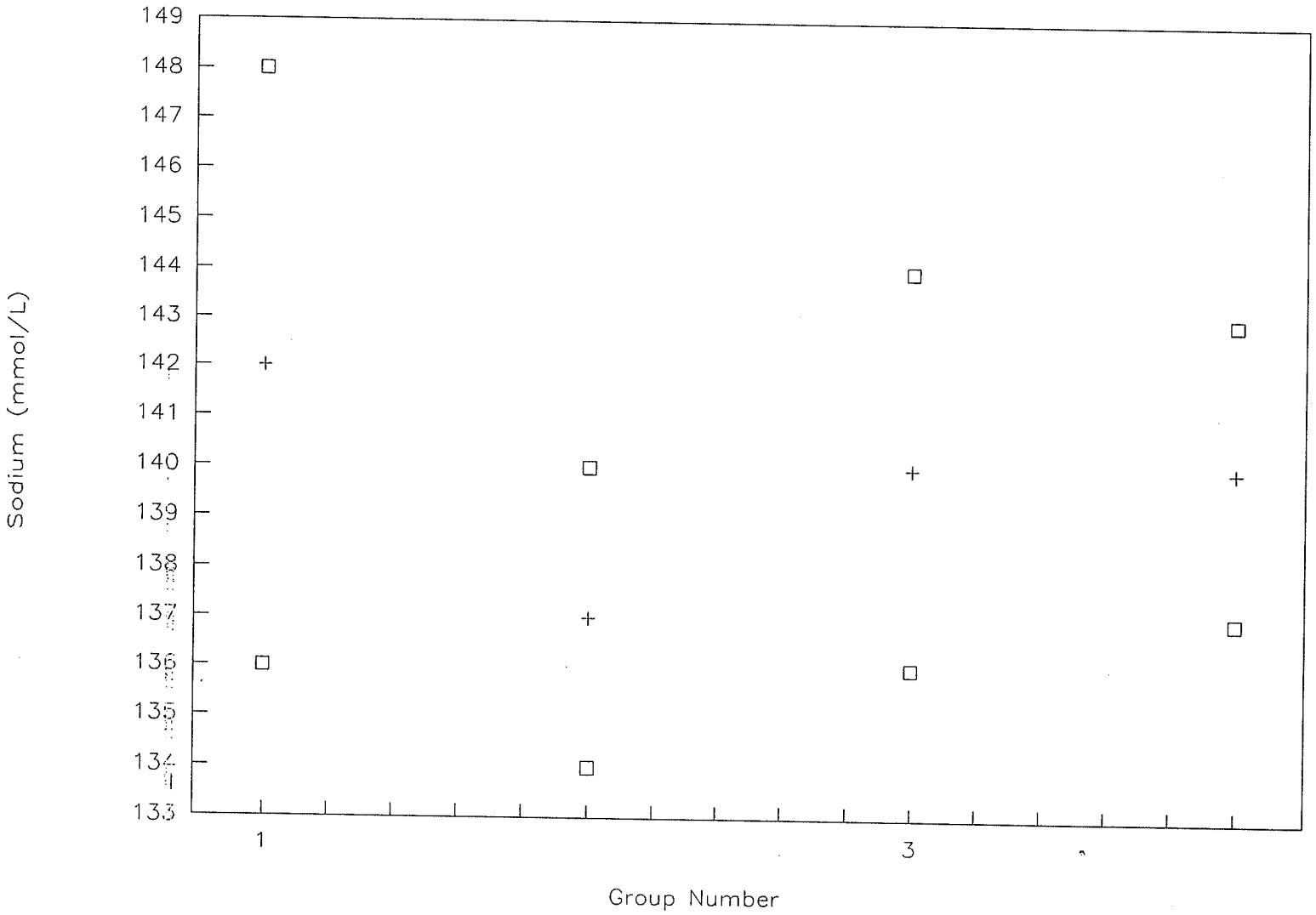

149
148
147
146
145
144
143
142
141
140
139
138
137
136
135
134
133
Sodium (mmol/L)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xiv. Potassium

This figure has demonstrated the relationship between age and gender category and mean serum potassium concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Potassium concentration did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

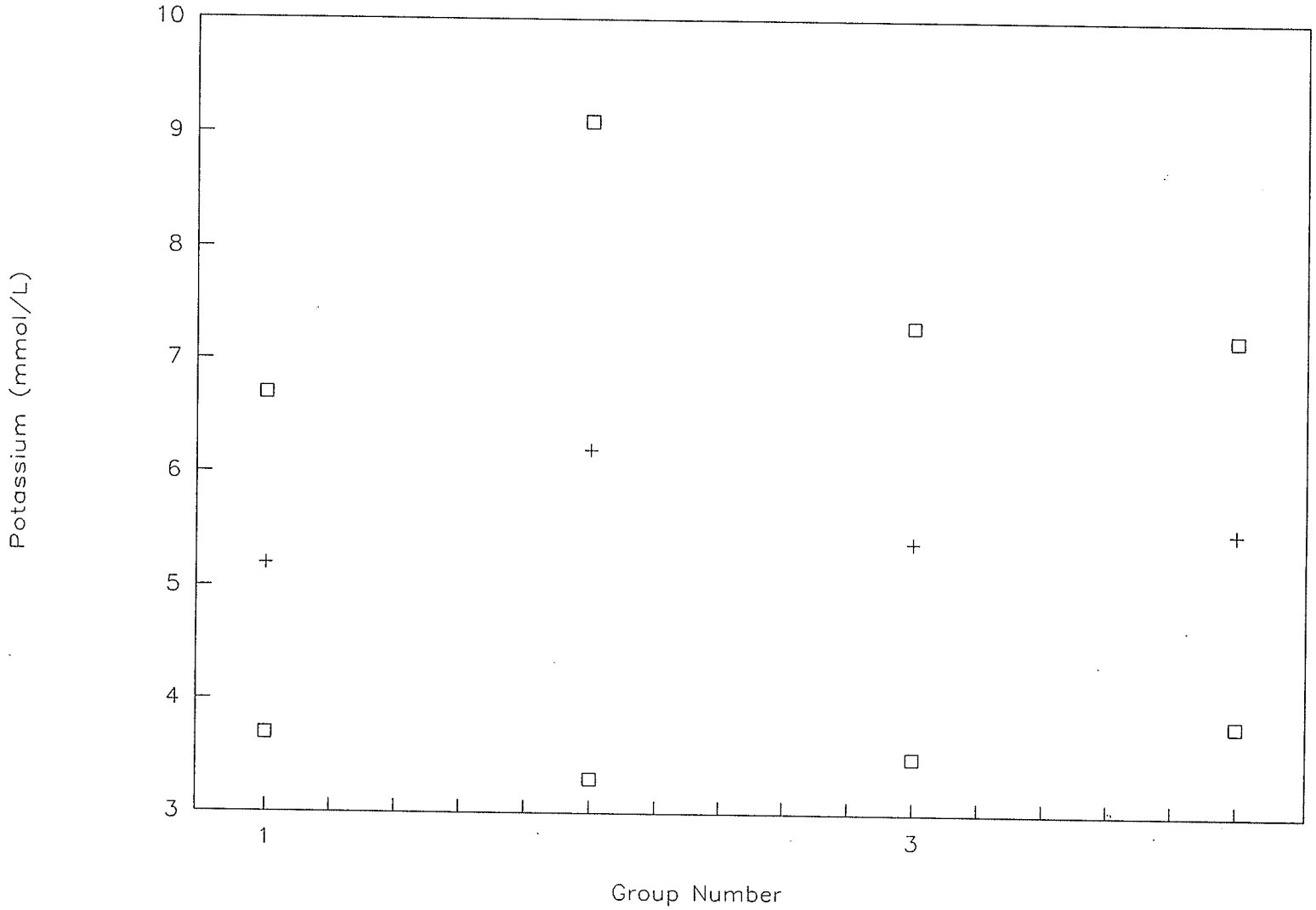

10
9
8
7
6
5
4
3
Potassium (mmol/L)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xv. Chloride

This figure has demonstrated the relationship between age and gender category and mean serum chloride concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Chloride concentration did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

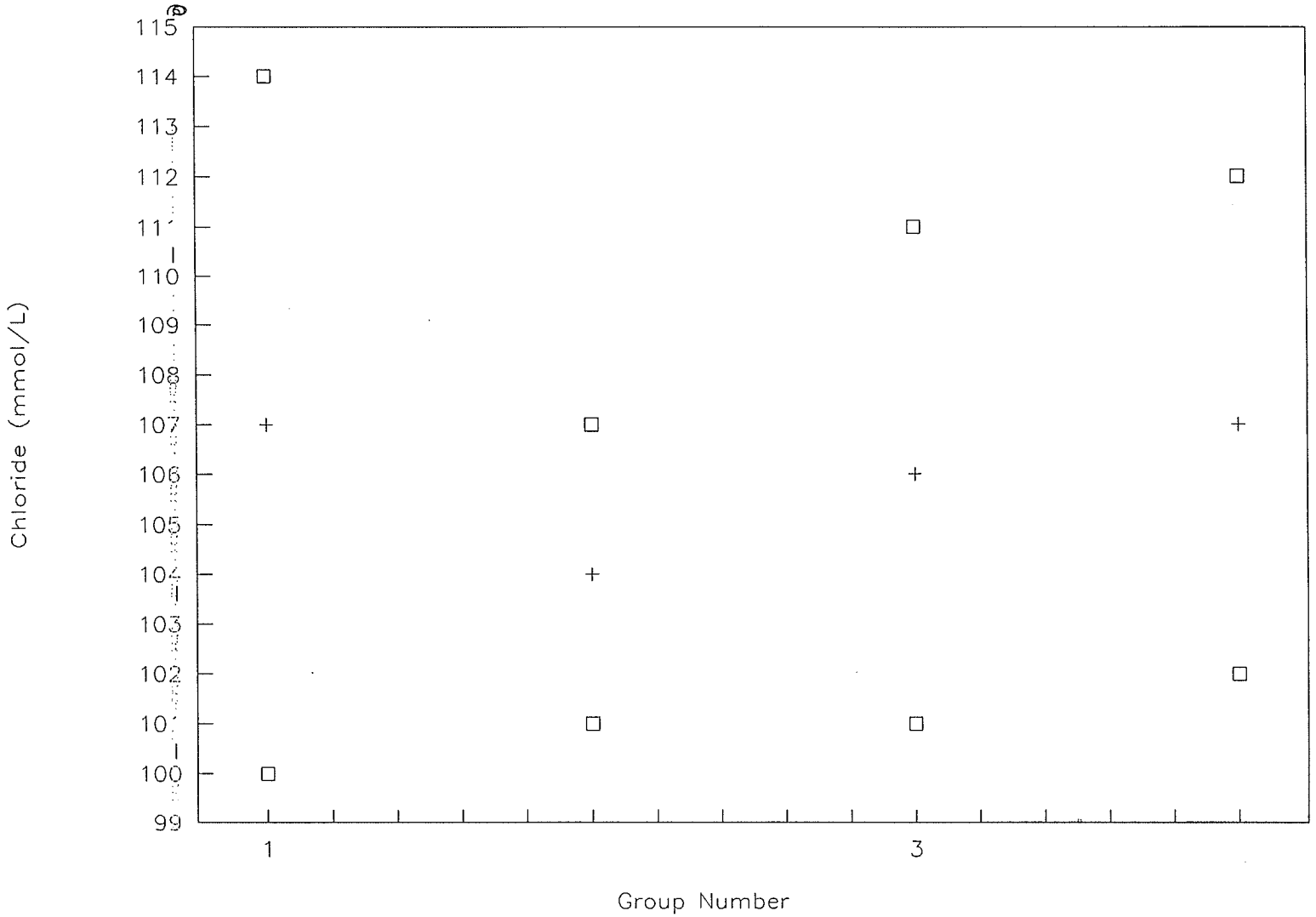

Chloride (mmol/L)
115
114
113
112
111
110
109
108
107
106
105
104
103
102
101
100
99
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xvi. Total CO_2 content

This figure has demonstrated the relationship between age and gender category and mean serum total carbon dioxide content, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Total carbon dioxide content differed significantly between younger males and females according to Tukey's multiple comparison test at $p = 0.05$.

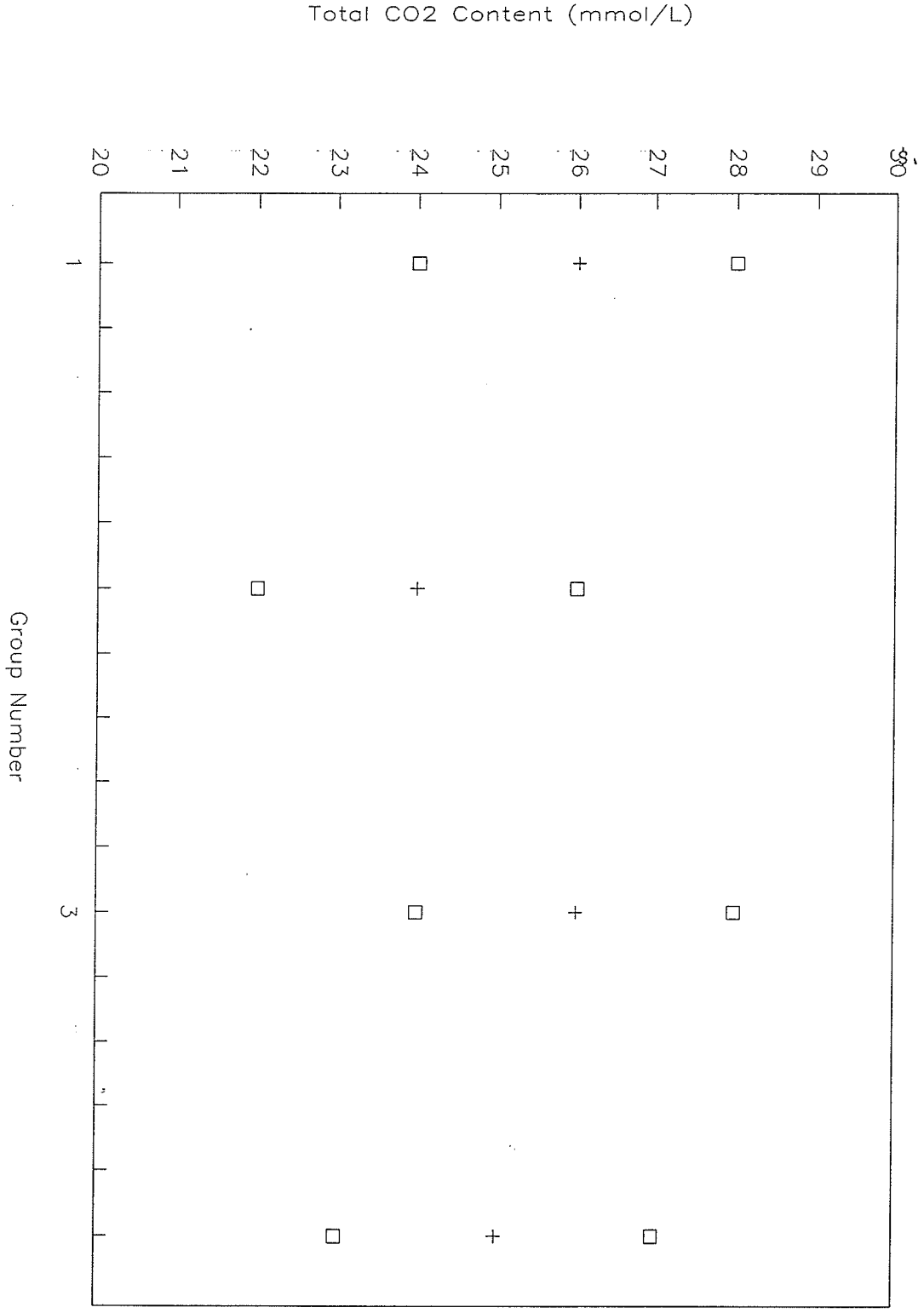

Total CO2 Content (mmol/L)
20
21
22
23
24
25
26
27
28
29
30
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline

p:rbc Ratio

xvii. Calcium

This figure has demonstrated the relationship between age and gender category and mean serum calcium concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Calcium concentration differed significantly between younger and older males according to Tukey's multiple comparison test at $p = 0.05$.

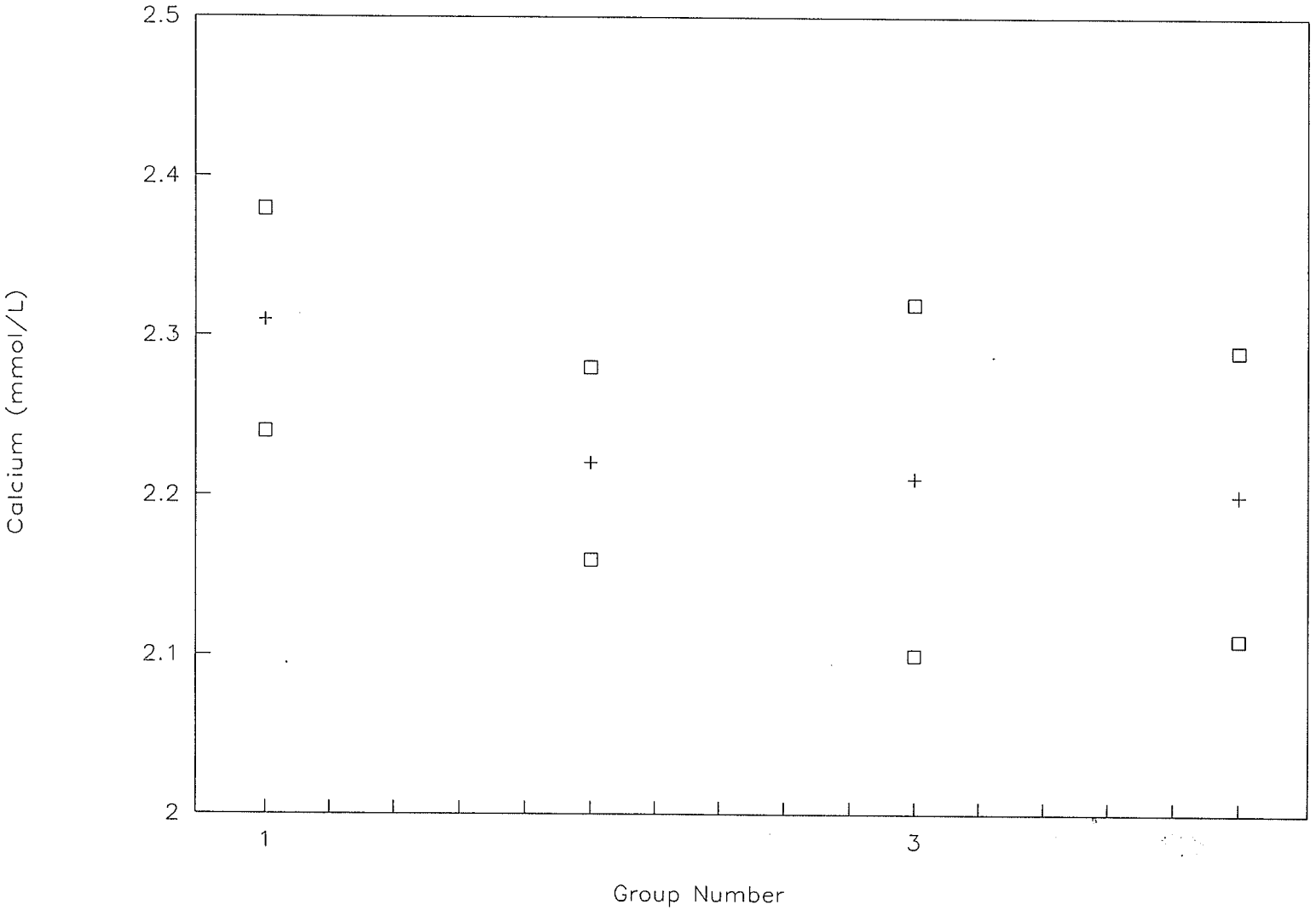

2.5
2.4
2.3
2.2
2.1
2
Calcium (mmol/L)
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline

p:rbc Ratio

xviii. Phosphate

This figure has demonstrated the relationship between age and gender category and mean serum phosphate concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Phosphate concentration differed significantly between younger and older males, older males and females, and younger females and older males, according to Tukey's multiple comparison test at $p = 0.05$.

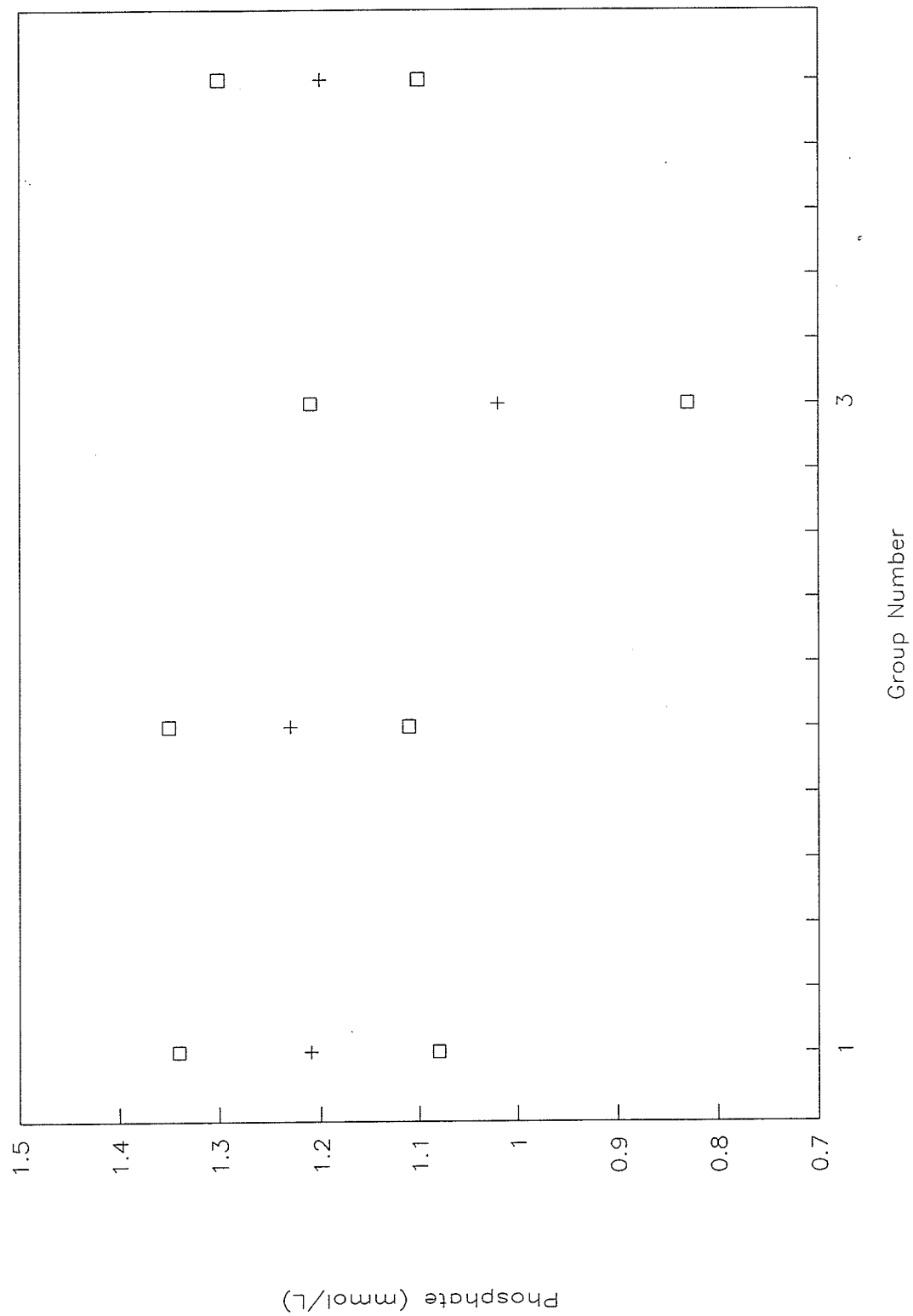

Phosphate (mmol/L)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xix. Magnesium

This figure has demonstrated the relationship between age and gender category and mean serum magnesium concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Magnesium concentration did not differ significantly among age and gender categories according to Tukey's multiple comparison test at $p = 0.05$.

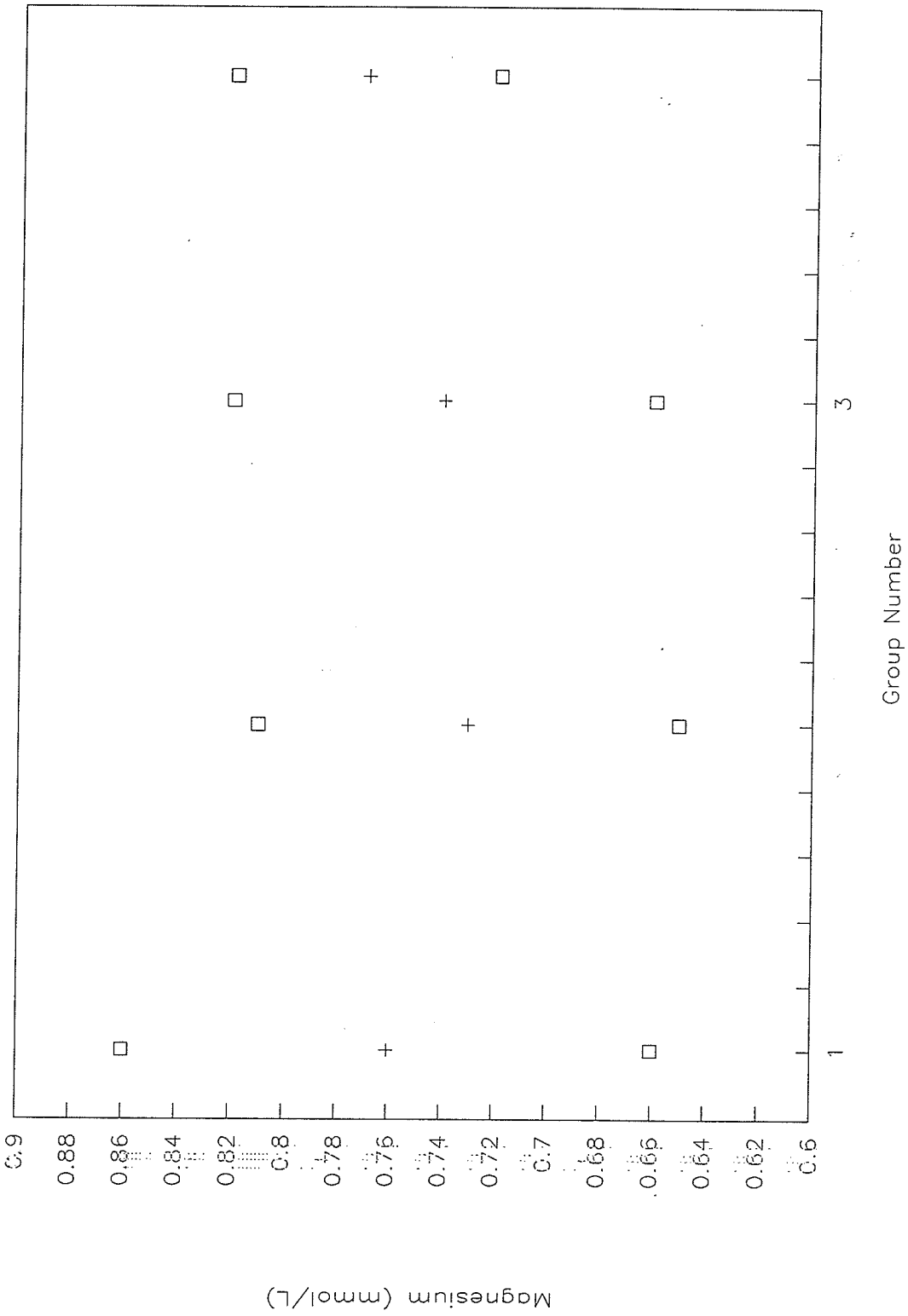

Magnesium (mmol/L)
0.9
0.88
0.86
0.84
0.82
0.8
0.78
0.76
0.74
0.72
0.7
0.68
0.66
0.64
0.62
0.6
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xx. Glucose

This figure has demonstrated the relationship between age and gender category and mean serum glucose concentration, measured in mmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Glucose concentration differed significantly between younger and older females, and between younger females and older males, according to Tukey's multiple comparison test at $p = 0.05$.

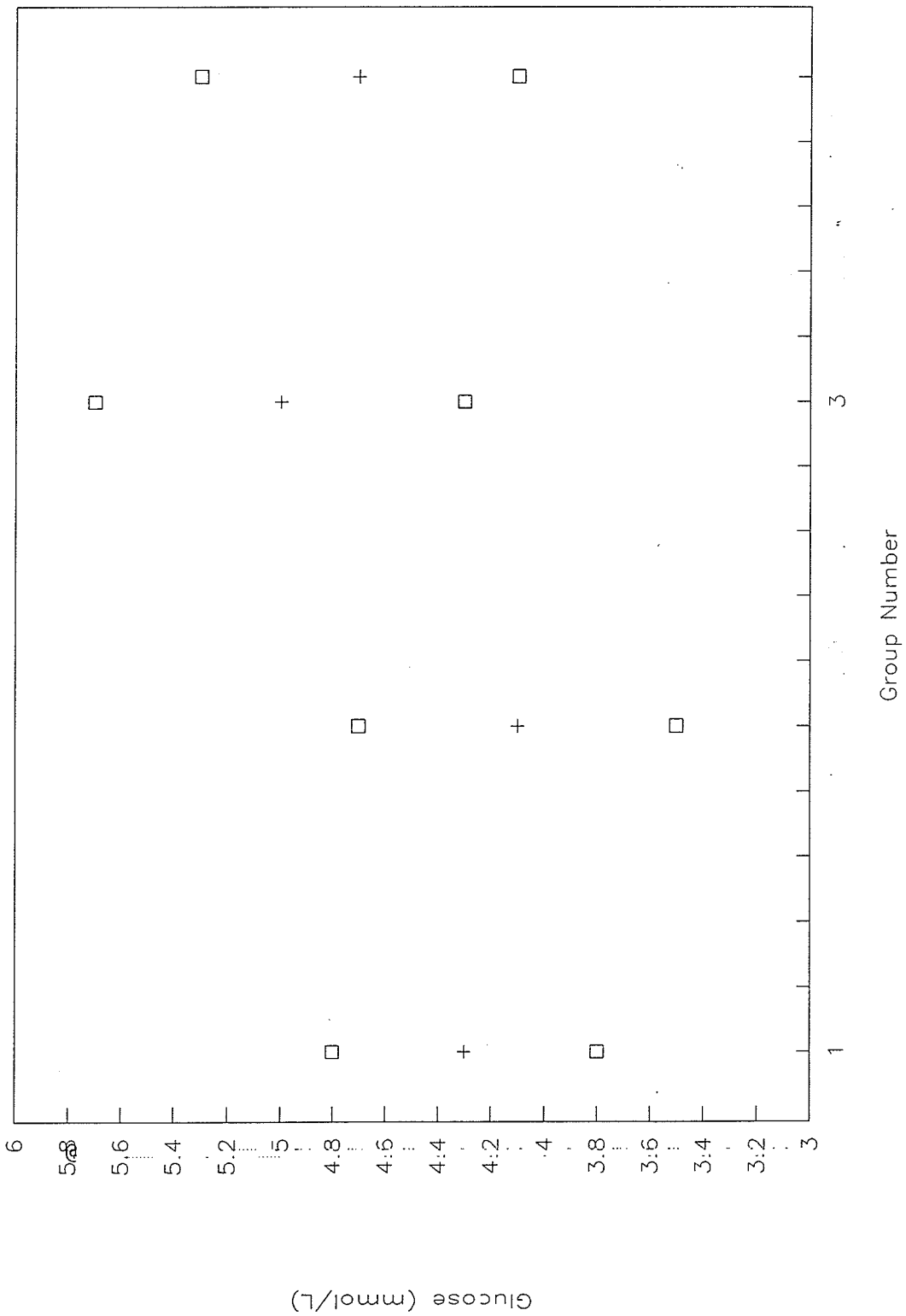

Glucose (mmol/L)
6
5.8
5.6
5.4
5.2
5
4.8
4.6
4.4
4.2
4
3.8
3.6
3.4
3.2
3
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xxi. Uric Acid

This figure has demonstrated the relationship between age and gender category and mean serum uric acid concentration, measured in μmol/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Uric acid concentration differed significantly between younger males and females and between younger females and older males, according to Tukey's multiple comparison test at $p = 0.05$.

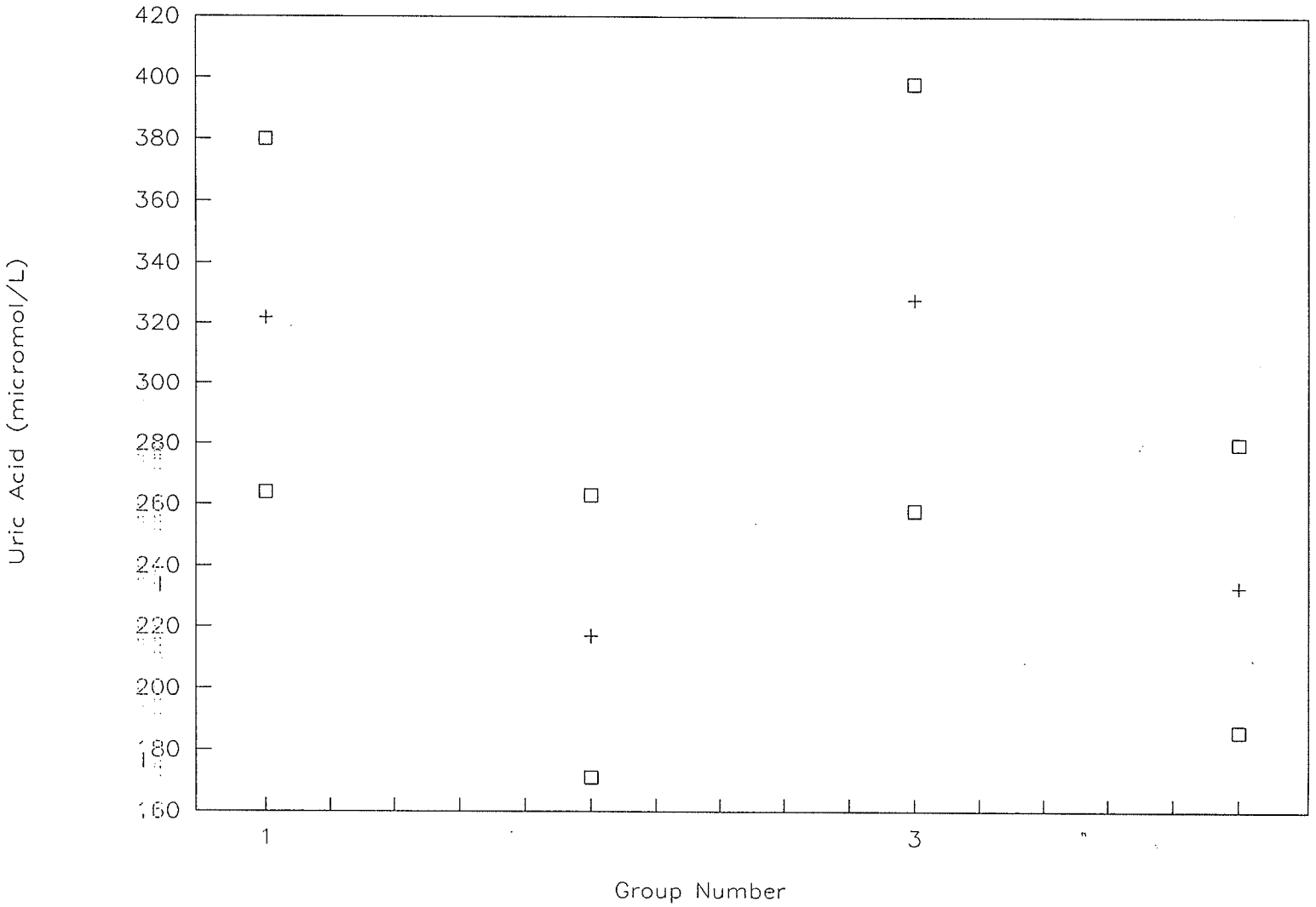

Uric Acid (micromol/L)
420
400
380
360
340
320
300
280
260
240
220
200
180
160
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xxii. Alanine Transaminase

This figure has demonstrated the relationship between age and gender category and mean serum alanine transaminase concentration, measured in U/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Alanine transaminase concentration differed significantly between younger males and older females, according to Tukey's multiple comparison test at $p = 0.05$.

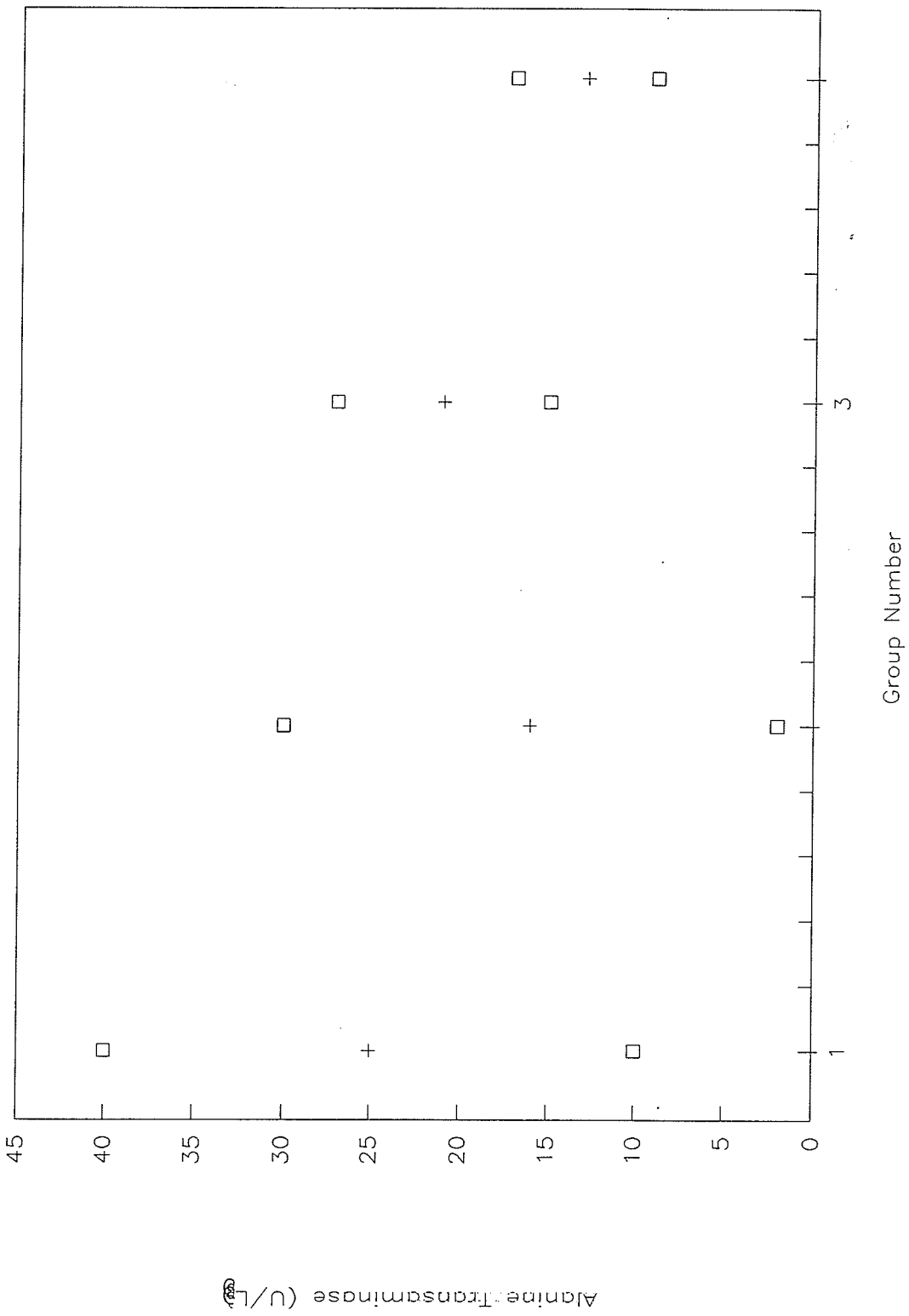

Alanine Transaminase (U/L)
45
40
35
30
25
20
15
10
5
0
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xxiii. Aspartate Transaminase

This figure has demonstrated the relationship between age and gender category and mean serum aspartate transaminase concentration, measured in U/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Aspartate transaminase concentration did not differ significantly among age and gender categories, according to Tukey's multiple comparison test at $p = 0.05$.

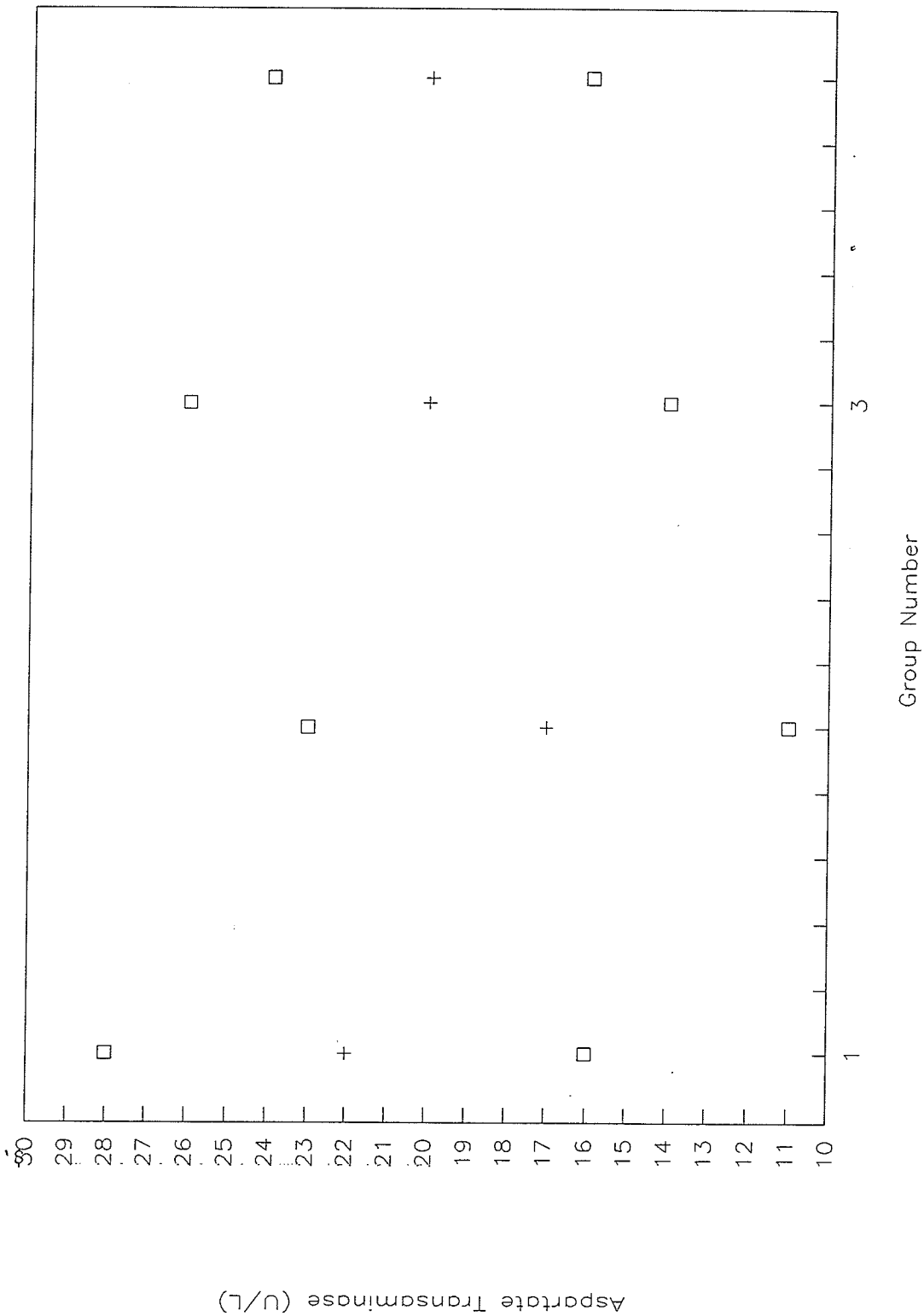

Aspartate Transaminase (U/L)
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
1
3
Group Number

Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xxiv. Gamma Glutamyl Transferase

This figure has demonstrated the relationship between age and gender category and mean serum gamma glutamyl transferase concentration, measured in U/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Gamma glutamyl transferase concentration differed significantly between younger females and older males, according to Tukey's multiple comparison test at $p = 0.05$.

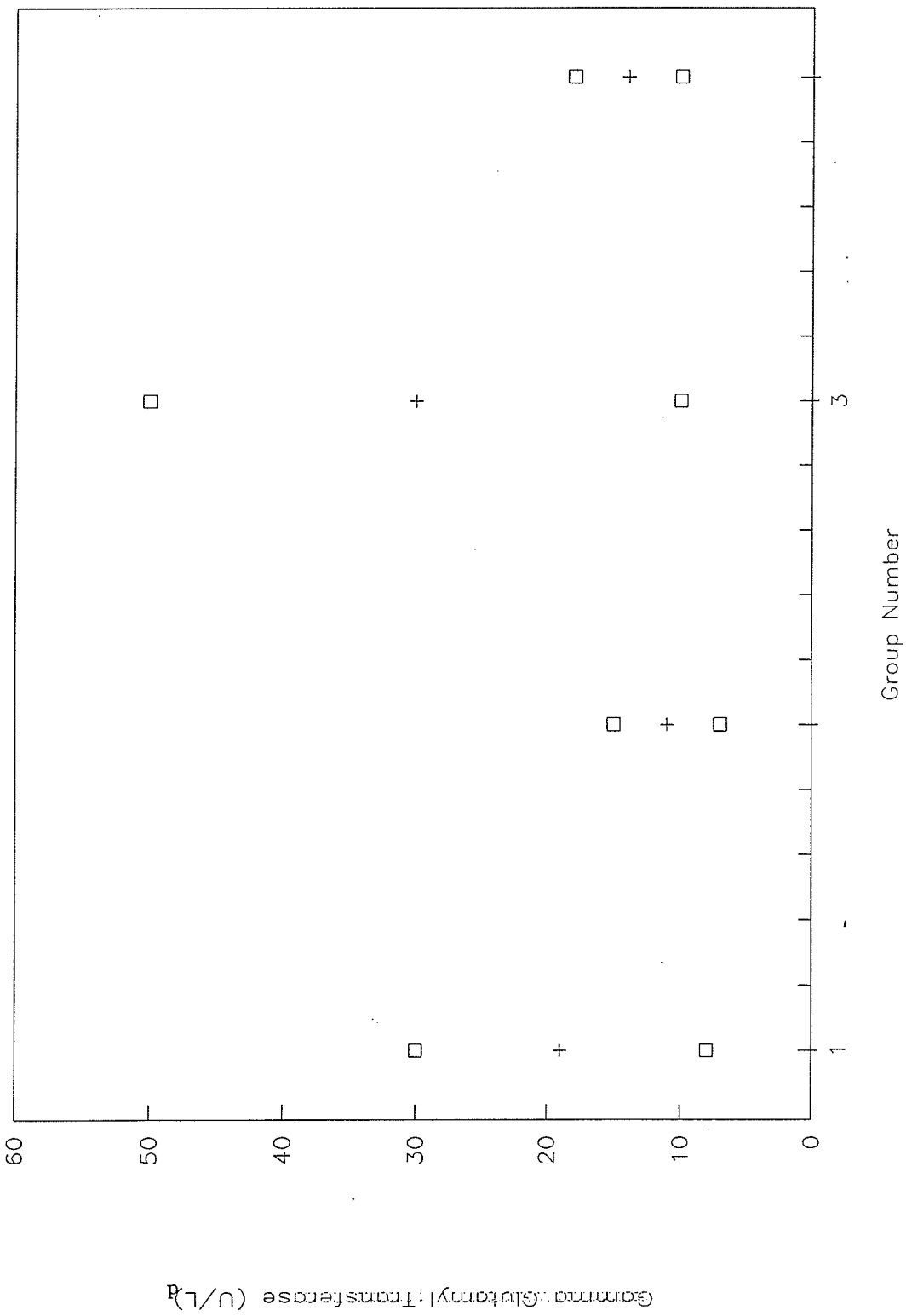

Gamma-Glutamyl Transferase (U/L)
60
50
40
30
20
10
0
1
3
Group Number

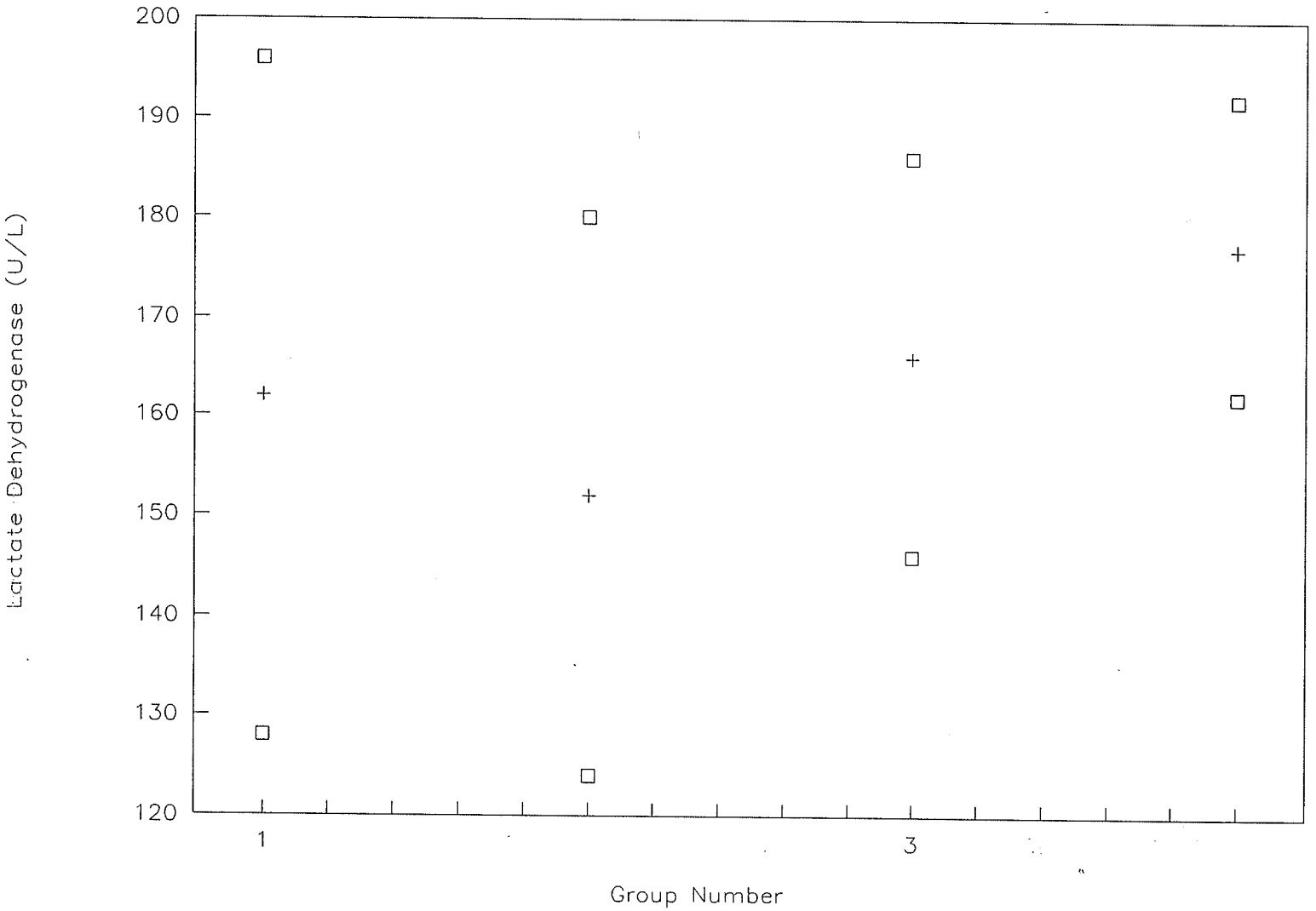
Figure 1 Theophylline Partitioning, Hematological and Biochemical Data, by Age and Gender Group

d. Hematological and Biochemical Parameters Not Examined for Correlation with Theophylline p:rbc Ratio

xxv. Lactate Dehydrogenase

This figure has demonstrated the relationship between age and gender category and mean serum lactate dehydrogenase concentration, measured in U/L and represented as a cross bracketed by two squares, signifying one standard deviation above and one below the cross. The age and gender categories were defined as follows: Group 1: younger males; 2: younger females; 3: older males; 4: older females.

Lactate dehydrogenase concentration differed significantly between younger and older females, according to Tukey's multiple comparison test at $p = 0.05$.


200
190
180
170
160
150
140
130
120
Lactate Dehydrogenase (U/L)
1
3
Group Number

statistical significance. Albumin was lower in elderly females than in other groups, varying according to gender in the young but not the elderly. Creatinine was higher in males than females, and in older males as compared to younger females. Bilirubin was higher in younger males than older females, but no other significant difference was found. Blood urea nitrogen was higher in older females than younger people. Too few observations of blood types b or ab were available to enable conclusions to be drawn about possible association with age or gender, but no differences were observed in the distribution of types a and o among age groups (Table 1a,b).

Among the parameters not selected for potential relevance to drug distribution (Tables 2b,c), minor significant differences existed among means, as for phosphate, which varied among genders in the elderly and between older men and younger subjects.

B. Equilibration Studies

Calibration curves demonstrated a linear relationship (Figure 2) between amounts of drug added and peak height ratio. Incubations (0-60 min) reached cell-plasma equilibrium in 5 min (Figure 3). An incubation time of 30 min was chosen for the definitive experiment, as being convenient, sufficient to allow a margin of safety regarding the validity of the initial quality control estimates, but insufficient for decomposition of blood. Drug concentrations in blood and

Figure 2 Standard Curve for Theophylline

A linear relationship was found between the concentration of theophylline added to plasma samples and the ultraviolet absorbance at 280 nanometers of the sample containing theophylline. This relationship permitted calculation of the amount of theophylline in an unknown sample after measuring its ultraviolet absorbance at 280 nanometers.

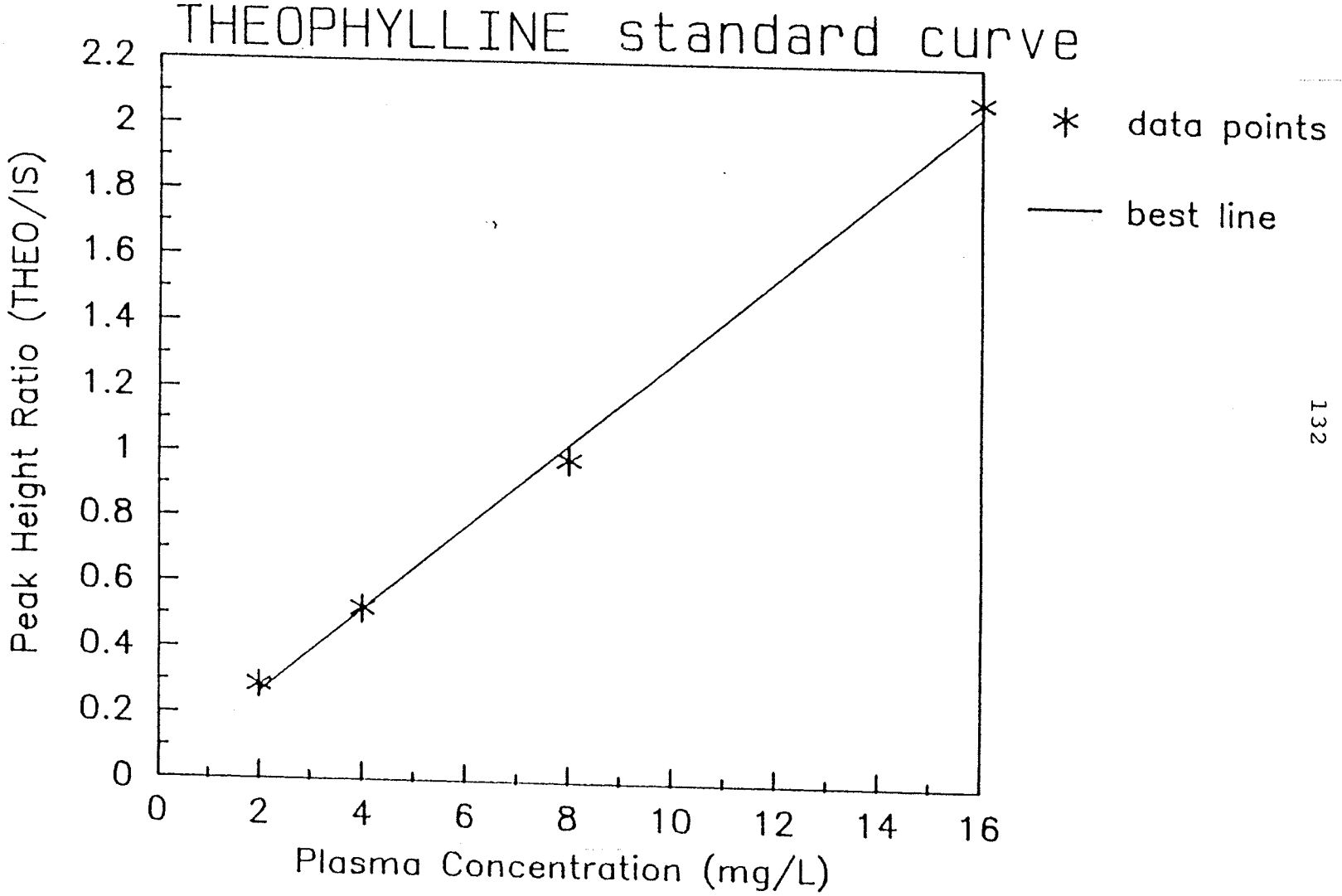
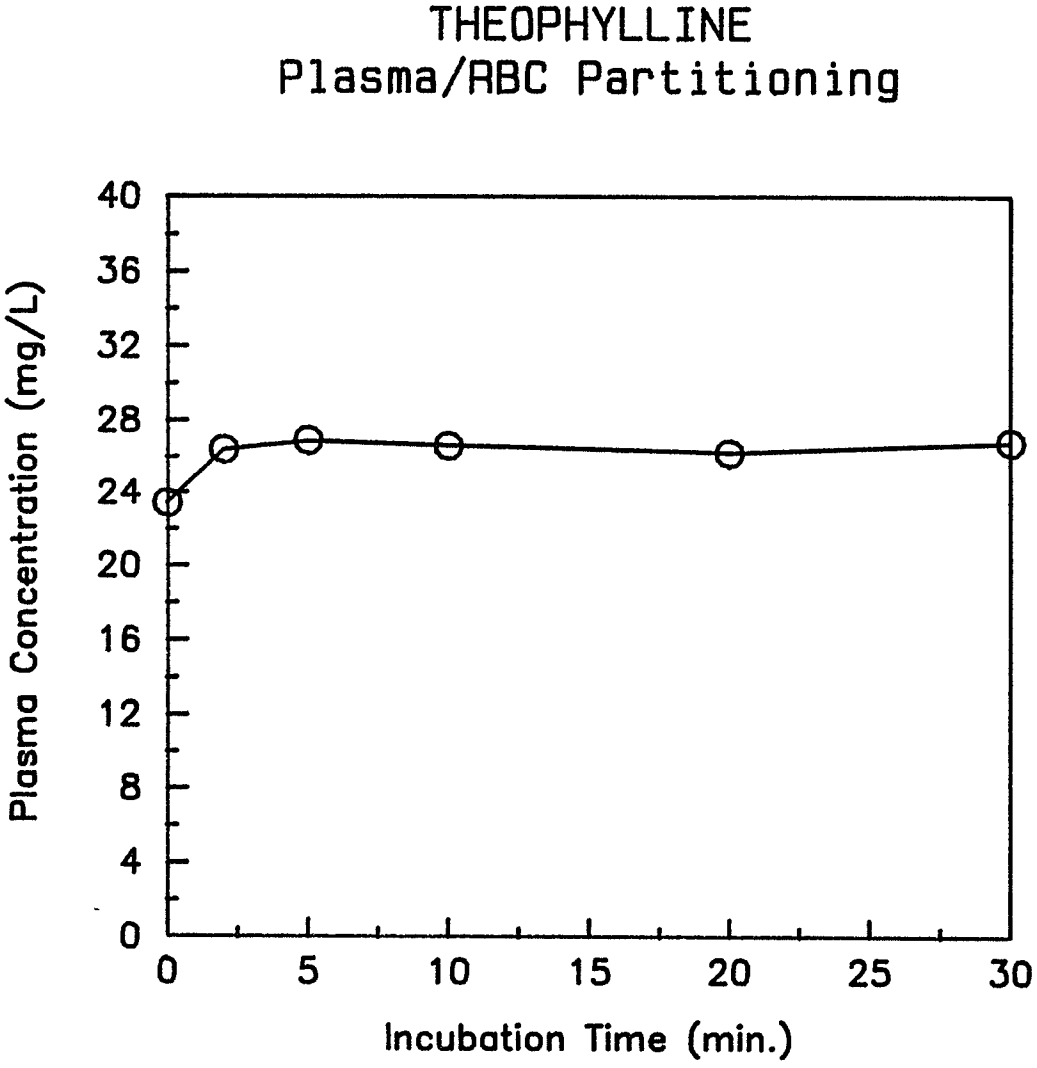

THEOPHYLLINE standard curve
Peak Height Ratio (THEO/IS)
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
12
14
16
Plasma Concentration (mg/L)
* data points
best line

Figure 3 Incubation of Theophylline in Whole Blood for Varying Time Intervals

After incubation of a constant concentration of theophylline in whole blood, with mixing, at 37° C for time intervals from 0 to 60 minutes, the concentration of theophylline in plasma was found to rapidly reach a constant value. An incubation time of 20 minutes was selected as being ample to allow theophylline concentrations to reach equilibrium across the red cell membrane.


THEOPHYLLINE
Plasma/RBC Partitioning
Plasma Concentration (mg/L)
40
36
32
28
24
20
16
12
8
4
0
0
5
10
15
20
25
30
Incubation Time (min.)

plasma were linearly related after incubations of varying theophylline concentrations with blood, ranging from 5-20 mg/L theophylline.

C. Analysis of Results

1. Plasma: Red Blood Cell Partitioning Ratio

The theophylline p:rbc ratio was approximately 1.5, with considerable individual variation (Table 1). The distribution was skewed: the mean exceeded the median and a few disproportionately high values were observed. Such a distribution has been not uncommon among biological parameters. A prospective analysis revealed that the p:rbc ratio showed an age-gender interaction, varying differently with age depending on gender (Figure 1a).

2. Explanation of the Variation in Theophylline Partitioning

Post hoc analyses were performed in order to identify parameters associated with the variation in p:rbc ratio. The Pearson correlation matrix yielded exceedingly low correlation coefficients. As the gender-associated changes in p:rbc ratio were in opposite directions, the data were analysed separately by gender; larger correlations were obtained in the Pearson matrix (Table 4a).

Multiple stepwise regression was performed on each gender category for age, cholesterol, triglyceride, bilirubin, blood urea nitrogen, albumin, and creatinine. In men, the most

Table 4 Statistical Data Analysis

a Excerpts from Pearson Correlation Matrix[1]

	Both Genders		Males		Females	
	p:rbc	ln(p:rbc)	p:rbc	ln(p:rbc)	p:rbc	ln(p:rbc)
albumin	0.09	0.10	0.39	0.39	-0.21	-0.22
	0.45	0.41	0.02	0.02	0.20	0.19
triglyceride	0.12	0.13	0.04	0.04	0.24	0.26
	0.31	0.26	0.82	0.79	0.14	0.12
bilirubin	-0.13	-0.12	0.16	0.20	-0.40	-0.40
	0.23	0.32	0.32	0.23	0.01	0.01
age	0.02	0.02	-0.41	-0.41	0.37	0.37
	0.85	0.87	0.01	0.01	0.03	0.03

[1]This matrix determined the correlation of various parameters with the theophylline plasma: red blood cell concentration ratio (p:rbc) or with the log transformation of this ratio. The upper number in each pair was the correlation coefficient r; the lower was p to test the significance of r.

important factors predictive of p:rbc ratio were age (inversely), cholesterol, and creatinine, with an r^2 of 0.27; in women, predictive factors were bilirubin (inversely) and age, at an r^2 of 0.22 (Table 4b, Figure 1a,b). Addition of further potential factors did not significantly improve the variance while maintaining an acceptable probability that the relationship was valid.

Table 4 Statistical Data Analysis

b Prediction of Theophylline Partitioning (p:rbc Ratio)

Multiple stepwise linear regression

males	Step	Parameter	r^2
ln (p:rbc)	1	age	0.17
	2	cholesterol	0.25
females			
ln (p:rbc)	1	bilirubin	0.16
	2	age	0.22

Analysis of variance in ln (p:rbc)

Sex	Parameter	D.F.	Estimate	p	p of the model
m	constant	1	0.214	0.127	0.006
	age	1	-0.004	0.002	
	cholesterol	1	0.055	0.053	
f	constant	1	0.354	0.011	0.015
	age	1	0.002	0.138	
	bilirubin	1	-0.015	0.069	

IV. DISCUSSION AND CONCLUSIONS

A. Design of Study

The design of this experiment permitted comparison of directly observed data concerning the p:rbc ratio with reported data concerning V_d. Although this design had the advantage of relative convenience, more informative data would have been obtained by direct comparison of the p:rbc ratio and V_d in the same study population. Such a design would have permitted not only determination of similarity of the parameters correlated to the p:rbc ratio and V_d, but also of direct investigation of the existence of a correlation between the p:rbc ratio and V_d in the same subjects. In this way, more definitive evidence might have been obtained regarding the potential value of p:rbc ratio in prediction of V_d. Another possible improvement on the design of the present study might have been determination of the subjects' body weight, a variable known to influence V_d, and investigation of any correlation between weight and the p:rbc ratio. Further study could permit investigation of these topics.

B. Demographic, Hematologic, and Biochemical Data

The general health of the volunteers was considered satisfactory, as the majority of biochemical and hematological data were normal. Measuring a large number of parameters has, as would be expected, predisposed the results to random findings of occasional unusual values. The high potassium

concentrations were most likely related to hemolysis associated with difficulties in rapid transport of samples to the Health Sciences Centre laboratory. Hemolysis of blood samples has also been reported as capable of affecting lactate dehydrogenase, aspartate transaminase, gamma glutamyl transferase, and possibly magnesium. In this sample, means and standard deviations of these parameters were within normal limits. The effect of hemolysis on the biochemical and hematological specimens analysed in the hospital laboratory was apparently not serious, as estimated by alterations in these parameters. The mean gamma glutamyl transferase in older men was, however, raised by three isolated high values, one associated with a high potassium and likely related to hemolysis. Long-term alcohol consumption might have accounted for the other high values; other biochemical data did not suggest very recent alcohol consumption. The relatively low mean red cell count in older subjects might possibly reflect the greater osmotic fragility with subject age reported by Araki and Rifkind (1980), Bowdler et al (1981), and Hussain and Voaden (1984). As the majority of individuals had hematocrit and hemoglobin values within normal limits, the importance of the low mean red cell count was most likely not remarkable. The hematocrit differed by gender, as predicted by the reference values. Cholesterol increased with age, a common finding possibly related to diet. Albumin decreased with age, a common finding which has been attributed to poorer

nutrition and widespread subclinical pathology in the elderly. Bilirubin was higher in younger men than older women, but the effects of alcohol were less likely because the gamma glutamyl transferase did not differ significantly among these two groups. The raised blood urea nitrogen in older women compared to younger subjects, and raised creatinine in older men compared to younger women, was not necessarily indicative of disease. Creatinine clearance might well have been normal. This study group as a whole appeared to be relatively normal by biochemical screening.

Among the hematological and biochemical parameters not selected for regression with p:rbc ratio, phosphate means changed among age and gender groups in a manner somewhat similar to the p:rbc ratio, but phosphate shared very little variation with p:rbc ratio in a correlation matrix. In a Pearson correlation matrix using the data for both sexes combined, the variables in this category most closely correlated with p:rbc ratio were white cell count, calcium concentration, and platelet count. The correlation coefficients were approximately 0.1 to 0.2, comparable to or greater than coefficients in the same matrix for several variables selected for potential relevance to drug partitioning. The design of this experiment was not capable of thorough investigation of a large number of relationships in only 75 subjects. Further study would be necessary to determine the significance, if any, of these findings.

Analysis of significance of differences among group means by Tukey's multiple comparison test should be performed on groups of equal size with equal standard deviations in each group. This was a fair approximation in most cases. Group sizes, although consistent among the parameters selected for potential relevance to drug distribution, were not consistent among all parameters for reasons including laboratory policy regarding which parameters were to be analysed in a given case, and difficulty in obtaining a sufficient sample initially or after unexpected misadventure. Further investigation would be needed to make definitive statements about data concerning the relationships of age and gender to parameters whose overall sample size was smaller than expected.

C. HPLC Assay

Satisfactory precision and accuracy were obtained for the determinations of theophylline by HPLC assay, as previously demonstrated in the present laboratory and currently verified by the initial quality control work. The linear calibration curve between known added concentration of drug and peak height ratio, and the linear relationship between whole blood and plasma concentration of theophylline, were consistent with the data of Peat et al (1977). These data indicated that at increasing drug concentrations, both in blood to which drug had been added and in 15 patients taking

theophylline, increasing amounts of drug entered blood cells. The effect was marked at high concentrations, such as 30 μg/ml (Peat et al, 1977). Such high concentrations were not used in the present study, but limited numbers of blood samples to which varying drug concentrations had been added revealed a similar trend. This corroboration of the present study has lent support to its data, although further work would be desirable. Because of the linearity of the relationship between known added drug concentration and peak height ratio, quantitative determination of unknown concentrations of theophylline was possible from the peak height ratio. Similarly, the linear relationship between whole blood and plasma theophylline concentrations permitted selection of a quantity of theophylline to be added to a blood sample in order to yield a plasma concentration within the therapeutic range, and a p:rbc of potential clinical relevance.

The observation of rapid equilibration of theophylline with red cells (Figure 3) was consistent with previous reports on equilibration of theophylline in the body (Ogilvie, 1978, Chiou et al, 1981). Drug interactions with blood cells and with tissue were both rapid, providing evidence for similarity of interactions with both types of cells. Drug might also have been transported in blood cells with ready availability of blood cell drug to tissues to supplement the F, resulting in especially rapid tissue uptake. The interactions of theophylline with red cells as observed in this study appeared

consistent with reported data and with the potential value of whole blood in investigations of drug-tissue interactions.

D. Procedural Validity

Various potential problems with the procedure have been noted. As with any **in vitro** procedure, direct extrapolation of results to **in vivo** conditions has not been possible. Temperature effects on theophylline red cell partitioning have not previously been investigated, but incubation of theophylline with blood at 25°C and at 37°C was not found to differ. Slight hemolysis by methanol, the vehicle for theophylline, was observed as a color change of plasma, but 60 μl of methanol solution in 6 ml were considered such a small amount that a significant effect of hemolysis would not occur. Hemolysis would be expected to lower the volume of red cells available for theophylline entry, thus raising the concentration observed in plasma. The previously reported blood: plasma ratio of 0.82 +/- 0.10 for four subjects to whom drug had been administered (Mitenko and Ogilvie, 1973) would not have been confounded by possible effects of the solvent methanol. For comparison, the present study yielded an overall mean blood:plasma ratio of 1.0. This was equivalent to a p:rbc ratio of 1.4, for a hematocrit of 0.4. Sheehan and Haythorn (1976) found, in five patients given theophylline, blood:serum ratios ranging from 0.8 to 1.2. Peat et al (1977) obtained blood:plasma ratios of approximately 1 in the range

of 10-20 μg/l for fifteen patients on theophylline therapy. The published values were consistent with the results of the present study. Another potential problem was the use of blood anticoagulated with heparin, which might have generated free fatty acids competing for drug binding to albumin (Wilkinson, 1987), but the small amounts in the blood collection tubes should have had very little effect. Plasticizers from the stoppers of the "Vacutainer" blood collection tubes might also have displaced drugs from protein binding, but recent modifications in construction of these tubes might have minimized the disturbance. Such effects should have been significant for highly protein-bound drugs, at least 90% bound, whereas theophylline was bound on average 53-65% in the plasma of healthy adults (Ogilvie, 1978). Another confounding factor might have been drug accumulation by formed elements of blood other than red cells. This possibility has not been extensively investigated, although O'Neill et al (1986) have not shown theophylline to be concentrated significantly by alveolar macrophages. The differences, if any, between alveolar macrophage drug accumulation and circulating white cell and platelet accumulation have not been clarified. The correlation of white cell and platelet counts with p:rbc in the Pearson matrix might have indicated such an effect, although the sample size was slightly less than that for the remainder of the data and the p:rbc ratio was not separated according to gender. Separation of the data according to

gender could decrease correlation coefficients, as seen for triglyceride in males in Table 4a. Like triglyceride concentration, white cell and platelet counts showed no significant differences among age and gender groups when compared by Tukey's multiple comparison test and would thus not be expected to explain an age-dependent gender effect. Although further study would be needed to clarify this issue, the experimental design was apparently, to a first approximation, adequate.

E. Interpretation of Age-Dependent Gender Effect

The primary effect of age and gender on p:rbc ratio was significant. The **post hoc** attempts to explain this change by examining other parameters had less power but could be confirmed and clarified by future investigations.

The age effect on theophylline p:rbc ratio in males was opposite to that in females, resulting in a net cancellation of effect that might have been missed if the sample had been examined as a whole. Age-dependent gender effects on red cell partitioning have evidently not previously been described, although the literature in the field has been scanty, sometimes misleading, and sometimes incapable of detecting such effects. The studies of Garver et al (1976, 1977) on red cell butaperazine concentrations do not mention the gender or age of their subjects.

Gender effects on red cell partitioning have been

examined by few investigators. No effect of gender was found to alter tricyclic red cell partitioning (Linnoila et al, 1978). However, the age and gender distribution of the sample of 59 depressed inpatients was not specified by Linnoila et al (1978). No gender effect was found on phenytoin red cell partitioning by Kurata and Wilkinson (1974) in six male and six female normal volunteers of unspecified age. The absence of data has precluded evaluation of the possibility of confounding effects of gender and age in these studies. If an age-dependent gender effect existed, these studies might have failed to detect it. Langer et al (1980) observed an effect of age but not gender on imipramine binding to platelets in volunteers and hospital patients, 18 males and 17 females, 17-97 years old. Although no psychoactive medication had been given, other medications may have affected platelet properties. Platelet properties may have differed from the red cell properties shown by the present study.

Age effects on red cell partitioning have been described, but often for small samples with various confounding factors, sometimes including gender. Red cell drug concentrations have seldom been measured more than once per drug, but controversy has arisen. The existence of an age effect on erythrocyte pethidine reported by Chan et al (1975) in volunteers of unspecified gender, four under 30 and four 65 years old, was contradicted by Holmberg et al (1982) and Herman et al (1985). Holmberg et al (1982) compared 19-25 year-old healthy

volunteers, six men and six women, to 63-86 year-old inpatients, five of one gender and six of the other. The presence of various diseases, and pharmacologic therapy if any, might have affected erythrocyte drug binding. Herman et al (1985) compared seven healthy 60-79 year-old men to the six healthy 23-31 year-old men described by Verbeeck et al (1981): the temporal difference may have introduced error. An age effect might have been more readily detected if the age difference between groups had been greater, and an age-dependent gender effect could not have been detected in an exclusively male sample. Nation et al (1977) reported a difference in blood cell chlormethiazole between a group of one female and five males 25-28 years old, compared to three females and three males 71-86 years old. A confounding factor in addition to gender was attainment of final whole blood concentrations, after **in vitro** drug addition, of 5.5 μg/ml in two young subjects but 2 μg/ml in all others. The significance of the findings of Nation et al (1977) have remained unclear. Chapron et al (1985) noted a difference in blood cell acetazolamide in healthy volunteers, two men and two women 27-44 years old, as compared to four women 76-82 years old, one of whom was receiving chronic systemic medication. These findings, in small samples, have been confounded by unequal gender distribution and the presence of other medication. A larger sample size of 18 men and 17 women was used by Langer et al (1980) to demonstrate an age effect on imipramine

platelet binding. However, this study involved inpatients whose pathology and pharmacology may have interfered with the results. Although no age-dependent gender effect on blood cell drug partitioning has previously been demonstrated, many previous studies were incapable by design of detecting such an effect. The nature of the effect on p:rbc ratio observed by the present study and the possibility of such an effect for other drugs should be investigated further.

Post hoc analysis in the present study has revealed several factors predictive of change in the theophylline p:rbc ratio: age, cholesterol, and creatinine for men, and bilirubin and age for women. Although cholesterol might have affected membrane and plasma lipid composition, and bilirubin could conceivably have altered binding to albumin, the mechanism of the age-dependent gender effect has not yet been clarified. Age-dependent gender changes in red cell partitioning of drug might be related to similar changes in physiology. Naylor et al (1977, 1980) demonstrated an age-dependent change in the erythrocyte membrane of women but not men. The mechanism of the age-dependent gender effect on theophylline p:rbc ratio would be an intriguing subject for investigation, and might contribute to understanding of aging mechanisms.

The age-dependent gender effect on theophylline p:rbc ratio has not been previously reported. It could prove useful in dose prediction in the elderly, who have been described as being at increased risk of theophylline toxicity (Jacobs et

al, 1976; Weinberger et al, 1976; Jusko et al, 1977; Nielsen-Kudsk et al, 1978) although controversy exists (Fox et al, 1983). A drug equilibrating rapidly across membranes, as does theophylline (Ogilvie, 1978, Belknap et al, 1987), should be available to tissues in greater quantities in the presence of drug-containing blood cells (Shand, 1973). The effect would be expected to be enhanced at high blood-cell drug concentrations. Canada et al (1986) noted an age and gender difference in theophylline V_d at steady state in rabbits, and suggested that one explanation for this observation might have been a gender and age difference in the fraction of blood to tissue theophylline. Altered p:rbc ratios such as those found by the present study could have contributed to such a difference. A promising topic for investigation might be to what extent such a contribution could explain the findings of Canada et al (1986).

Another possible mechanism suggested by Canada et al (1986) for the gender and age difference in the fraction of blood to tissue theophylline was altered drug uptake by tissue cells as well as by erythrocytes. This interpretation would have been consistent with the hypothesis that similar factors, in this case gender and age, affected both p:rbc and V_d. However, the gender and age difference described by Canada et al (1986) consisted of a pronounced decrease in V_d among mature female rabbits as compared to other gender and age groups, in contrast to the findings of the present study of a

lower p:rbc ratio in older men than women without other differences among gender and age groups. Both V_d and p:rbc ratio were found to exhibit an age-dependent gender effect among older individuals, but these effects differed in nature and might well have been unrelated. Comparability of rabbits and humans has also been doubtful. The applicability of the paper of Canada et al (1986) to the present study has thus been unclear.

Age-dependent gender effects on kinetic parameters, most strikingly on Cl but also on V_d, have been demonstrated to be of significance for desmethyldiazepam (Allen et al, 1980), clobazam (Greenblatt et al, 1981), and diazepam (Greenblatt et al, 1980). Such effects have not been shown to apply to theophylline V_d in man, implying a possible difference between factors associated with theophylline p:rbc ratio and with V_d. Conflicting data have been reported regarding age effects on theophylline V_d. A decrease with age among adults was described by Nielsen-Kudsk et al (1978) and Antal et al (1981). Fox et al (1983) obtained similar data relating age and the ratio of V_d/bioavailability, since V_d could not be measured after an oral dose of theophylline. The unknown effects of age and gender on bioavailability rendered interpretation of the study of Fox et al (1983) difficult. Unfortunately, the above studies suffered from small subject numbers, lack of gender stratification, drug administration under clinical rather than controlled experimental conditions,

and the presence of other medications or confounding influences such as cigarette smoking. In contrast to these researchers, Powell et al (1978) found no relationship between age and V_d in 31 normal volunteers, mean age 29.6 +/- 20.5 years, or in 26 patients with acute airway obstruction, mean age 51.5 +/- 17.2 years. Disease and therapy would have been expected to involve complicated confounding factors. Among the healthy subjects of Powell et al (1978), subject numbers might have been inadequate to detect small effects on V_d: 28 of the volunteers were 20-39 years old whereas only 3 were 65-70 years old. Au et al (1985) found no influence of age on V_d in 38 men with stable chronic obstructive pulmonary disease, 21 of whom were 50-60 years old and 18 of whom were 60 years or older. Nineteen subjects had concurrent cardiovascular disease; confounding variables included cardiovascular and pulmonary pathology. Three studies involving only one gender also failed to demonstrate age changes. No significant age difference in theophylline V_d was detected by Vestal et al (1987) in 40 healthy men categorized into equal groups according to smoking habits. Crowley et al (1988) found, in a sample of 40 men divided into equal subgroups according to age (20-33 years old and 64-88 years) and smoking habits, no significant change in theophylline V_d with age. Shin et al (1988) reported no significant effect of age on V_d of the central compartment or V_d at steady state. The sample size of Shin et al (1988) was smaller than that of Vestal et al (1987)

or Crowley et al (1988), consisting of 6 men 20-27 years old and 6 men 65-77 years old; one older man out of the 6 was receiving chronic medication for diabetes. None of the above studies has considered the effects of passive smoking, shown by Matsunga et al (1989) to affect theophylline Cl similarly to active smoking. Although Matsunga et al (1989) found no effect of passive smoking on V_d, Hunt et al (1976) reported increased theophylline V_d in smokers. Other possibly relevant variables not always taken into account were dietary protein and exercise patterns. Several studies attempted to eliminate confounding effects of gender by selecting only males. The largest age- and gender-related difference might not lie between younger and older men, and a small alteration in V_d might not be detected without a large subject group. The present study has indicated, by analysis of variance and Tukey's multiple comparison test, a statistically significant difference in p:rbc ratio only between older men and women. Other differences in p:rbc ratio observed among the four age and gender groups in this sample might have been due to random variation. Previous studies of factors correlated with V_d might have been flawed because of failure to control for relevant variables and poor choice of study group size, age range, or gender. The best-designed of these studies have not demonstrated an age effect on theophylline V_d. As age-related changes in theophylline V_d have not been unequivocally demonstrated, factors correlated with the p:rbc ratio would

appear to differ from those correlated with theophylline V_d and the hypothesis proposed by the present investigation to be false. However, these studies of theophylline V_d may not have been capable of detection of an age-dependent gender effect. Indirect comparison of p:rbc and V_d derived from different subject groups under different conditions would be expected to be less informative than simultaneous comparison of these parameters in the same sample of individuals. Such comparison has not been performed, although Mitenko and Ogilvie (1973) have measured the inverse of the p:rbc ratio in a very small subset of a sample from which V_d had been determined. Although the present study has provided no evidence for correlation of similar factors to the theophylline p:rbc ratio and to V_d, more reliable data would be obtained by comparison of these parameters in the same sample. Further studies should be performed to clarify the issue and explore related questions such as the mechanism of the changes in p:rbc ratio.

F. Opportunities for Investigation

The contribution of platelets to drug association with the cellular fraction of blood could be estimated by incubating duplicate samples of blood with drug, processing one sample of each pair as in the present experiment, and transferring the second sample, after incubation with drug, to a serum tube in order to promote coagulation and permit comparison of serum and plasma drug concentrations. Addition

of protamine sulfate might be necessary in order to neutralize the anticoagulant effect of heparin mixed with the blood prior to incubation. Serial fractionation of blood by repeated centrifugation would permit separation of all the cellular elements of blood at the expense of a longer processing time, allowing potential re-equilibration during fractionation.

The effects of alterations in bilirubin, cholesterol, and creatinine on theophylline V_d have not been reported but would be expected to be associated with, and easily confounded by, alterations in liver or kidney function or in diet and widespread physiologic or pathologic effects. In man, distinguishing between the effects of concentrations of these substances and associated physiologic and pathologic conditions could be exceedingly difficult. In experimental animals, tissue drug concentrations could be compared to V_d after controlled alteration of bilirubin, cholesterol, or creatinine in the absence of pre-existing pathologic conditions. Determination of the effects of body weight and smoking on theophylline p:rbc ratio would also clarify the issue of similarity of factors correlated to p:rbc ratio and V_d. In a single large sample, equally divided according to gender, age, and confounding factors, p:rbc ratio and V_d could be investigated with respect to any effects of bilirubin, cholesterol, creatinine, history of passive and active smoking, and body weight, as well as with respect to any correlation between p:rbc ratio and V_d. Such a study could be

performed both in healthy volunteers under controlled experimental conditions and in the population of practical interest, patients receiving therapy.

The present data on p:rbc ratio, as compared to previous reports concerning V_d, have not indicated similar predictive factors for these parameters. The data have not been conclusive, due to flaws in numerous previous reports on theophylline V_d and to lack of comparison of both parameters with each other in a single subject group under the same conditions. If a study which circumvented these difficulties showed a correlation between p:rbc ratio and V_d, such a relationship might permit convenient prediction of optimal drug dosage for a particular individual before the onset of drug therapy. If p:rbc ratio and V_d did not both show an age-dependent gender effect, the possibility of correlation between p:rbc and V_d might remain valid within a given age or gender group, or for other drugs than theophylline. The effects of age and gender on the p:rbc ratios of morphine and diazepam could be compared in blood samples from volunteers; the age changes in V_d have been found to be sizable and opposite. The p:rbc ratio and V_d could also be compared in man for various drugs during modifications of physiological or pathological conditions liable to influence V_d, such as weight loss in the obese or surgical removal of the spleen or parts of the gastrointestinal tract. The onset or conclusion of pregnancy might offer an opportunity to observe physiological

conditions which have altered the V_d of certain drugs, but investigation in man would be limited to determination of p:rbc ratio in order to avoid possible teratogenicity. In animals, p:rbc ratios and V_d could be directly compared during pregnancy. Such studies might clarify the relationship, if any, of p:rbc ratios, V_d, and factors influencing them under various conditions.

The mechanism of the age-dependent gender effect on the theophylline p:rbc ratio would certainly be worthy of further study. The p:rbc ratios could be compared in animals, volunteers, and patients during alteration in the possible predictive factors identified in the present experiment, namely bilirubin, cholesterol, and creatinine; an example might be the therapy of hypercholesterolemia. Spontaneous changes in bilirubin and creatinine in a patient would most likely involve altered drug metabolism and excretion, confounding measurement of tissue or blood cell drug concentrations. Controlled alteration of these factors might more appropriately be performed in experimental animals, animal tissue preparations, or human blood samples.

Human studies by Nielsen-Kudsk et al (1978) and Antal et al (1981) have suggested an effect of age on theophylline V_d, unlike the human studies of Powell et al (1978), Au et al (1985), Vestal et al (1987), Crowley et al (1988), and Shin et al (1988); a rabbit study by Canada et al (1986) has demonstrated an age-dependent gender effect on theophylline

V_d. The present experiment has demonstrated an age-related gender effect on theophylline p:rbc ratio not previously described and unlike most reported data on theophylline V_d. The present data have not supported the hypothesis of correlation of similar factors to theophylline p:rbc ratio and V_d. Further study could be performed in order to investigate the possibility of correlation between theophylline p:rbc ratio and V_d, as well as to examine the mechanism of the age-dependent gender effect on theophylline p:rbc ratio.

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REFERENCES

Abbey, M., P.M. Clifton, E.J. McMurchie, G.I. McIntosh, and B.J. Nestel. Effect of a high fat/cholesterol diet with or without eicosapentanoic acid on plasma lipids, lipoprotein and lipid transfer protein activity in the marmoset. Atherosclerosis 81: 163-174, 1990

Abernethy, D.R., D.J. Greenblatt, M. Divoli, J.S. Harmatz and R.I. Shader. Alterations in drug distribution and clearance due to obesity. The Journal of Pharmacology and Experimental Therapeutics 217: 681-685, 1981

Abernethy, D.R., D.J. Greenblatt, M. Divoli, and R.I. Shader. Prolongation of drug half-life due to obesity: studies of desmethyldiazepam (clorazepate). Journal of Pharmaceutical Sciences 71: 942-944, 1982

Aggeler, P.M., R.A. O'Reilly, L. Leong, and P.E. Kowitz. Potentiation of anticoagulant effect of warfarin by phenylbutazone. The New England Journal of Medicine 276: 496-501, 1967

Alafuzoff, I., R. Adolfsson, I. Grundke-Iqbal, and B. Winblad. Blood-brain barrier in Alzheimer dementia and in non-demented elderly. Acta Neuropathologica (Berlin) 73: 160-166, 1987

Alafuzoff, I., R. Adolfsson, I. Grundke-Iqbal, and B. Winblad. Perivascular deposits of serum proteins in cerebral cortex in vascular dementia. Acta Neuropathologica (Berlin) 66: 292-298, 1985

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson. Differentiated cells and the maintenance of tissues. In Molecular Biology of the Cell. Second Edition. Garland Publishing, Inc., New York, pp.951-1000, 1989

Alexander, L.R. and J.B. Justice. Fatty acid composition of human erythrocyte membranes by capillary gas chromatography-mass spectrometry. Journal of Chromatography 342: 1-12, 1985

Allen, M.D., D.J. Greenblatt, J.S. Harmatz, and R.I. Shader. Desmethyldiazepam kinetics in the elderly after oral prazepam. Clinical Pharmacology and Therapeutics 28: 196-202, 1980

Anonymous. Drugs in the elderly. Editorial. British Medical Journal 1: 1168, 1978

Anonymous. Waters Associates Liquid Chromatography School. Waters Scientific Limited, unknown publication date.

Antal, E.J., P.A. Kramer, S.A. Mercik, D.J. Chapron, and I.R. Lawson. Theophylline pharmacokinetics in advanced age. British Journal of Clinical Pharmacology 12: 637-645, 1981

Aoki, F.Y. and D.S. Sitar. Amantadine kinetics in healthy elderly men: implications for influenza prevention. Clinical Pharmacology and Therapeutics 37: 137-144, 1985

Aoki, F.Y., V.K. Hildahl, G.W. Large, P.A. Mitenko, and D.S. Sitar. Aging and heavy drug use: a prescription survey in Manitoba. Journal of Chronic Disease 36: 75-84, 1983

Araki, K. and J.M. Rifkind. Age dependent changes in osmotic hemolysis of human erythrocytes. Journal of Gerontology 35: 499-505, 1980

Au, W.Y.W., A.K. Dutt, and N. DeSoyza. Theophylline kinetics in chronic obstructive airway disease in the elderly. Clinical Pharmacology and Therapeutics 37: 472-478, 1985

Bandyopadhyay, S. and P. Somani. A comparison of plasma, white blood cell, red blood cell, and tissue distribution of amiodarone and desethylamiodarone in anesthetized dogs. Journal of Cardiovascular Pharmacology 10: 379-388, 1987

Belknap, S.M., J.E. Nelson, T.I. Ruo, M.C. Frederiksen, E.M. Worwag, S-G. Shin and A.J. Atkinson. Theophylline distribution kinetics analyzed by reference to simultaneously injected urea and inulin. Journal of Pharmacology and Experimental Therapeutics 243: 963-969, 1987

Benowitz, N., R.P. Forsyth, K.L. Melmon, and M. Rowland. Lidocaine disposition kinetics in monkey and man: I. Prediction by a perfusion model. Clinical Pharmacology and Therapeutics 16: 87-98, 1974a

Benowitz, N., R.P. Forsyth, K.L. Melmon, and M. Rowland. Lidocaine disposition kinetics in monkey and man: II. Effects of hemorrhage and sympathomimetic drug administration. Clinical Pharmacology and Therapeutics 16: 99-109, 1974b

Bergqvist, Y. and B. Domej-Nyberg. Distribution of chloroquine and its metabolite desethylchloroquine in human blood cells and its implication for the quantitative determination of these compounds in serum and plasma. Journal of Chromatography 272: 137-148, 1983

Berkowitz, B.A., K.V. Cerreta and S. Spector. The influence of physiologic and pharmacologic factors on the disposition of morphine as determined by radioimmunoassay. Journal of Pharmacology and Experimental Therapeutics 191: 527-534, 1974

Bilej, M. and V. Vetvicka. The transmembrane and intracellular transport of drugs: interactions with the cytoskeletal network. Critical Reviews in Therapeutic Drug Carrier Systems 6: 163-191, 1989

Bjorksten, J. and H. Tenhu. The cross-linking theory of aging: added evidence. Experimental Gerontological 25: 91-95, 1990

Borga, O., K-E. Andersson, L-E. Edholm, P-O. Fagerstrom, E. Lunell, and C.G. Persson. Emprofylline kinetics in healthy subjects after single doses. Clinical Pharmacology and Therapeutics 34: 799-804, 1983

Borondy, P., W.A. Gill, T. Chang, R.A. Buchanan, and A.J. Glazko. Effect of protein binding on the distribution of 5,5-diphenylhydantoin between plasma and red cells. Annals of the New York Academy of Sciences 226: 82-87, 1973

Bourn, D.M., R.S. Gibson, and A.C. MacDonald. Selected nutrient intakes of a cohort of Canadian post-menopausal women estimated from three-day dietary records: sources of variance in nutrient intakes and probability estimation of their adequacy. Nutrition Research 10: 391-401, 1990

Bourre, J.M., M. Piciotti, O. Dumont, G. Pascal, and G. Durand. Dietary linoleic acid and polyunsaturated fatty acids in rat brain and other organs. Minimal requirements of linoleic acid. Lipids 25: 465-472, 1990

Bowdler, A.J., R.M. Dougherty and N.C. Bowdler. Age as a factor affecting erythrocyte osmotic fragility in males. Gerontology 27: 224-231, 1981

Bredesen, J.E. and P. Kierulf. Relationship between alpha-1 acid glycoprotein and plasma binding of disopyramide and mono-N-dealkyldisopyramide. British Journal of Clinical Pharmacology 18: 779-784, 1984

Butler, T.C. The distribution of drugs. In Fundamentals of Drug Metabolism and Drug Distribution. Editors B.N. LaDu, H.G. Mandel, and E.L. Way. The Williams and Wilkins Company, Baltimore, 1971, ch. 3, pp. 44-62

Canada, A., J. Wilson, and E. Calabrese. Elimination kinetics of theophylline in the rabbit: effect of age and sex. Drug Metabolism and Disposition 14: 372-374, 1986

Castleden, C.M. and C.F. George. Increased sensitivity to benzodiazepines in the elderly. In Drugs and the Elderly. Editors J. Crooks and I.H. Stevenson. University Park Press, Baltimore, 1979, ch. 15, pp. 169-178

Chan, G.L.C., D.M. Canafax, and C.A. Johnson. The therapeutic use of azathioprine in renal transplantation. Pharmacotherapy 7: 165-177, 1987

Chan, K., M.J. Kendall, M. Mitchard, W.D.E. Wells, and M. D. Vickers. The effect of ageing on the plasma pethidine concentration. British Journal of Clinical Pharmacology 2: 297-302, 1975

Chapel, H.H, M.M.Esirl, and G.K. Wilcock. Immunoglobulin and other proteins in the cerebrospinal fluid of patients with Alzheimer's disease. Journal of Clinical Pathology 37: 697-699, 1984

Chapron, D.J., K.R. Sweeney, P.U. Feig, and P.A. Kramer. Influence of advanced age on the disposition of acetazolamide. British Journal of Clinical Pharmacology 19: 363-371, 1985

Chen, M-L., M.G. Lee, and W.L. Chiou. Pharmacokinetics of drugs in blood III: metabolism of procainamide and storage effect of blood samples. Journal of Pharmaceutical Sciences 72: 463-584, 1983

Chesrow, E.J., D. Bronsky, E. Orfei, H. Dyniewicz, A. Dubin, and J. Musci. Serum proteins in the aged. Geriatrics 13: 20-24, 1958

Chiou, W.L. A new model-independent physiological approach to study hepatic drug clearance and its applications. International Journal of Clinical Pharmacology, Therapy and Toxicology 22: 577-590, 1984

Chiou, W.L., G. Lam, M-L. Chen, and M.G. Lee. Instantaneous input hypothesis in pharmacokinetic studies. Journal of Pharmaceutical Sciences 70: 1037-1039, 1981

Ciuffetti, G., M. Mercuri, M.T. Rizzo, S.E. Lennie, and G.D.O. Lowe. Age and blood cell rheology. Gerontology 35: 31-35, 1989

Cohn, V.H. Transmembrane movement of drug molecules. In Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins Company, Baltimore, 1971, ch. 1, pp. 3-21

Cristofalo, V.J. Biological mechanisms of aging: an overview. In Principles of Geriatric Medicine and Gerontology. Second Edition. Editors W.R. Hazzard, R. Andres, E.L. Bierman, and J.P. Blass. McGraw-Hill Information Services Co., Health Professions Division, New York, pp. 3-14, 1990, ch. 1, pp. 3-14

Crouthamel, W.G. New areas of research. In The Effect of Disease States on Drug Pharmacokinetics. Editor L.Z. Benet. American Pharmaceutical Association, Washington, D.C., 1976, ch. 1, pp.1-9

Crowley, J.J., B.J. Cusack, S.G. Jue, J.R. Koup, B.K. Park, and R.E. Vestal. Aging and drug interactions. II. Effect of phenytoin and smoking on the oxidation of theophylline and cortisone in healthy men. Journal of Pharmacology and Experimental Therapeutics 245: 513-523, 1988

Dalmark M. Characteristics of doxorubicin transport in human red blood cells. Scandinavian Journal of Clinical Laboratory Investigation 41: 633-639, 1981

David, B.M., K.F. Ilett, E.G. Whitford and N.S. Stenhouse. Prolonged variability in plasma protein binding of disopyramide after acute myocardial infarction. British Journal of Clinical Pharmacology 15: 435-441, 1983

Dayton, P.G., Z.H. Izraili, and J.M. Perel. Influence of binding on drug metabolism and distribution. Annals of the New York Academy of Sciences 226: 172-194, 1973

DeLucchi, C., M.L. Pita, M.J. Faus, J.A. Molina, R. Uauy, and A. Gil. Effects of dietary nucleotides on the fatty acid composition of erythrocyte membrane lipids in term infants. Journal of Paediatric Gastroenterology and Nutrition 6: 568-574, 1987

De Simone, G., R.B. Devereux, S. Chien, M.H. Alderman, S.A. Atlas, and J.H. Laragh. Relation of blood viscosity to demographic and physiologic variables and to cardiovascular risk factors in apparently normal adults. Circulation 81: 107-117, 1990

Dickson, C., M.S. Schwartzman, and J.S. Bertino. Factors affecting aminoglycoside disposition: effects of circadian rhythm and dietary protein intake on gentamicin pharmacokinetics. Clinical Pharmacology and Therapeutics 39: 325-328, 1986

Dollery, C.T., D. Emslie-Smith and D.F. Muggleton. Action of chlorothiazide on the distribution, excretion and hypotensive effect of pempidine in man. British Journal of Pharmacology 17: 488-506, 1961

Duhm, von B., W. Maul, H. Medenwald, K. Patzschke, and L.A. Wagner. Tierexperimentelle Untersuchungen zur Pharmacokinetik und Biotransformation von radioaktiv markiertem 4-(2'-nitrophenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarbonsauredimethylester. Arzneimittel Forschung 22: 42-53, 1972

Dybkaer, R., M. Lauritzen and R. Krakauer. Relative reference values for clinical chemical and hematological quantities in "healthy" elderly people. Acta Medica Scandinavica 209: 1-9, 1981

Eap, C.B., C. Cuendet, and P. Baumann. Binding of d-methadone, l-methadone, and dl-methadone to proteins in plasma of healthy volunteers: Role of the variants of α_1-acid glycoprotein. Clinical Pharmacology and Therapeutics 47: 338-346, 1990

Erdmann, E. and W. Hasse. Quantitative aspects of ouabain binding to human erythrocyte and cardiac membranes. Journal of Physiology 251: 671-682, 1975

Eskelinen, S. The kinetics of drug-membrane interactions in human erythrocytes. Canadian Journal of Physiology and Pharmacology 65: 2373-2378, 1987

Essassi, D., R. Zini, C. Hamberge, S. Urien, C. Rougeot, A. Uzan and J-P. Tillement. Binding **in vitro** of pipequaline (45319RP) onto plasma proteins and blood cells in man. Biochemical Pharmacology 36: 3501-3507, 1987

Essassi, D., S. Urien, R. Zini, and J-P. Tillement. Pipequaline transport from blood to brain and liver: role of plasma protein-bound drug. Journal of Pharmacy and Pharmacology 41: 595-600, 1989

Evans, G.H., A.S. Nies, and D.G. Shand. The disposition of propranolol III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog and rat. Journal of Pharmacology and Experimental Therapeutics 186: 114-122, 1973

Fagan, T.C., T. Walle, M.J. Oexmann, U.K. Walle, S.A. Bai, and T.E. Gaffney. Increased clearance of propranolol and theophylline by high-protein compared with high-carbohydrate diet. Clinical Pharmacology and Therapeutics 41: 402-406, 1987

Feldman, R.D., L.E. Limbird, J. Nadeau, D. Robertson and A.J.J. Wood. Alterations in leukocyte beta receptor affinity with aging: a potential explanation for altered beta-adrenoceptor sensitivity in the elderly. The New England Journal of Medicine 310: 815-819, 1984

Fleck, A. Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. Proceedings of the Nutrition Society 48: 347-354, 1989

Fox, R.W., S.Samaan, and S.C. Bukantz. Theophylline kinetics in a geriatric group. Clinical Pharmacology and Therapeutics 34: 60-67, 1983

Fraser, C.G., S.P. Wilkinson, R.G. Neville, J.D.E. Knox, J.F. King, R.S. MacWalter. Biologic variation of common hematologic laboratory quantities in the elderly. American Journal of Clinical Pathology 92: 465-470, 1989

Frederiksen, M.C., T.I. Ruo, M.J. Chow, and A.J. Atkinson. Theophylline pharmacokinetics in pregnancy. Clinical Pharmacology and Therapeutics 40: 321-328, 1986

Gal, P., W.J. Jusko, A.M. Yurchak, and B.A. Franklin. Theophylline disposition in obesity. Clinical Pharmacology and Therapeutics 23: 438-444, 1978

Garry, P.J., J.S. Goodwin, and W.C. Hunt. Iron studies and anemia in the elderly: new findings and a review of previous studies. Journal of the American Geriatric Society 1: 389-399, 1983

Gibaldi, M. and M. Schwartz. Apparent effect of probenecid on the distribution of penicillins in man. Clinical Pharmacology and Therapeutics 9: 345-349, 1968

Garver, D.L., J.M. Davis, H. Dekirmenjian, F.D. Jones, R. Casper, and J. Haraszti. Pharmacokinetics of red blood cell phenothiazine and clinical effects. Archives of General Psychiatry 33: 862-866, 1976

Garver, D.L., H. Dekirmenjian, J.M. Davis, R. Casper and S. Ericksen. Neuroleptic drug levels and therapeutic response: preliminary observations with red blood cell bound butaperazine. American Journal of Psychiatry 134: 304-307, 1977

Gietl, Y., H. Spahn, and E. Mutschler. Enantioselective disposition of R-(-)- and S-(+)-prenylamine in the rat. Arzneimittel-Forschung 39: 853-856, 1989

Goldstein, A., L. Aronow, and S.M. Kalman. Principles of drug action: the basis of pharmacology. Second edition. John Wiley and Sons, New York, 1974

Goresky, C.A., G.G. Bach, and B.E. Nadeau. Red cell carriage of label. Its limiting effect on the exchange of materials in the liver. Circulation Research 36: 328-351, 1975

Gorodischer, R., W.J. Jusko, and S.J. Yaffe. Tissue and erythrocyte distribution of digoxin in infants. Clinical Pharmacology and Therapeutics 19: 256-263, 1976

Greenblatt, D.J., M.D. Allen, J.S. Harmatz, and R.I. Shader. Diazepam disposition determinants. Clinical Pharmacology and Therapeutics 27: 301-312, 1980

Greenblatt, D.J., M. Divoli, S.K. Puri, I. Ho, M.A. Zinny and R.I. Shader. Clobazam kinetics in the elderly. British Journal of Clinical Pharmacology 12: 631-636, 1981

Gross, A.S., J.A. Baird-Lambert and K.F. Brown. Disposition of ritodrine in pregnant and non-pregnant women. British Journal of Clinical Pharmacology 25: 155P-156P, 1988

Guezennec, C.Y., J.F. Nadaud, P. Satabin, F. Leger and P. Lafargue. Influence of polyunsaturated fatty acid diet on the hemorrheological response to physical exercise in hypoxia. International Journal of Sports Medicine 10: 286-291, 1989

Hahn, F.E., R.L. O'Brien, J. Ciak, J.L. Allison, and J.G. Olenick. Studies on modes of action of chloroquine, quinacrine, and quinine and on chloroquine resistance. Military Medicine 131S: 1071-1089, 1966

Harnes, J.R. Normal values with increasing age. Journal of Chronic Disease 33: 593-594, 1980

Heckers, H., F.W. Melcher, and Platt, D.D. The structure of lipids in erythrocytic membranes in young and old people. Fette Seifen Anstrichmittel 80: 440, 1978

Herman, R.J., C.B. McAllister, R.A. Branch, and G.R. Wilkinson. Effects of age on meperidine disposition. Clinical Pharmacology and Therapeutics 37: 19-24, 1985

Hoffbrand, A.V. and J.E. Pettit. Essential Haematology. Second Edition. Blackwell Scientific Publications, Oxford, 1985

Holley, F.O., K.. Ponganis, and D.R. Stanski. Effects of cardiac surgery with cardiopulmonary bypass on lidocaine disposition. Clinical Pharmacology and Therapeutics 35: 617-626, 1984

Holmberg, L., I. Odar-Cederlof, L.O. Boreus, L. Heyner, and M. Ehrnebo. Comparative disposition of pethidine and norpethidine in old and young patients. European Journal of Clinical Pharmacology 22: 175-179, 1982

Holmes, R.P., M. Mahfouz, B.D. Travis, N.L. Yoss. and M.J. Keenan. The effect of membrane lipid composition on the permeability of membranes to Ca++. Annals of the New York Academy of Sciences 414: 44-56, 1983

Holt, D.W., G.C.A. Storey, W.J. McKenna, P.C. Adams, and R.W.F. Campbell. Unwanted effects of amiodarone in relation to tissue concentrations. Journal of the American College of Cardiology 1: 630, 1983b (Abstract)

Holt, D.W., G.T. Tucker, P.R. Jackson and G.C.A. Storey. Amiodarone pharmacokinetics. American Heart Journal 106: 840-847, 1983a

Hunt, S.N., W.J. Jusko, and A.M. Yurchak. Effect of smoking on theophylline disposition. Clinical Pharmacology and Therapeutics 19: 546-551, 1976

Hurdle, A.D.F. and A.J. Rosin. Red cell volume and red cell survival in normal aged people. Journal of Clinical Pathology 15: 343-345, 1962

Hurwitz, N. Admissions to hospital due to drugs. British Medical Journal 1: 539-540, 1969b

Hurwitz, N. Predisposing factors in adverse reactions to drugs. British Medical Journal 1: 536-539, 1969a

Hussain, A.A. and M.J. Voaden. Erythrocyte osmotic fragility in man: variation with age and sex. British Journal of Haematology 57: 716-718, 1984

Jacobs, M.H., R.M. Senior, G. Kessler. Clinical experience with theophylline. Relationships between dosage, serum concentration, and toxicity. Journal of the American Medical Association 235: 1983-1986, 1976

Jusko, W.J., J.R. Koup, J.W. Vance, J.J. Schentag, and P. Kuritzky. Intravenous theophylline therapy: nomogram guidelines. Annals of Internal Medicine 86: 400-404, 1977, ch. 7, pp. 99-123

Jusko, W.J., M.J. Gardner, A. Mangione, J.J. Schentag, J.R. Koup, and J.W. Vance. Factors affecting theophylline clearances: age, tobacco, marijuana, cirrhosis, congestive heart failure, obesity, oral contraceptives, benzodiazepines, barbiturates, and ethanol. Journal of Pharmaceutical Sciences 68: 1358-1366, 1979

Jusko, W.J. Pharmacokinetics in disease states changing protein binding. In The Effect of Disease States on Drug Pharmacokinetics. Editor L.Z. Benet. American Pharmaceutical Association, Washington, D.C., 1976

Kaiser, V., G.M.E. Janssen, J.W.J. van Wersch. Effect of training on red blood cell parameters and plasma ferritin: a transverse and a longitudinal approach. International Journal of Sports Medicine 10: S169-175, 1989

Kampmann J.P. and J.E. Molholm Hansen. Serum protein binding of propylthiouracil. British Journal of Clinical Pharmacology 16: 549-552, 1983

Kaye, C.M., R.E. Haddock, P.F. Langley, G. Mellows, T.C.G. Tasker, B.D. Zussman, and W.H. Greb. A review of the metabolism and pharmacokinetics of paroxetine in man. Acta Psychiatrica Scandinavica 80: S60-S75, 1989

Keberle, H., P. Loustalot, R.K. Maller, J.W. Faigle, K. Schmid. Biochemical effects of drugs on the mammalian conceptus. Annals of the New York Academy of Sciences 123: 252-262, 1965

Kelly, A. and L. Munan. Haematologic profile of natural populations: red cell parameters. British Journal of Haematology 35: 153-160, 1977

Kenney, R.A. Physiology of aging. Clinics in Geriatric Medicine 1: 37-59, 1985

Kitagawa, T., and Y. Akamatsu. Modulation of passive permeability by external ATP and cytoskeleton-attacking agents in cultured mammalian cells. Biochemica et Biophysica Acta 734: 25-32, 1983

Klein, J.O., M. Finland. Nafcillin: antibacterial action **in vitro** and absorption and excretion in normal young men. American Journal of the Medical Sciences 246: 10-26, 1963

Kleine, T.O., L. Weber, and P. Zofel. Changes with age in leucocyte counts, protein and IgG content of lumbar cerebrospinal fluid (CSF) of patients with inflammatory, ischemic, and hemorrhagic diseases or tumors of the central nervous system (CNS). Zeitschrift fur Gerontologie 21: 102-105, 1988

Klotz, U., G.R. Avant, A. Hoyumpa, S. Schenker, and G.R. Wilkinson. The effects of age and liver disease on the disposition and elimination of diazepam in adult man. Journal for Clinical Investigation 55: 347-359, 1975

Knoppert, D.C., M.S. Spino, R. Beck, J.J. Thiessen, and S.M. MacLeod. Cystic fibrosis: enhanced theophylline metabolism may be linked to the disease. Clinical Pharmacology and Therapeutics 44: 254-264, 1988

Kochenour, N.K., M.G. Emery, and R.J. Sawchuk. Phenytoin metabolism in pregnancy. Obstetrics and Gynecology 56: 577-582, 1980

Koch-Weser, J., D.J. Greenblatt, E.M. Sellers and R.I. Shader. Drug disposition in old age. New England Journal of Medicine 306: 1081-1088, 1982

Kohn, R.R. and V.M. Monnier. Normal aging and its parameters. Clinical Pharmacology in the Elderly. Editor C. Swift. Marcel Dekker, Incorporated, New York, 1987, ch. 1, pp. 3-30

Korpela, R. and K.T. Olkkola. Pharmacokinetics of intravenous diclofenac sodium in children. European Journal of Clinical Pharmacology 38: 293-295, 1990

Korten, K. and K.W. Miller. Erythrocyte ghost-buffer partition coefficients of phenobarbital, pentobarbital, and thiopental support the pH-partition hypothesis. Canadian Journal of Physiology and Pharmacology 57: 325-328, 1978

Koysooko, R., E.F. Ellis, and G. Levy. Relationship between theophylline concentration in plasma and saliva of man. Clinical Pharmacology and Therapeutics 15: 454-460, 1974

Krauer, B., F. Krauer, and F.E. Hytten. Drug disposition and pharmacokinetics in the maternal-placental-fetal unit. Pharmacology and Therapeutics 10: 301-328, 1980

Kremer, J.H., J. Wilting, and L.H.M. Janssen. Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacological Reviews 40: 1-47, 1988

Kroening, B.H. and M. Weintraub. Age-associated changes in tissue distribution and uptake of ^{3}H-digoxin in mice and guinea pigs. Pharmacology 20: 21-26, 1980

Kuntzman, R., M. Jacobson, I. Tsai, and J.J. Burns. Certain aspects of drug binding to nonplasma proteins as illustrated by studies with cyclizine, chlorcyclizine, and polymyxin B. Annals of the New York Academy of Sciences 226: 131-147, 1973

Kurata, D. and G.R. Wilkinson. Erythrocyte uptake and plasma binding of diphenylhydantoin. Clinical Pharmacology and Therapeutics 16: 355-362, 1974

Lalonde, R.L., D.M. Tenero, B.S. Burlew, V.L. Herring, and M.B. Bottorff. Effects of age on the protein binding and disposition of propranolol stereoisomers. Clinical Pharmacology and Therapeutics 47: 447-455, 1990

Langer, S.Z., M.S. Briley, R. Raisman, J-F. Henry, and P.L. Morselli. Specific ^{3}H-imipramine binding in human platelets. Influence of age and sex. Archives of Pharmacology 313: 189-194, 1980

Lee, H-J and W.L. Chiou. Erythrocytes as barriers for drug elimination in the isolated rat liver. I. Doxorubicin. Pharmaceutical Research 6: 833-839, 1989

Lin, T. and J.H. Lin. Effects of protein binding and experimental disease states on brain uptake of benzodiazepines in rats. Journal of Pharmacology and Experimental Therapeutics 253: 45-50, 1990

Linnoila, M., F. Dorrity, and K. Jobson. Plasma and erythrocyte levels of tricyclic antidepressants in depressed patients. American Journal of Psychiatry 135: 557-561, 1978

Lipschitz, D.A. Aging of the hematopoietic system. In Principles of Geriatric Medicine and Gerontology. Second Edition. Editors W.R. Hazzard, R. Andres, E.L. Bierman, and J.P. Blass. McGraw-Hill Information Services Co., Health Professions Division, New York, pp. 655-661, 1990

Machard, B., P. Misslin and M. Lemaire. Influence of plasma protein binding on the brain uptake of an antifungal agent, terbinafine, in rats. Journal of Pharmacy and Pharmacology 41: 700-704, 1989

Machleidt, H., S. Roth, and P. Seeman. The hydrophobic expansion of erythrocyte membranes by the phenol anesthetics. Biochimica et Biophysica Acta 255: 178-189, 1972

Magnani, M., L. Rossi, V. Stocchi, L. Cucchiarini, G. Piacentini, and G. Fornaini. Effect of age on some properties of mice erythrocytes. Mechanisms of Ageing and Development, 42: 37-47, 1988

Mancardi, G.L., F. Perdelli, C. Rivano, A. Leonardi, and O. Bugiani. Thickening of the basement membrane of cortical capillaries in Alzheimer's disease. Acta Neuropathologica (Berlin) 49: 79-83, 1980

Manku, M.S., D.F. Horrobin, Y-S. Huang and N. Morse. Fatty acids in plasma and red cell membranes in normal humans. Lipids 18: 906-908, 1983

Marchant, B., B.E.W. Brownlie, D. McKay Hart, P.W. Horton, and W.D.Alexander. The placental transfer of propylthiouracil, methimazole and carbimazole. Journal of Clinical Endocrinology 45: 1187-1193, 1977

Marcus, F.I. Clinical pharmacology of amiodarone. In Clinical aspects of life-threatening arrhythmias. Annals of the New York Academy of Sciences 427: 112-125, 1984

Marshall, E.K. and Dearborn, E.H. The relation of the plasma concentration of quinacrine to its antimalarial activity. Journal of Pharmacology and Experimental Therapeutics 88: 142-153, 1946

Martyn, J.A.J., D.R. Abernethy, and D.J. Greenblatt. Plasma protein binding of drugs after severe burn injury. Clinical Pharmacology and Therapeutics 35: 535-539, 1984

Mather, L.E., G.T. Tucker, A.E. Pflug, M.J. Lindop, and C. Wilkerson. Meperidine kinetics in man. Intravenous injection in surgical patients and volunteers. Clinical Pharmacology and Therapeutics 17: 21-30, 1975

Matsunga, S.K., P.M. Plezia, M.D. Karol, M.D. Katz, A.E. Camilli, and N.L. Benowitz. Effects of passive smoking on theophylline clearance. Clinical Pharmacology and Therapeutics 46: 399-407, 1989

McGuire, G.M., G.J.A. Macphee, G.G. Thompson, M.R. Moore, and M.J. Brodie. Effects of sodium valproate on haem biosynthesis in man: effects for seizure management in the porphyric patient. European Journal of Clinical Investigation 18: 29-32, 1988

Meuldermans, W.E.G., R.M.A. Hurkmans and J.J.P. Heykants. Plasma protein binding and distribution of fentanyl, sufentanil, alfentanil and lofentanil in blood. Archives internationales de pharmacodynamie et de therapie 257: 4-19, 1982

Miller, K.W. and S-C. T. Yu. The dependence of the lipid bilayer membrane: buffer partition coefficient of pentobarbitone on pH and lipid composition. British Journal of Pharmacology 61: 57-63, 1977

Miskolczi, P., L. Vereczkey, L. Szalay, and C. Gondocs. Effect of age on the pharmacokinetics of vinpocetine (Cavinton) and apovincaminic acid. European Journal of Clinical Pharmacology 33: 185-189, 1987

Mitenko, P.A. and R.I. Ogilvie. Pharmacokinetics of intravenous theophylline. Clinical Pharmacology and Therapeutics 14: 509-513, 1973

Mitenko, P.A., D.S. Sitar, and F.Y. Aoki. Heavy drug use among the elderly: prescription surveys in Manitoba. In Clinical Pharmacology in Psychiatry. Editors L.F. Gram, E. Usdin, S.G. Dahl, P. Kragh-Sorensen, F. Sjoqvist, and P.L. Morselli. Macmillan Press, Limited, London, 1983, section 1, pp. 32-41

Monks, T.J., J. Caldwell, and R.L. Smith. Influence of methylxanthine-containing foods on theophylline metabolism and kinetics. Clinical Pharmacology and Therapeutics 26: 513-524, 1979

Montamat, S.C. and A.O. Davies. Physiological response to isoproterenol and coupling of beta-adrenergic receptors in young and elderly human subjects. Journal of Gerontology 44: M100-M105, 1989

Montgomery, P.R. and D.S. Sitar. Hepatic uptake of indocyanine green and perfusion rate in rats: effect of age and albumin concentration. Canadian Journal of Physiology and Pharmacology 66: 592-595, 1987

Morgan, D.B., H.M.V. Newton, C.J. Schorah, M.A. Jewitt, M.R. Hancock, and R.P. Hullin. Abnormal indices of nutrition in the elderly: a study of different clinical groups. Age and Ageing 15: 65-76, 1986

Morgan, D.B. The impact of ageing--present and future. Annals of Clinical Biochemistry 20: 257-261, 1983

Morgan, D.J., C.T. Toh, G.L. Blackman and J.D. Paull. Plasma binding of thiopentone in late pregnancy. British Journal of Clinical Pharmacology 15: 121-123, 1983

Morse, E.E. Toxic effects of drugs on erythrocytes. Annals of Clinical and Laboratory Science 18: 13-18, 1988

Murphy, J.J. and M.P. Ryan. The effects of propranolol on 86-Rb effluxes in rat thymocytes and human peripheral blood lymphocytes. British Journal of Pharmacology 84: 26P, 1985

Murray, T.G., S.T. Chiang, H.H. Koepke and B.R. Walker. Renal disease, age, and oxazepam kinetics. Clinical Pharmacology and Therapeutics 30: 805-809, 1981

Nation, R.L., J. Vine, E.J. Triggs, and B. Learoyd. Plasma level of chlormethiazole and two metabolites after oral administration to young and aged human subjects. European Journal of Clinical Pharmacology 12: 137-145, 1977

Naylor, G.J., D.A.T. Dick, E.P. Worrall, E.G. Dick, P. Dick and L. Boardman. Changes in the erythrocyte sodium pump with age. Gerontology 23: 256-261, 1977

Naylor, G.J., E.G. Dick, A.H.W. Smith, D.A.T. Dick, A.M. McHarg and C.A. Chambers. Changes in erythrocyte membrane cation carrier with age in women. Gerontology 26: 327-329, 1980

Nicolson, G.L., I.J. Fidler, and G. Poste. Effect of tertiary amine local anesthetics on the blood-borne implantation and cell surface properties of metastatic mouse melanoma cells. Journal of the National Cancer Institute 76: 511, 1986

Nielsen-Kudsk, F., I. Magnussen, and P. Jakobsen. Pharmacokinetics of theophylline in ten elderly patients. Acta Pharmacologica et Toxicologica 42: 226-234, 1978

Norris, A.H. and N.W. Shock. Aging and variability. Annals of the New York Academy of Sciences 134: 591-601, 1966

Ogilvie, R.I. Clinical pharmacokinetics of theophylline. Clinical Pharmacokinetics 3: 267-293, 1978

O'Neill, S.J., D.S. Sitar, D.J. Klass, V.A. Taraska, W. Kepron, and P.A. Mitenko. The pulmonary disposition of theophylline and its influence on human alveolar macrophage bactericidal function. American Review of Respiratory Disease 134: 1225-1228, 1986

Owen, J.A., D.S. Sitar, L. Berger, L. Brownell, P.C. Duke, and P.A. Mitenko. Age-related morphine kinetics. Clinical Pharmacology and Therapeutics 34: 364-368, 1984

Owen, J.A., S.L. Nakatsu, J. Fenemore, M. Condra, D.H.C. Surridge, and A. Morales. The pharmacokinetics of yohimbine in man. European Journal of Clinical Pharmacology 32: 577-582, 1987

Peat, M.A., T.A. Jennison, and D.M. Chinn. Analysis of theophylline in serum and whole blood samples by high-pressure liquid chromatography. Journal of Analytical Toxicology 1: 204-207, 1977

Perl, W. Red cell permeability effect on the mean transit time of an indicator transported through an organ by red cells and plasma. Circulation Research 36: 352-357, 1975

Phillips, R.A., V.P. Dole, P.B. Hamilton, K. Emerson, R.M. Archibald, and D.D. van Slyke. Effects of acute hemorrhagic and traumatic shock on renal function of dogs. American Journal of Physiology 145: 314-336, 1945-6

Piafsky, K.M. and R.I. Ogilvie. Dosage of theophylline in bronchial asthma. New England Journal of Medicine 292: 1218-1222, 1975 in Reprints from The New England Journal of Medicine, Drug Therapy, vol 2, Massachusetts Medical Society, 1976

Piafsky, K.M., D.S. Sitar, R.E. Rangno, and R.I. Ogilvie. Theophylline disposition in patients with hepatic cirrhosis. New England Journal of Medicine 296: 1495-1497, 1977a

Piafsky, K.M., D.S. Sitar, R.E. Rangno, and R.I. Ogilvie. 1978). Theophylline kinetics in acute pulmonary edema. Clinical Pharmacology and Therapeutics 21: 310-316, 1977b

Piomelli, S., D.G. Nathan, J.F. Cummins, and F.H. Gardner. The relationship of total red cell volume to total body water in octogenarian males. Blood 19: 89-98, 1962

Powell, J.R., S. Vozeh, P. Hopewell, J. Costello, L.B. Sheiner, and S. Riegelman. Theophylline disposition in acutely ill hospitalized patients. The effect of smoking, heart failure, severe airway obstruction, and pneumonia. American Review of Respiratory Disease 118: 229-238, 1978.

Purcell, Y. and B. Brozovic. Red cell 2,3-diphosphoglycerate concentration in man decreases with age. Nature 251: 511-512, 1974

Pussard, E., F. Verdier, F. Faurisson, F. Clavier, F. Simon, and Claude Gaudebout. Disposition of amopyroquin in rats and rabbits and **in vitro** activity against **Plasmodium falciparum**. Antimicrobial Agents and Chemotherapy 32: 568-572, 1988

Rahman, A., H.L. Komiskey, W.L. Hayton, and W.P. Weisenburger. Aging: changes in distribution of diazepam and metabolites in the rat. Drug Metabolism and Disposition 14: 299-302, 1986

Reidenberg, M.M., M. Levy, H.Warner, C.B. Courinho, M.A. Schwartz, G. Yu, and J. Cheripko. Relationship between diazepam dose, plasma level, age, and central nervous system depression. Clinical Pharmacology and Therapeutics 23: 371-374, 1978

Reitz, R.C. Effect of chronic ethanol ingestion on membrane lipids. Proceedings of the Western Pharmacology Society 23: 437-440, 1980

Rinne, U.K. Parkinson's disease as a model for changes in dopamine receptor dynamics with aging. Gerontology 28 (Suppl. 1): 35-52, 1982

Rodgers, D.H. Developing an Analytical Method by High-Efficiency Liquid Chromatography. Perkin-Elmer Corporation, Norwalk, Connecticut, 1974

Rondel, R.K. General aspects of the registration of geriatric drugs. Gerontology 28: 49-58, 1982

Roth, S. and P. Seeman. The membrane concentrations of neutral and positive anesthetics (alcohols, chlorpromazine, morphine) fit the Meyer-Overton rule of anesthesia; negative narcotics do not. Biochimica et Biophysica Acta 255: 207-219, 1972

Roth, S., P. Seeman, S.B.A. Akerman and M. Chau-Wong. The action and adsorption of local anesthetic enantiomers on erythrocyte and synaptosome membranes. Biochimica et Biophysica Acta 255: 199-206, 1972

Routledge, P.A. The plasma protein binding of basic drugs. British Journal of Clinical Pharmacology 22: 499-506, 1986

Rowland, M., T.F. Blaschke, P.J. Meffin, and R.L. Williams. Pharmacokinetics in disease states modifying hepatic and metabolic function. In The Effect of Disease States on Drug Pharmacokinetics. Editor L.Z. Benet. American Pharmaceutical Association, 1976, ch. 4, pp. 53-75

Russell, J.W., V.J. Whiterock, D. Marrero, and L.J. Klunk. Disposition in animals of a new anti-HIV agent: 2',3'-didehydro-3'deoxythymidine. Drug Metabolism and Disposition 18: 153-157, 1990.

Saija, A., P. Princi, R. De Pasquale, and D. Costa. Arecoline, but not haloperidol, induces changes in the permeability of the blood-brain-barrier in the rat. Journal of Pharmacy and Pharmacology 42: 135-138, 1990

Sawchuk, R.J., J. Robayo and K.W. Miller. The distribution of propranolol between blood and plasma in hypertensive patients. British Journal of Clinical Pharmacology 1: 440-442, 1974

Schram, S.B. The LDC Basic Book on Liquid Chromatography. Milton Roy Company, St. Petersburg, Florida, 1981

Scott, P.H. Sustained-release theophylline for childhood asthma: evidence for circadian variation of theophylline pharmacokinetics. Journal of Pediatrics 99: 476-479, 1981

Sedek, G.S., I.R. Tsuen, M.C. Frederiksen, J.W. Frederiksen, S-R. Shih and A.J. Atkinson, Jr. Splanchnic tissues are a major part of the rapid distribution spaces of inulin, urea, and theophylline. Journal of Pharmacology and Experimental Therapeutics 251: 1026-1031, 1990

Seiter, D., D. Nagel, H. Frank, P. Hellstern, C. Leitzmann, and K. Jung. Effects of long-distance running on iron metabolism and hematological parameters. International Journal of Sports Medicine 10: 357-362, 1989

Seeman, P. The membrane actions of anesthetics and tranquilizers. Pharmacological Reviews 24: 583-655, 1972

Shand, D.G. Biological determinants of altered pharmacokinetics in the elderly. Gerontology 28 (Suppl. 1): 8-17, 1982

Shand, D.G. Discussion paper: results with propranolol. Annals of the New York Academy of Sciences 226: 267-268, 1973

Shaw, L.M., L. Fields, and R. Mayock. Factors influencing theophylline serum protein binding. Clinical Pharmacology and Therapeutics 32: 490-496, 1982

Sheehan, T.M., and P. Haythorn. Rapid gas chromatographic determination of underivatized theophylline in whole blood. Journal of Chromatography 117: 392-398, 1976

Shin, S-G., D. Juan, and M. Rammohan. Theophylline pharmacokinetics in normal elderly subjects. Clinical Pharmacology and Therapeutics 44: 522-530, 1988

Singh, A.K., U. Mishra, M. Ashraf, E.H. Abdennebi, S. Granley, D. Dombrovskis, D. Hewetson and C.M. Stowe. Analysis of detomidine in horse blood, plasma and urine samples utilizing a sensitive gas chromatography-mass spectrometry method. Journal of Chromatography 404: 223-232, 1987

Sitar, D.S., F.Y. Aoki, D.J. Hoban, K-G. Hidinger, P.R. Montgomery, and P.A. Mitenko. Enprofylline disposition in the presence and absence of amoxycillin or erythromycin. British Journal of Clinical Pharmacology 24: 57-61, 1987

Sitar, D.S., P.C. Duke, J.L. Benthuysen, T.J Sanford, and N.T. Smith. Aging and alfentanil disposition in healthy volunteers and surgical patients. Canadian Journal of Anaesthesiology 36: 149-154, 1989

Smith, R.A. and M.J. Wade. Ribavirin: a broad spectrum antiviral agent. In Studies With a Broad Spectrum Antiviral Agent. Editor Thomas Stapleton. International Congress and Symposium Series no. 108. Royal Society of Medicine Services, London, pp.3-23, 1986

Smith, S.E. and M.D. Rawlins. Variability of the Human Drug Response. Butterworths, London, 1973

Stewart, P.A., M.Magliocco, K. Hayakawa, C.L. Farrell, R.F. Del Maestro, J. Girvin, J.C.E. Kaufmann, H.V. Vinters, and J. Gilbert. A quantitative analysis of blood-brain barrier ultrastructure in the aging human. Microvascular Research 33: 270-282, 1987

Stewart, R.B., L.E. Cluff, and J.R. Philp. Drug Monitoring: A Requirement for Responsible Drug Use. The Williams and Wilkins Co., Baltimore, 1977

Storiko, K. Normal values for 23 different human plasma proteins determined by single radial immunodiffusion. Blut 16: 200-208, 1968

Swift, C.G. and E.J. Triggs. Clinical pharmacokinetics in the elderly. In Clinical Pharmacology in the Elderly. Editor C.G. Swift. Marcel Dekker, Inc., New York, 1987, ch. 2, pp. 31-82

Swift, C.G. Pharmacodynamics: changes in homeostatic mechanisms, receptor and target organ sensitivity in the elderly. British Medical Bulletin 46: 36-52, 1990

Takahashi, H. and H. Ogata. Plasma protein binding and blood cell distribution of propranolol enantiomers in rats. Biochemical Pharmacology 39: 1495-1498, 1990

Takikawa, H., A. Stolz, S. Kuroki, and N. Kaplowitz. Oxidation and reduction of bile acid precursors by rat hepatic 3α-hydroxysteroid dehydrogenase and inhibition by bile acids and indomethacin. Biochimica et Biophysica Acta 1043: 153-156, 1990

Taylor, F.A. and P. Turner. The distribution of propranolol, pindolol and atenolol between human erythrocytes and plasma. British Journal of Clinical Pharmacology 12: 543-548, 1981

Tsuji A., H. Sato, I. Tamai, H. Adachi, T. Nishihara, M. Ishiguro, N. Ohnuma, and T. Noguchi. Physiologically based pharmacokinetics of a new penem, SUN5555, for evaluation of **in vivo** efficacy. Drug Metabolism and Disposition 18: 245-252, 1990

Verbeeck, R.K., R.A. Branch, and G.R. Wilkinson. Meperidine disposition in man: Influence of urinary pH and route of administration. Clinical Pharmacology and Therapeutics 30: 619-628, 1981

Vestal, R.E., B.J. Cusack, G.D. Mercer, G.W. Dawson, and B.K. Park. Aging and drug interactions. I. Effect of cimetidine and smoking on the oxidation of theophylline and cortisol in healthy men. The Journal of Pharmacology and Experimental Therapeutics 241: 488-500, 1987

Vickers, S., C.A. Duncan, I.W. Chen, A. Rosegay, and D.E. Duggan. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metabolism and Disposition 18: 138-145, 1990.

Wagner, J.G. Half-life and volume of distribution. In Biopharmaceutics and Relevant Pharmacokinetics. Drug Intelligence Publications, Hamilton, Illinois, 1971, ch. 32, pp. 232-259

Wahle, K.W.J. Fatty acid modification and membrane lipids. Proceedings of the Nutritional Society 42: 273-287, 1983

Weinberger, M.W., R.A. Matthay, E.J. Ginchansky, C.A. Chidsey, and T.L. Petty. Intravenous aminophylline dosage. Use of serum theophylline measurement for guidance. Journal of the American Medical Association 235: 2110-2113, 1976

Welling, P.G. and W. A. Craig. Pharmacokinetics in disease states modifying renal function. In The Effect of Disease States on Drug Pharmacokinetics. Editor L.Z. Benet. American Pharmaceutical Association, 1976, ch. 10, pp. 155-187

White, H.L. Observations on the behavior of diodrast in the dog. American Journal of Physiology 130: 454-463, 1940

Whittam, R. Transport and Diffusion in Red Blood Cells. Edward Arnold Limited, London, 1964

Wilkinson, G.R. Clearance approaches in pharmacology. Pharmacological Reviews 39: 1-47, 1987

Wilkinson, G.R. The effects of ageing on the disposition of benzodiazepines in man. In Drugs and the Elderly. Editors J. Crooks and I.H. Stevenson. University Park Press, Baltimore, 1979, ch. 10, pp. 103-116

Wilkinson, G.R. and Shand. A physiological approach to hepatic drug clearance. Clinical Pharmacology and Therapeutics 18: 377-390, 1975

Williamson, J. Adverse reactions to prescribed drugs in the elderly. In Drugs and the Elderly. Editors J. Crooks and I.H. Stevenson. University Park Press, Baltimore, 1979, ch. 22, pp. 239-246

Yee, G.C., T.P. Lennon, D.J. Gmur, M.S. Kennedy, and H.J. Deeg. Age-dependent cyclosporine: pharmacokinetics in marrow transplant recipients. Clinical Pharmacology and Therapeutics 40: 438-443, 1986

Yost, R.L. and C.L. DeVane. Diurnal variation of alpha 1-acid glycoprotein concentration in normal volunteers. Journal of Pharmaceutical Sciences 74: 777-779, 1985

Yusuf, H.K.M. and J.W.T. Dickerson. Elevated level and underdeveloped fatty acid composition of brain cholesterol esters in malnourished children. Neurochemistry International 16: 157-162, 1990

Zingales, I.A. Diazepam metabolism during chronic medication. Unbound fraction in plasma, erythrocytes and urine. Journal of Chromatography 75: 55-78, 1973