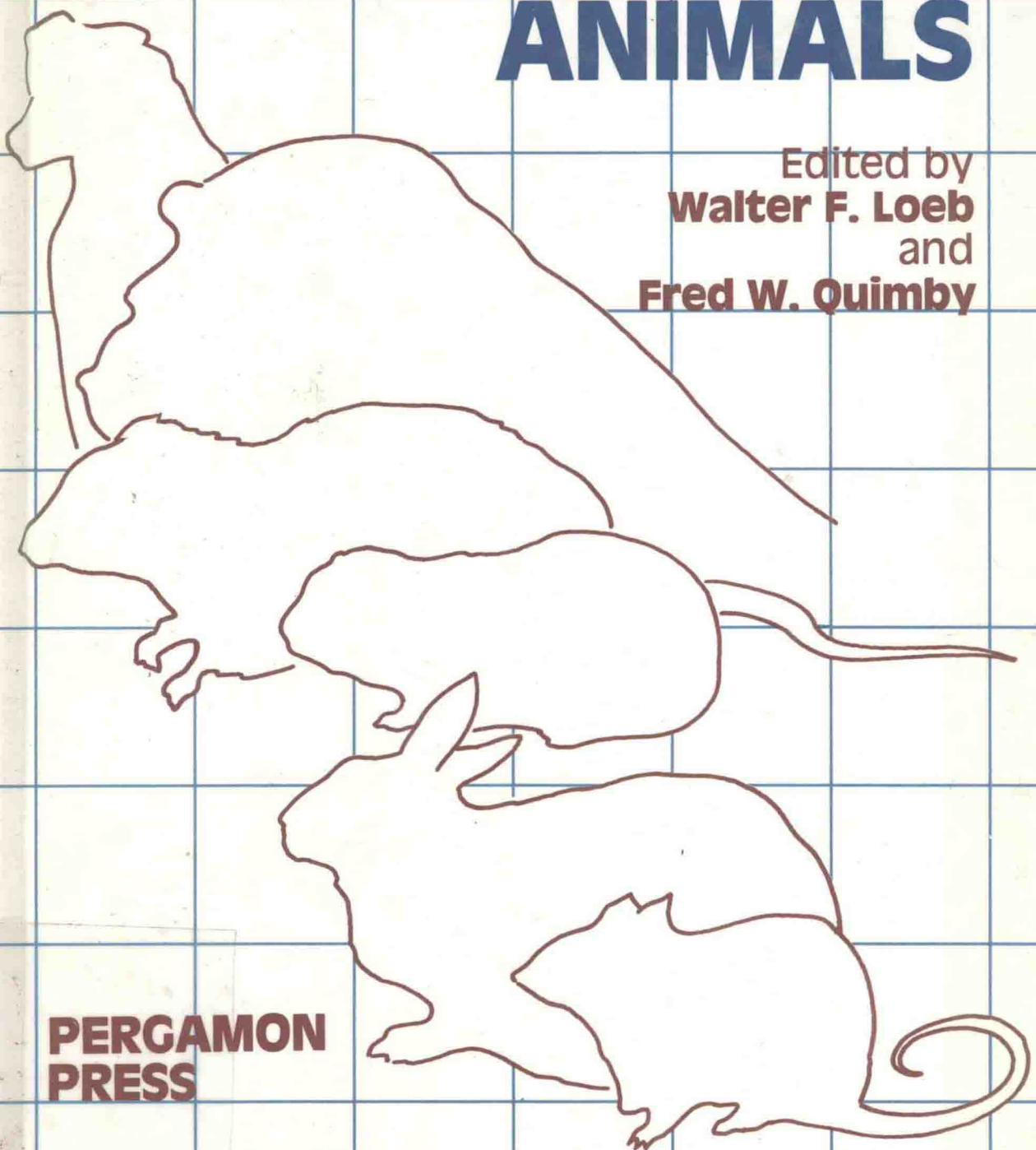
THE CLINICAL CHEMISTRY OF LABORATORY ANIMALS

Edited by
Walter F. Loeb
and
Fred W. Quimby



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Preface

Millions of individual clinical chemistry determinations are performed annually on specimens from rats, mice, and other laboratory animal species in the course of biomedical studies. The information obtained is used in a plethora of journal publications and reports to regulatory agencies, which, as a whole, tend to be inaccessible to and unread by much of the user community responsible for interpretation of the data so generated. No single text is available to guide the scientist in the selection and interpretation of clinical chemical analyses on laboratory animals. In compiling this book, our principal objective was to bring this information to the user. It is our hope and belief that this book will be a valuable resource to laboratory animal veterinarians, clinical pathologists and clinical chemists, experimental toxicologists, and the host of other biomedical researchers, technologists, and technicians responsible for the optimal interpretation of clinical laboratory data from animals in research.

Each contributing author of this book has knowledge of both laboratory animals and clinical chemical evaluation, a rarely found combination. Each is a well-informed authority in his or her discipline and was given free rein with respect to the content, organization, and style of his or her chapter. As a result there is considerable variation among the chapters, reflecting both the available body of information and the author's vantage point. This brings us to the second objective of this book: to familiarize the reader with areas that have been well studied and areas in which information is lacking, fruitful new areas for investigation.

Internationally, the biomedical professions have agreed to use a system of units termed *Système International* (SI) built upon molar concentrations and metric units. Although most of the world has adopted this system, the United States has been slow to do so. Consequently, most of the values in this book are in conventional units. An introduction to SI is given in Chapter 15.

This book represents the participation and unselfish contributions of innumerable persons. To each we express our gratitude.

To the reader go our thanks for your support and for sharing our interest in upgrading the study of laboratory animals by the tools of clinical chemistry. In the future, we or others will revise this book, or others will be able to write a better book because this beginning was made. We will be grateful for your feedback, for calling to our attention the errors and omissions of this book which surely exist, and for sharing with us the information that will improve subsequent editions.

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Contents

PART A

Clinical Chemical Studies Applicable to Laboratory Animal Species and Their Interpretation

1

The Mouse

FRED W. QUIMBY, VMD, PhD

In the United States, the mouse is the most widely used mammalian species in biomedical research, with 8,500,000 mice being used in 1983 (127). It is estimated that the use of mice exceeds twofold that of rats (next most popular mammal). Mice represented 61 per cent of all vertebrate mammals used in 1983. In addition, although the number of other commonly used research animals is declining, the number of mice used during the 1980s is increasing (127). Since 1901 when the first Nobel prize for physiology and medicine was awarded, 11 Nobel laureates have received this award based on work specifically involving mice (119). Many characteristics of mice, such as small body size, ease of care, availability in a disease-free state, inexpensiveness, and genetic purity, make this species well suited for biomedical research; however, perhaps the greatest advantage of this species over other animals used in research is its well-described genetic composition.

Often, in biomedical research, there is a need to understand if the observations recorded are due to genetically or nongenetically determined factors. This type of discrimination is possible using the mouse, because of development of inbred strains, congenic lines, and recombinant inbred strains. Inbreeding has been used successfully to produce strains of mice with genetic homogeneity. The most common method employs 20 successive generations of brother by sister matings, which leads to progeny with a theoretical inbreeding coefficient of 0.986 (4); this coefficient measures the probability that both alleles at a locus are identical by descent. Inbreeding gains tend to plateau before the inbreeding coefficient reaches 1 because of the countering effects of new mutations. However, the theoretical probability of reaching total homozygosity and genetic fixation takes place between inbred generations 35 to 40. Two practical implications arise from these observations: (1) following 20 inbred generations, each animal within the strain is essentially uniform in genotype, and (2) a strain maintained in isolation by inbreeding after 20 generations should be designated as an individual subline because continued mutations may allow this stock to become genetically divergent.

Once inbred strains are established, additional lines and strains can be generated through genetic manipulation. A congenic line may be developed when an inbred strain is bred to an individual bearing a distinctive gene, and the progeny bearing this gene are selectively backcrossed to the inbred strain. If backcrossing continues, eventually all genes except the distinctive gene are homozygous and identical to the original inbred strain. These congenic lines can then be used to evaluate the effect of a single locus (or closely linked loci) on a similar background. Inbred strains and congenic lines have

allowed investigators to map the various murine chromosomes, recognize mutations, determine if isoenzymes are the result of multiple alleles or multiple loci, and explore such complex phenomena as immunoglobulin gene rearrangement, genetic control of immune responsiveness, and changes in the phenotypic expression of a single gene product associated with its interaction with other genes such as the expression of thymic alymphoplasia associated with the nude (nu) mutation in various inbred strains.

A third type of inbred animal, the recombinant inbred strain (RI), results from crossing two inbred strains and systematically inbreeding the first generation progeny by brother by sister matings for 20 generations. Each substrain, repeatedly inbred from a single brother by sister cross, will have a reassortment of loci with a fixed allele at each locus which is derived from one of the two original parental strains. The pattern of inheritance for the various genetic loci will be unique for each RI strain if no selection takes place during inbreeding. These RI strains have been particularly useful for assigning new traits to linkage groups (4).

Mutations at a single locus may have profound biochemical and physiologic effects on the host. Proper identification and characterization of mutations in the mouse have led to the development of a large number of murine models for some of man's most serious afflictions. In fact, the number of models for human disease available for study in the mouse is unparalleled by any other species. The Jackson Laboratory maintains 260 mutant genes in 400 different strains and stocks (68).

Because of the complexity of murine inbred strains, lines, and substrains, as well as the recognition of multiple alleles at single loci and mutant alleles, a standardized nomenclature has been developed. By following the rules of nomenclature, researchers can easily identify individually maintained sublines, indicate special procedures used in the maintenance of a strain, such as foster nursing, ova transfer, and ovary transplant, and give precise information relating to specific genes, such as allele, recessive or dominant mutation, and wild type (4). Lists of inbred strains (155), congenic lines, and segregation inbred strains and recombinant inbred strains (54,63) are also available.

Given the tremendous genetic complexity of the mouse, it is not surprising that mice that are not genetically pure occasionally make their way into the research laboratory (49). The reasons include residual heterozygosity, recent mutations (not fixed by inbreeding), and pedigree errors or contamination. To ensure the genetic purity of breeding colony mice, a system of genetic monitoring should be employed. Such programs have employed mandible shape analysis (172), biochemical and serologic markers (54,116), and DNA restriction enzyme analysis (85).

The amount of clinical chemistry information available on the mouse is tremendous, relates in large part to investigations of murine models of human disease, and is impossible to review completely in this chapter. However, in the discussion of specific tests, clinical chemistry data will be presented in several popular murine model systems.

SAMPLING TECHNIQUE

One problem that has prevented the mouse from being used more widely in the evaluation of serum chemistries is its small body size and the difficulty in obtaining an adequate blood sample. However, the recent development of micromethodology for many chemistry assays allows the mouse to be used for sequential small blood sampling. Three techniques used for blood collection in the mouse are retroorbital plexus sampling (69), tail vein sampling (31), and heart puncture (56,152). In each procedure, 0.5 to 0.7 ml of blood may be obtained from adult mice as a survival procedure that can be repeated. The orbital plexus may be used successfully to collect small volumes (30 to 80 μl) from mice 14 to 16 days old. Larger volumes may be obtained from the tail vein if a vacuum apparatus is used (124). General anesthesia is recommended for orbital plexus and heart puncture techniques (31). Lewis et al. (96) found that heparinization of the mouse before tail bleeding increases the yield. Prewarming the mouse under a lamp or by immersion in warm water facilitates the bleeding procedure.

Disadvantages associated with tail bleeding techniques include stimulation of the sympathetic nervous system, significant differences between samples and between tail vein blood and orbital plexus blood, and mixing of venous and arterial blood (147).

Loeb (99) found that when mice were bled via

the orbital plexus, there was an elevation of the enzymes alanine aminotransferase, aspartate aminotransferase, and lactic dehydrogenase on subsequent bleeding from the contralateral orbital sinus. The effect was observed on rebleeding within 1 minute or as long as 48 hours after the initial collection.

Patrick et al. (133) compared the values of several routine clinical chemistry measurements conducted on N:NIH(S) mice submitted to intracardiac or jugular vein collection. They found that the sample collection technique greatly affected two chemical parameters and that intracardiac collection was associated with a higher glucose and a lower creatine phosphokinase (CPK) level.

The anesthetic or physical restraint method used during blood collection may also lead to increased variation among certain analytes; this problem has been reviewed by Pfeiffer and Muller (136).

Four additional bleeding procedures that yield greater volumes of blood have been described, but each requires anesthesia and all are terminal. These are jugular vein (5), abdominal aorta (100), brachial artery (170), and exposed heart bleeding techniques (30,114). The technique of Young and Chambers (170) is reputed to be associated with minimal hemolysis.

The author has found that the use of heparized microhematocrit tubes, when combined with either tail vein or orbital plexus procedures, has the added advantage of immediately separating plasma from cellular components after centrifugation and breaking the capillary tube above the buffy coat line (140). This procedure maximizes the retrievable plasma volume and minimizes hemolysis.

A variety of inhalation and injectable anesthetics are available and safe for use in mice (31). Several anesthetics, such as ether, chloral hydrate, and tribromoethanol, have been associated with increased mortality in mice and rats caused by respiratory irritation, which may lead to airway obstruction (for ether), and the development of adynamic ileus after intraperitoneal injection of chloral hydrate and tribromoethanol (53,159). However, tribromoethanol is the anesthetic of choice at Jackson Laboratory (31). Halothane, methoxyflurane, and pentobarbital sodium have all been widely used and are safe; however, very little is known concerning their effects on clinical chemistry values. Carbon dioxide narcosis provides sedation and analgesia for 1 to 2 minutes and is a good anesthetic for orbital bleeding (31). Fowler et al. (55) found no alterations in packed cell volume, urea, prothrombin, or alanine aminotransferase when rats anesthetized with CO_2 were compared with rats anesthetized with ether; however, CO_2 did alter plasma glucose levels. Naturally CO_2 anesthesia would be unsatisfactory when blood gases are being measured. Ether anesthesia in mice is not associated with hematologic changes (31).

Restraint devices allowing for collection of blood from the tail of a conscious mouse have been described (156), as have devices designed for collection of urine from mice for periods in excess of 24 hours (153,165).

Hemolysis is a continuing problem associated with serum collection in mice. Hemolyzed samples are associated with elevations in various enzymes. Everett and Harrison (42) have addressed this problem, providing advice on collection sites and animal numbers, and recommend heparinized plasma for routine chemical determinations.

REFERENCE RANGES

Variables

The usefulness of compiled control data will depend on controlling a large number of variables known to influence chemistry determinations. Included among those variables known to affect the host adversely are environmental factors, pathogens, and shipment. In addition, nutrition, time of sample collection, and storage techniques may all contribute to variability.

Everett and Harrison (42) illustrate the effect of strain, sex, and age differences on selected chemistry determinations. Finch and Foster (50), evaluating the effect of age on 16 hematologic and electrolyte values, found no alterations in electrolytes providing the aged population were free of gross pathologic lesions. However, significant age-associated changes in serum calcium determinations have been reported in certain strains of mice (9). Profound differences in chemistry determinations may be seen between strains of mice (173). Strain-associated changes appearing in healthy animals

have been documented for complement components (61), cholesterol (37,111), testosterone (79), cortisol-binding protein (62), and serum protein (14). The sex of the mouse is known to exert an effect on many parameters (9), and the increased variability seen in certain analyte levels of female mice is thought to be the effect of estrus (42).

The degree of hydration, exposure to noise, degree of confinement, and environmental temperatures have all been shown to affect the metabolism and toxicity of drugs in mice (23); therefore, it is not surprising that similar environmental factors influence serum chemistry determinations (12,15,80,98,149).

Diet is known to influence the blood levels of many plasma (serum) analytes. Perhaps the best studied of these is the effect of atherogenic diets on serum cholesterol (117). Similarly, significant differences in both serum cholesterol and blood urea nitrogen are seen in mice maintained on a semipurified (AIN-76) diet (64). The mouse is unique among mammals because it carries neither carnosine nor anserine (18). Because carnosine probably serves as a resource for histidine when its intake is low, one may speculate that the mouse is more sensitive than other animals to dietary histamine (130).

The presence or absence (axenic) of intestinal microbial flora is associated with dramatic changes in immunoglobulin levels (163) and may be associated with changes in other analytes as well (161). Certainly, the presence of pathogens is associated with dramatic changes in various analytes (76,137), even when infection by those agents is subclinical (143).

These effects are particularly well known for lactic dehydrogenase-elevating virus in which infection is associated with major elevations in lactic dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, aspartate aminotransferase, and glutathione reductase (142, 143,145).

Alterations in clinicopathologic parameters can be attributed to stress in mice. Landi et al. (91,92) found that plasma corticosterone concentrations in mice tested on arrival or 24 or 48 hours after arrival (by plane or truck transport) were significantly higher than those of control mice. Mice sensitized on arrival with sheep red cells as an antigen had significantly lower antibody titers, fewer plaque-forming cells, and a decreased delayed type of hypersensitivity reaction when compared with normal mice allowed to acclimate to the facility for 48 hours.

The effect of serum storage at various temperatures and for varying periods has been reported in several domestic (84) and laboratory (114) species. Falk et al. (44) evaluated the effect of storage time (after freezing) on 20 serum analytes in 6 laboratory species. They found that in the mouse, only creatinine phosphokinase activity changed significantly with storage up to 28 days.

Quality Assurance

Everett and Harrison (42) stressed the importance of a clinical pathology quality assurance program that would include regular assays of pooled and commercially prepared preassayed sera. In addition, they encourage participation of the laboratory in a subscription quality assurance program. The within-day and day-to-day coefficient of variation should be known for each analyte being measured.

Because of the tremendous number of variables known to influence clinical chemistry values in mice, it is often prudent to test adequate numbers of control specimens along with the experimental samples. This technique is often impractical when tests are being conducted strictly for diagnostic purposes, and in those situations, compiled values controlled for as many variables as possible may be sufficient. Compilation of murine clinical chemistry data was accomplished by the Laboratory Animal Data Bank (National Technical Information Service).

Statistics

The normal values that define a reference range for a particular analyte in mice may be described using several methods. For values that fall into a Gaussian distribution, parametric methods, such as mean and standard deviation, are appropriate. Regardless of the distribution of data, it is generally useful to describe the limits that include 95 per cent of the test results in a disease-free population. For Gaussian distributed data, this is the range that includes 2 standard deviations above and 2 below the mean (167).

Certain murine analytes have non-Gaussian distributions and must be evaluated using nonparametric methods. A variety of methods are

available and include the percentile method (42,105) and logarithm-transformed data analyzed using parametric methods (16).

The method of percentile estimates is more vulnerable to bias because of extreme values (outliers) than is the log-transformed parametric method. Boyd (16) asserts that a sample size of at least 120 is required to give 90 per cent confidence intervals using the percentile method, whereas a sample size of 50 may give reliable ranges if parametric analyses are used. Neither statistical method just described will replace raw data in certain situations.

SPECIFIC TESTS

Glucose

The concentration of blood glucose has been studied intensely in mice because of the availability of several murine models of diabetes mellitus (27,59,77,83). Glucose determinations generally are conducted on fresh serum; however, if some delay before analysis is anticipated, fluoride should be used as an anticoagulant and preservative because it inhibits glycolysis (114). Blood glucose in mice has been measured using a modification of Faulkner's method (59), the Somogyi-Nelson method (21), and the o-toluidine method (42). Each appears to give reliable results.

Glucose tolerance tests (GTT) have also been conducted in mice. A 1-hour GTT was performed by comparing preinjection serum to serum collected 1 hour following the administration of 2 mg/g (2 g/kg) of glucose intraperitoneally (128). A 4-hour GTT has also been described in which mice are given 10 ml/kg of 10 per cent glucose orally (59). This assay was very sensitive in measuring the differences in glucose tolerance following implantation of normal islet cells in New Zealand obese mice.

The hypoglycemic response to insulin also has been described in mice (21).

Serum Protein

Total serum proteins have been evaluated in mice using the Lowry (14,50) and Biuret (42,114) techniques. The Lowry method can accurately measure protein in a sample size of only 2 μl (50). Hyperproteinemia may be seen in mice during severe dehydration (42) and hypoproteinemia is associated with increased renal loss of protein as seen in glomerulonephritis (73).

Electrophoresis has been used to evaluate the major classes of serum (or plasma) protein. Cellulose acetate electrophoresis in barbital buffer is the most popular method used and gives excellent separation of α_1-, α_2-, β- and γ-globulins (50,114,150). This method has been used to demonstrate the murine myeloma monoclonal spike (95), alterations in serum proteins in mice afflicted with immune complex glomerulonephritis (157), and protein alterations in mice of the mottled locus with an intestinal copper transport defect (150).

The major serum protein, albumin, has been measured accurately in mice using the radial immunodiffusion technique with goat anti-mouse albumin antibody (14,73). This technique also has sufficient sensitivity to measure urinary albumin in mice with glomerulonephritis (73).

Zizkovsky (173) identified three specific murine fetal serum proteins using immunoelectrophoresis. These fetal proteins correspond to α_1-fetoprotein, lipoprotein esterase, and a counterpart to rat α_2-slow globulin. Alpha-1-fetoprotein was also found in the serum of mice receiving a transplantable hepatoma (1).

Three murine pregnancy-associated serum proteins, PAMP-1, PAMP-2, and PAMP-4, which correspond to human pregnancy zone protein, human pregnancy-specific β_1-glycoprotein, and α-fetoprotein, respectively, may be demonstrated using a sensitive line immunoelectrophoresis technique (66). The dependency of these serum proteins on pregnancy was demonstrated by Lin et al. (97).

Lipids

Total serum cholesterol in mice has been measured using the enzymatic oxidation method of Roschlay (111), the Lipid Research Clinics Program protocol (117), and the Abell technique (114). These methods have the necessary sensitivity to measure differences in serum cholesterol associated with H-2 haplotype (111) and diet (117,132). Dunnington et al. (37) also used serum cholesterol determinations as a factor for genetic selection to produce hypercholesterolemic, high activity mice. Mark et al. (103) measured changes in blood cholesterol in autoimmune disease-prone mice on various diets.

Serum triglycerides have been measured using

the Lipid Research Clinics Program protocols, and normal as well as abnormal triglyceride levels have been reported (19,103,117).

The distribution and characterization of murine serum lipoproteins and apoproteins have been reported (20). Using density gradient ultracentrifugation, nine subfractions of murine lipoproteins were identified and later characterized by electrophoretic, immunologic, chemical, and morphologic analyses. Similar methods were used to evaluate changes in serum lipoproteins during diet-induced atherosclerosis in mice (117).

Electrolytes

Sodium and potassium levels are easily measured in murine serum using flame photometry (lithium reference) (42,50). Serum sodium levels are slightly higher in mice than in most other mammalian species with reported values of 174 ± 23 (SD) mEq/l (50) and 147 ± 15 (SD) mEq/l (42). No differences in serum sodium were seen during aging (50). Serum chloride has been measured using mercuric thiocyanate (42) and the chloridometric (114) techniques, and inorganic phosphorus in mice has been measured using the phosphomolybdate technique (42, 114).

Calcium

Total serum calcium in mouse serum has been measured using the sodium alizarin sulfonate technique (42) or atomic absorption spectrometry (9,114). Two reports, using different techniques, list similar reference ranges of 9 ± 1 (SD) mg/dl (9,42), whereas a third report lists reference ranges of 5.6 ± 0.4 (SD) mg/dl for male and 7.4 ± 0.50 (SD) mg/dl for female albino mice (114). The latter values reported for male mice were significantly lower than those for six inbred strains of mice in two different age groups using the same technique (9). Likewise, no differences associated with sex were reported by Everett and Harrison (42) who also examined random bred albino mice. However, Bonella et al. (13) demonstrated a significantly higher level in female Swiss albino mice.

Total calcium levels reflect both ionized (active) calcium and protein-bound calcium; therefore, mice with decreased serum albumin levels are expected also to have decreased total calcium levels. Alcock and Shils (3) found that in mice fed magnesium-deficient diets hypocalcemia developed as it did in mice receiving intramuscular injections of heparin (13). Bonella et al. (13) demonstrated significantly elevated calcium levels when blood was collected by orbital puncture versus cardiac puncture.

Enzymes

Alkaline Phosphatase (AP)

This production enzyme (in which serum concentrations change as a result of synthesis) is found in the highest concentrations in the kidney and intestine in mice (25) with virtually no detectable activity in the liver when measured using the Hausamen technique (67). Kinnett and Wilcox (88) assayed murine AP by measuring the rate of hydrolysis of p-nitrophenyl phosphate in the presence of isoenzyme (52). They found evidence of two isoenzymes of AP in mouse liver, both cytosolic in location and each requiring different pH and manganese requirements for optimal measurement. Fassati et al. (45) measured AP fluorometrically using 2-naphthyl phosphate as a substrate and quantitated two liver isoenzymes of AP that changed in activity in the liver during the course of mouse hepatitis virus infection with the appearance of the anodic isoenzyme in the plasma after 48 hours. Intestinal AP activity was shown to vary fourfold between two strains of Swiss mice (123). This difference in activity was under polygenic control and was influenced by a strain-specific factor in milk (122). Whether these differences reflect changes in catalytic activity or tissue concentration is unknown. In most species, changes in the serum activity of AP do not occur in hepatocellular disease unless accompanied by cholestasis (42). Clampitt and Hart (25) stress that the method used to measure AP in mice may be important.

Alanine Aminotransferase (ALT, GPT)

Alanine aminotransferase is a leakage enzyme (reflects alterations in cell membrane function) found in highest concentration in the liver of mice; however, tissue activity can also be demonstrated in intestine, kidney, heart, muscle, brain (25), skin, pancreas, spleen, and erythrocytes (24). Activity in spleen and erythrocytes

was not found by others (38). Ruscak et al. (146) demonstrated that most species have two proteins with ALT activity, one located in the cytosol and the other in mitochondria. Despite its widespread tissue distribution, ALT is a useful analyte to measure as an index of hepatocellular damage (42). An 11,000 per cent increase in serum ALT activity has been reported following infection with mouse hepatitis virus (33).

Aspartate Aminotransferase (AST, GOT)

Aspartate aminotransferase is a leakage enzyme associated with both cytosol and mitochondria in the mouse (36). Dooley (36) found that 59 per cent of the total activity of liver AST was mitochondria associated in the mouse. The tissue distribution of AST includes liver, blood vessels, brain, intestine, kidney, lung, testes, and cardiac and skeletal muscles (129); however, the activity in lung, kidney, intestine, and skeletal muscle was very low (25) as measured by the technique of Bergmeyer and Bernt (11). With histochemical techniques, AST was shown to be nonuniformly distributed in the liver, with periportal hepatocytes containing greater enzymatic activity (129). The highest specific activity of AST was found in mouse cardiac muscle, and skeletal muscle had the lowest activity of those tissues studied (70). Because of its widespread distribution, injury to several organs may be associated with serum elevations of AST in the mouse (42); however, the differential location of 2 ısozymes in liver cytosol and mitochondria has been used to assess the degree of hepatic injury in mice infected with mouse hepatitis virus (45). Reference values for murine AST have been reported (19,42).

Lactic Dehydrogenase (LDH)

Lactic dehydrogenase is a leakage enzyme that in mouse and man is characterized by five isozymes identified as LDH-1 through LDH-5 (46). In tissues LDH is under control of 2 structural genes that determine the A and B polypeptides. The rate of synthesis of each in various tissues determines the specific isozyme distribution (82). The enzyme itself is composed of multiple subunits constructed of either A or B polypeptides. During early fetal development, all murine tissues contain LDH-5 (more A subunits) activity. As the embryo matures, tissues develop shifts in A or B subunit production so that during adulthood, each tissue contains a characteristic LDH profile (46). In adult mice, the heart contains LDH-1 and LDH-2, and most other tissues have intermediate LDH-3. Skeletal muscle and liver fail to exhibit this developmental shift and are composed predominantly of LDH-5 (104). Destruction of a particular tissue is characterized by the presence of the tissue-specific activity in the blood. Erythrocyte LDH-B subunits are under partial control of the Ldr regulatory gene located on chromosome 6. The Ldr-B^{-} phenotype (negative isozyme phenotype) is present in the red cells of most mouse strains except SWR which contain LDH-4 (A^3B^1) in their red cells (46). The mouse has highest activity of LDH in skeletal muscle, with decreasing activity in the heart, liver, kidney and intestine, respectively (25). The sera and liver of normal mice contain each of the five isozymes with LDH-5 in highest concentration (42). LDH-5 rises in the blood within 72 hours after inoculation of mice with mouse hepatitis virus (45). Mice infected with the LDH virus (LDV) exhibit increased serum concentrations of LDH, isocitric dehydrogenase, malic dehydrogenase, phosphohexase isomerase, and AST (126). Notkins (126) showed that increased enzymatic activity was due to a decreased rate of endogenous clearance by infected mice. Decreased plasma protein turnover has also been associated with LDV in mice (142).

Ornithine Carbamoyltransferase (OCT)

Ornithine carbamoyltransferase is a mitochondrial enzyme found primarily in the liver of mice (115). Increases in the serum of mice reflect severe injury to hepatocytes, resulting in disruption of mitochondrial membranes (42). There is no simple method to assay for OCT; therefore, it is not frequently measured. An abnormal OCT has been described in mice having the sparse-fur mutation (32).

Creatine Phosphokinase (CPK)

Creatine phosphokinase participates in the production of creatine phosphate from adenosine triphosphate (ATP) and creatine. The reaction is reversible, and it is thought that high amounts of CPK in muscle and brain tissues may allow

maintenance of a constant ATP level and ensure immediate rephosphorylation of adenosine diphosphate (ADP) (39). The CPKs are dimeric enzymes with M and B subunits, and three dimeric combinations are found in mouse serum (42). In the mouse, the greatest activity of CPK is found in skeletal muscle with much less activity found in the heart and brain (25). The skeletal muscle contains the MM isozyme, cardiac muscle contains MM, MB, and BB isozymes, and brain contains the BB enzyme (2). No CPK activity is found in kidney, spleen, and liver; therefore, it is a useful marker enzyme for muscle injury.

The MM isozyme of CPK found in skeletal muscle cytosol is also localized in the thick filaments and on sarcoplasmic reticulum. Mitochondrial CPK is bound to the exterior aspect of the inner mitochondrial membrane (107). There is some evidence now that normal myocardium in man actually contains very little MB isozyme, but that during ischemia (myocardial infarction) there is expression of the fetal form of CPK (B-subunit), resulting in a greater tissue and serum concentration of the MB isozyme (78). Similar findings for other mammals have not been documented.

Aldolase

Aldolase is a cytosolic enzyme that can alter its distribution between soluble and particulate forms according to the metabolic status of the tissue (107). In adult mice, nine aldolase isozymes are known to occur in tissues with significant activities in muscle, brain, liver, kidney, and spleen (2). Everett and Harrison (42) report no apparent advantages in the measurement of aldolase over other enzymes known to have specific liver or muscle activity.

Sorbitol Dehydrogenase (SDH)

Sorbitol dehydrogenase is located primarily in the cyotplasm and mitochondria of liver, kidney, and seminal vesicles (25,34). The activity of SDH is usually low in the serum and rises during hepatic injury (34). A mechanized method for centifugal analyzer determination of SDH in the serum of mice has been developed which is based on conversion of D-fructose to sorbitol with simultaneous oxidation of NADH (34). Reference values in normal mice are 26.8 ± 2.1 (SD) U/L.

Amylase

Amylase is a gene product in mouse pancreas and salivary gland. Production is controlled by two distinct but closely linked loci in mice, designated Amy-1 (salivary) and Amy-2 (pancreatic) (112). The product of Amy-1 gene appears to be a single enzyme; however, mouse pancreatic amylase is complex with electrophoretic diversity. Recent evidence suggests that the Amy-2 gene region comprises multiple gene copies with some divergence of regulation (112). Based on electrophoretic patterns, pancreatic amylase isozymes of inbred strains may be assigned to four classes: A_1, A_2, B_1, and B_2.

Mackenzie and Messer (101) found that mouse serum predominantly contained amylase of salivary gland origin, and urine contained only pancreatic amylase. When pancreatic amylase was injected into the blood of mice, it rapidly cleared through the urine.

Ross et al. (144) reported two to threefold increases in serum amylase activity in mice infected with Coxsackie virus of salivary and pancreatic trophism. Alterations in the activity of specific pancreatic isozymes have been shown in streptozotocin-induced diabetes in mice (112).

Other Enzymes

Pancreatic lipase containing two isozymes has been measured in mice (17). The enzyme 5′-nucleotidase was measured in the serum of normal mice using a simple one-step kinetic method (35). A reference range of 10.9 ± 4.5 (SD) U/L has been recorded in 100 mice, and it is thought but not proven to be a good indicator of hepatic injury.

Glutamate dehydrogenase (GDH) has been measured in the tissues (25) and serum (19) of mice. The activity of GDH is fivefold greater in the liver than in the kidney and brain, and the authors speculated that its measurement would be a sensitive indicator of hepatic cell injury (25). Cranmer and Peoples (29) found that the substrate 3-dimethyl butyl acetate was not acceptable in the measurement of cholinesterase in mouse plasma in which high levels of nonspecific esterase complicate interpretation. Much is known concerning the function and