Chapter 6
BIOLOGIC VARIABILITY

SOURCES OF BIOLOGIC VARIABILITY

The two components of biologic variability are interindividual variability, which is the variability due to the heterogeneity of physiologic influences among individuals, and intraindividual variability, which is due to the variability in individuals over time. As discussed in Chapter 1, both components of biologic variability contribute to the distribution of study values that constitute reference frequency distributions.

Sex, race, age, and biorhythms are the sources of biologic variability that will be considered here. Sometimes one of these sources of variability can be identified as an important determinant of the reference frequency distribution. In such circumstances it is usually desirable to account for that source of variability when constructing the distribution. This can be done in two ways. If the source of variability has a discrete pattern of heterogeneity, the clinical population can be partitioned into subgroups based upon that pattern. For instance, sex shows discrete heterogeneity; if an individual's sex strongly influences the value of an analyte, two reference frequency distributions should be established, one for males and one for females. If the source of variability has a continuous pattern of heterogeneity, such as age, the reference frequency distribution can be constructed as a continuous function of that pattern.

Discrete heterogeneity

A number of different approaches can be taken to determine if a source of discrete heterogeneity should be taken into account when constructing reference frequency distributions. One approach is to decide that partitioning of the population into subgroups is justified if the difference in the location or width of the subgroup reference frequency distributions is statistically significant. Because a difference of any size, even a minute difference, can be made statistically significant as long as adequate numbers of individuals are studied, this approach is not satisfactory. A second approach is to utilize subgroup frequency distributions if the width of any of the separate frequency distributions is appreciably smaller than the width of the frequency distribution derived from the whole population. This criterion is appealing because it is based directly on the degree of variability attributable to an identifiable source of that variability. There is a practical problem with the criterion, however, in that the width of the population frequency distribution may not be much larger than the widths of the subgroup frequency distributions, even when the subgroup distributions are widely separated. This is illustrated for a two subgroup population in Figure 6.1.

Another approach to subgroup partitioning of reference frequency distributions is to base the decision to partition upon the magnitude of the effect that such partitioning would have upon the diagnostic performance of the study. If partitioning significantly improves the performance of a study, subgroup frequency distributions should be used. Consider, for example, the two subgroup population of Figure 6.1. At a study value that yields a specificity of 0.95 in the combined population, the likelihood ratios differ by 6.3-fold between the two subgroups. This is a large disparity, one that would clearly be clinically significant arising as it does in the range of study values that is frequently pertinent to diagnostic decision making. Thus, it would be preferable to utilize subgroup frequency distributions in this population.

Harris and Boyd (1990, 1991) consider this last approach to be the most appropriate clinically and suggest two decision rules based upon it: for a population with two subgroups, the frequency distribution for a study should be partitioned if either,

$$\frac{mean_1 - mean_2}{\sqrt{\frac{var_1}{n_1} + \frac{var_2}{n_2}}} > 3\sqrt{\frac{n_{avg}}{120}}$$

or

$$0.67 > \frac{SD_2}{SD_1} > 1.5$$

where n_1 and n_2, are the number of values in the frequency distribution arising from individuals in the first and second subgroups, respectively, n_{avg} is the

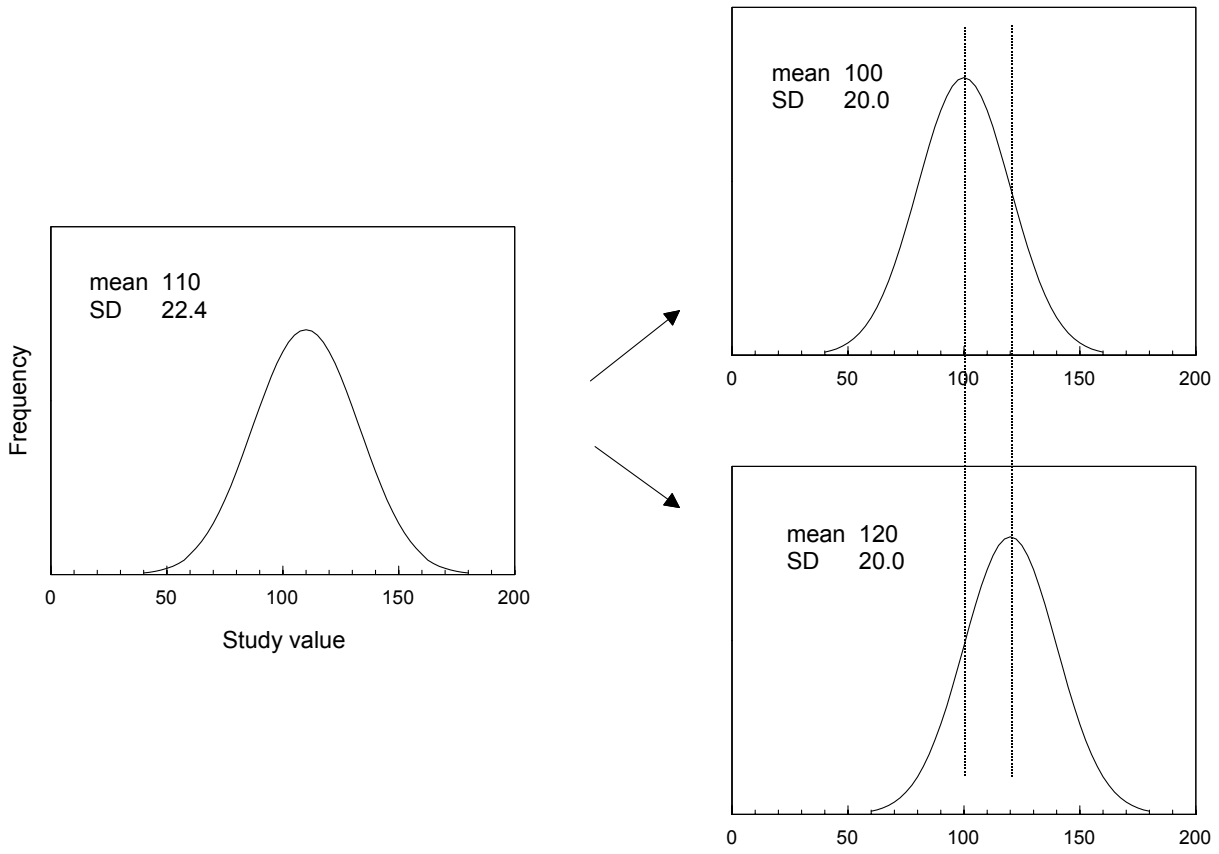

Figure 6.1 The hypothetical frequency distribution of a two subgroup population (left) and the frequency distributions of its constituent subgroups (right). The subgroups are equal in size and are normally distributed with the indicated means and standard distributions. The mean and standard deviation of the combined population, which is not normally distributed, is also indicated. The standard deviation of the combined population is only 12 percent larger than the standard deviations of the constituent subgroups.

average of n_1 and n_2, and SD_1 and SD_2 are the standard deviations of the frequency distributions for the first and second subgroups, respectively. These rules apply only to subgroup distributions that are normally distributed and those that after transformation are normally distributed.

A problem with these rules is that they do not arise from an explicit underlying diagnostic performance criterion. One criterion that could be suggested is the magnitude of the difference in likelihood ratios between the subgroup frequency distributions at some specified study value. A study value that yields a specificity of 0.95 in one of the subgroup frequency distributions seems a good choice because, as mentioned previously, such a value would likely be within the range of values most relevant to the making of diagnostic decisions based on the study results. In order to test the frequency distributions at both tails, the criterion must also include evaluation of the likelihood ratio difference at a study value that yields a specificity of 0.05. A graphical test for this two-tail criterion is illustrated in Figure 6.2 for a threshold value of a 3-fold difference in likelihood ratios. As with Harris and Boyd's rules, the graphical test is valid only when the subgroup frequency distributions are normally distributed or can both be transformed to yield normal distributions. Unlike Harris and Boyd's rules, however, the criterion can also be applied in a nonparametric fashion to subgroup frequency distributions having any shape. Simply determine the study values that yield a specificity of 0.95 and 0.05 in one of the subgroup frequency distributions and calculate the ratios of the frequencies associated with the respective study values in the two subgroups, using the larger frequencies as the numerators. Those ratios equal the multiplicative differences in the likelihood ratios at the two tails of the frequency distributions. If both of the ratios are less than 3, the frequency distribution for the combined population should be used; if the either ratio is greater than 3, subgroup frequency distributions should be used.

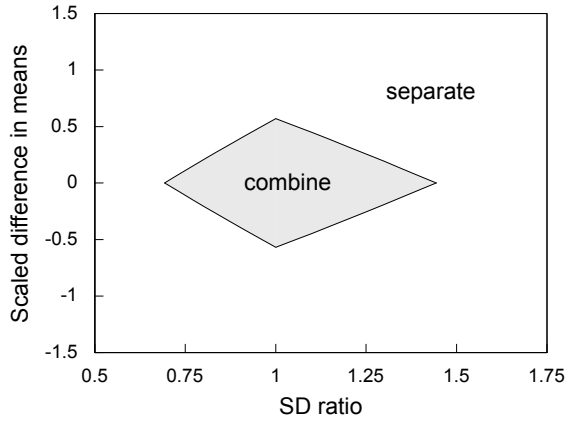

Figure 6.2 Graphical test of the heterogeneity of reference frequency distributions when both distributions are normal or can both be transformed to yield normal distributions. SD ratio is the standard deviation of subgroup 2 divided by the standard deviation of subgroup 1. The difference in the subgroup means is scaled by division by the standard deviation of subgroup 1.

Continuous heterogeneity

Two questions must be addressed when dealing with continuous heterogeneity. First, it must be decided if there is an interval over which the source of heterogeneity influences the frequency distribution of study values to a clinically significant degree. The most practical way to answer this question is to generate a scatterplot of study results from a large number of individuals who represent as broad as possible a range of values for the source of heterogeneity under consideration. The scatterplot is inspected visually to determine if there are intervals over which the distribution of data points appears to vary significantly. While visual examination is often adequate to detect a pattern in the width of a data distribution, it can be difficult to identify trends in the mean, especially when the data show appreciable variability. In such cases, the scatterplot should be sliced or nonparametric scatterplot smoothing should be performed to reveal how the central tendency of the data varies with respect to the source of heterogeneity (Jacoby 1997). Slicing a scatterplot means separating the data into several bins defined by equal intervals on the x axis, i.e. the axis representing the source of heterogeneity, and computing the frequency distributions of the data within each bin. The mean values of each distribution are then plotted against the mid-interval values of the bins. Nonparametric scatterplot smoothing techniques are elaborations of the slicing method in which numerous variably sized, partially overlapping bins are used

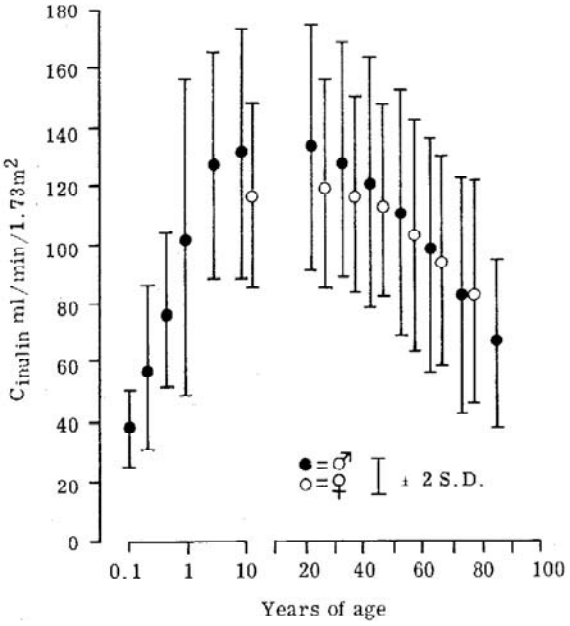

Figure 6.3 Inulin clearance rate as a function of age. Mean rates and standard deviations binned irregularly until 10 years and by decade thereafter. Reprinted from Avendano LH and Novoa JML. 1987. Glomerular filtration and renal blood flow in the aged. In Nunez JFM and Cameron JS (eds). *Renal Function and Disease in the Elderly.* Butterworths, London.

to generate a large number of local mean value estimates which are then connected by short line segments to yield a smooth curve.

The second question that arises when dealing with continuous heterogeneity is how best to present the reference frequency distribution. Two different approaches are in common use. The first and by far the most common approach is to present the frequency distribution as a sliced scatterplot. The median and central range of the data for each bin is plotted either as a boxplot or, if the data is normally distributed, as the mean with "error" bars. This method is illustrated in Figure 6.3 which shows the relationship between inulin clearance rate (a measure of glomerular filtration rate) and age. In this graph, the error bars represent plus and minus 2 standard deviations and hence directly demonstrate the central 95 percent range of the data within each bin. The second approach is to present the frequency distribution as smooth curves indicating the mean values of the distribution and the upper and lower limits of the central range of the distribution. This is done by modeling the relationship between the frequency distribution and the source of heterogeneity. The curve defining the mean values of the frequency distribution can be modeled nonparametrically using

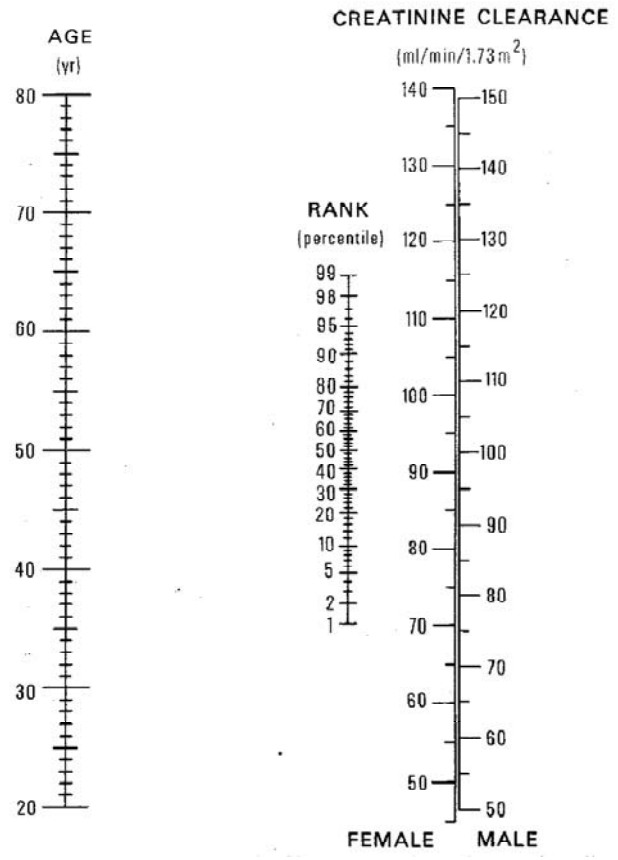

Figure 6.4 Nomogram for the determination of age-based percentile rank of creatinine clearance rate. Reprinted from Rowe JW, Andres R, Tobin JD, Nomis AH, and Shock NW. 1976. Age-adjusted standards for creatinine clearance. Ann Intern Med 84:567.

scatterplot smoothing or it can be modeled parametrically by fitting the trend of the data to a line or higher order polynomial (Royston 1991) using regression analysis. The curves defining the limits of the frequency distribution are constructed by modeling the scatter of the data around the curve that defines the mean values (Royston 1991, Wright and Royston 1997). A useful way to present a modeled frequency distribution is as a nomogram. It is much easier to determine a numerical value from a nomogram than it is from a graph, even if the graph is well-drawn and the curves have simple shapes. An example of a frequency distribution presented as a nomogram is found in a report by Rowe *et al.* (1976b) describing the relationship between age and creatinine clearance rate in adults. The nomogram, shown in Figure 6.4, is based on the following linear model of mean clearance rate and constant model of clearance rate standard deviation,

mean creatinine clearance rate (ml/min/1.73 m^2) =

133 - 0.64 age (in years)

Table 6.1
Laboratory analytes with sex-based reference frequency distributions

M > F
Alanine aminotransferase
Alkaline phosphatase
Aspartate aminotransferase
Creatine kinase
Creatinine
Ferritin
Hematocrit
Hemoglobin
Uric acid
F > M
Aldosterone
(Erythrocyte) sedimentation rate

and

standard deviation = 15 ml/min/1.73 m^2

for males. Creatinine clearance rates in females equal 0.93 times the value in age-matched males.

SEX

Sex-based heterogeneity in laboratory study results arises in three ways. First, the concentrations of sex organ-specific marker substances differ between the sexes. Most notably, prostatic acid phosphatase and prostate-specific antigen, which are moderately specific for the prostate, are present in only low concentrations in the plasma of females. Second, beginning at puberty, the plasma concentrations of the sex hormones and sex hormone-binding plasma proteins differ between the sexes. Third, also starting at puberty, secondary sexual differences in body composition and differences in various sex hormone-influenced aspects of metabolism cause the frequency distributions of many laboratory study results to differ between the sexes.

In addition to listing separate sex hormone reference frequency ranges for adult males and females, the sex-specific reference frequency distributions for the analytes listed in Table 6.1 are used at either the Massachusetts General Hospital (Jordan *et al.* 1992) or the Johns Hopkins Hospital (Noe and Rock 1994).

Pregnancy

Pregnancy has profound effects upon a woman's physiology and metabolism resulting in numerous alterations in laboratory study results (Young 1979).

For example, creatinine is among the many substances that show a change in plasma concentration during pregnancy. Beginning at about 10 weeks of gestation and continuing until term, renal blood flow increases with an accompanying increase in glomerular filtration rate. Consequently the clearance rate of creatinine increases and the plasma concentration of creatinine declines.

The plasma concentrations and urinary excretion rates of the sex hormones and their metabolites change dramatically as a result of pregnancy (Kandeel and Swerdloff 1994). The plasma concentration of progesterone increases 10-fold between early and late pregnancy due to the placental production of progesterone following the corpus luteum-placental shift in progesterone production at 7 to 9 weeks of gestation. Many other hormones produced by the placenta enter the maternal circulation; human chorionic gonadotropin is one that is especially important in the laboratory evaluation of pregnancy. It is detectable in maternal plasma within a week of conception, increases in concentration rapidly, reaching a peak at 9 to 13 weeks of gestation, then declines quickly to a plateau that is maintained throughout the remainder of the pregnancy. The prompt appearance of this placental hormone in the maternal plasma and urine make it an ideal early marker of pregnancy.

RACE

A race is a local geographic population distinguished as a more or less distinct group by genetically transmitted physical characteristics. The distinguishing characteristics most often thought of when referring to the human races are skin color and facial features but other characteristics—in particular, ones that affect laboratory study values—may also differ among the races.

There are generally thought to be between six and ten major geographic races, the exact number depending upon the criteria used by the taxonomist to make the classification. For instance, American Indians arose from migrations of Asiatics into the Americas so the two populations can be considered one. However, thousands of years have passed since the migrations and the geographically isolated American Indians have developed genetic differences from their forebears; thus, the populations can be considered distinct. Table 6.2 lists nine major races and their historic geographic locales. In addition to the major races there are numerous local races and microraces. These terms refer to distinctive but small populations of peoples such as the Capoid local race of Southern Africa which is comprised of two microraces, the San and the Khoikhoin.

Table 6.2 Major geographic races of man

African	Sub-Saharan Africa
American Indian	North and South America except western Alaska and the Aleutian Islands
Asiatic	Central, East, and Southeast Asia, western Alaska and the Aleutian Islands
Australian	Australia and Tasmania
European	Europe, North Africa, and the Middle East
Indian	Indian subcontinent
Melanesian	New Guinea
Micronesian	Yap, Pohnpei, and Guam
Polynesian	Islands from Easter Island to the Hawaiian Islands to New Zealand

Although originally settled by American Indians, most of the residents of North America are now of European and African extraction with appreciable but smaller numbers of persons of Asiatic and Hispanic (a European-American Indian admixture) extraction. Because the majority of the residents are of European racial stock, most reference frequency distributions have been derived from clinical populations consisting predominantly or entirely of individuals of the European race. Only recently have efforts begun to determine the appropriateness of these distributions when applied to individuals of other races. These efforts have not yet resulted in the appearance of any widely employed race-based reference ranges. The only analyte with race-based reference frequency distributions at the Johns Hopkins Hospital is red cell glucose-6-phosphate dehydrogenase. None of the analytes measured in the clinical laboratories at the Massachusetts General Hospital have race-based reference frequency distributions. It is almost certain, however, that some analytes will eventually be recognized to require the use of race-based reference frequency distributions

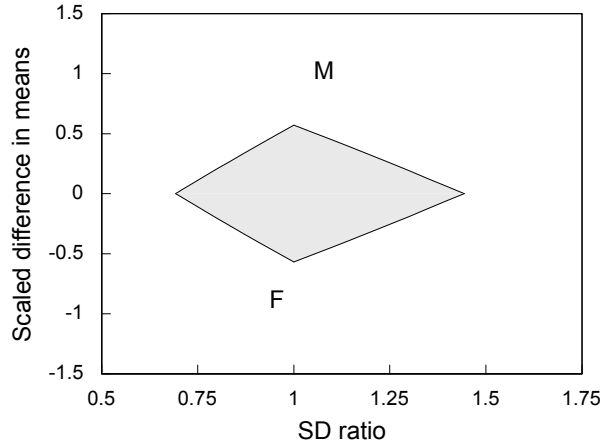

Figure 6.5 Graphical test of the heterogeneity of reference frequency distributions for creatine kinase based on race. M, comparison of "black" and "white" males; F, comparison of "white" and "black" females.

to allow for optimal medical decision making. One of the first analytes for which this recognition may come is creatine kinase. The plasma concentrations of creatine kinase tend to be much higher in "black" men and women than in "white" men and women, respectively. Using the data reported by Harris *et al.* (1991), race-based reference frequency distributions of creatine kinase are significantly different for both men and women (Figure 6.5). The large differences between the reference frequency distributions in "whites" and "blacks" has clear implications for the use of creatine kinase measurements in the diagnosis of myocardial infarction. For instance, using the critical value determined in "white" men, creatine kinase has a low specificity when used in the evaluation of chest pain in "black" men. The use of a critical value derived from a reference frequency distribution as found in "black" men would improve the diagnostic performance of creatine kinase in "black" men considerably.

Practical problems in constructing race-based reference frequency distributions

A considerable difficulty in establishing race-based reference frequency distributions in North America is the highly variable degree of racial admixing that is present in our society. For instance, many individuals descended from African slaves brought to the American Colonies are a mix of African and European races. A reference frequency distribution derived from such an admixed group would be expected to be intermediate between a reference frequency distribution derived from Europeans and a reference frequency distribution derived from a group of recent West African immigrants.

Another challenge in the establishment of reference frequency distributions based on race is determining the extent to which ethnic practices and socioeconomic conditions rather than race itself affect the analyte under study. As an example, it has been suggested that the higher plasma concentrations of creatine kinase in "black" men compared to "white" men may arise not from inherent racial differences but rather from their generally lower socioeconomic status (Young 1979). The logic behind this suggestion is that the members of lower socioeconomic groups are more likely to be employed in manual labor and to have greater muscular development resulting in increased plasma creatine kinase concentrations. This possibility, and other questions relating to ethnic practices and socioeconomic conditions, can been investigated scientifically by comparing racial reference groups that are matched for the supposed confounding variables. In the case of creatine kinase, it would be useful to compare office workers of African and European extraction and to compare manual laborers of the two races. Alternatively, the effects of muscle development on plasma creatine kinase concentrations could be investigated directly by quantifying the degree of muscle development of each individual in a reference population and determining the relationship, if any, between the measure of muscle development and the concentration of creatine kinase.

AGE

Five distinct periods of life are usually recognized when considering the effects of age upon laboratory study results. These are (1) the fetal period, (2) the newborn period, (3) childhood and adolescence, (4) adulthood, and (5) old age.

Laboratory studies conducted early in the fetal period are almost all performed with the intent of detecting genetic disease or developmental disorders. Direct genetic studies are not sensitive to fetal age but indirect genetic studies and developmental studies necessarily utilize marker substances that will depend upon fetal age. Prenatal screening for both trisomy 21 and for neural tube defects, for instance, use the concentration of alpha-fetoprotein in maternal plasma as one of a set of marker substances.

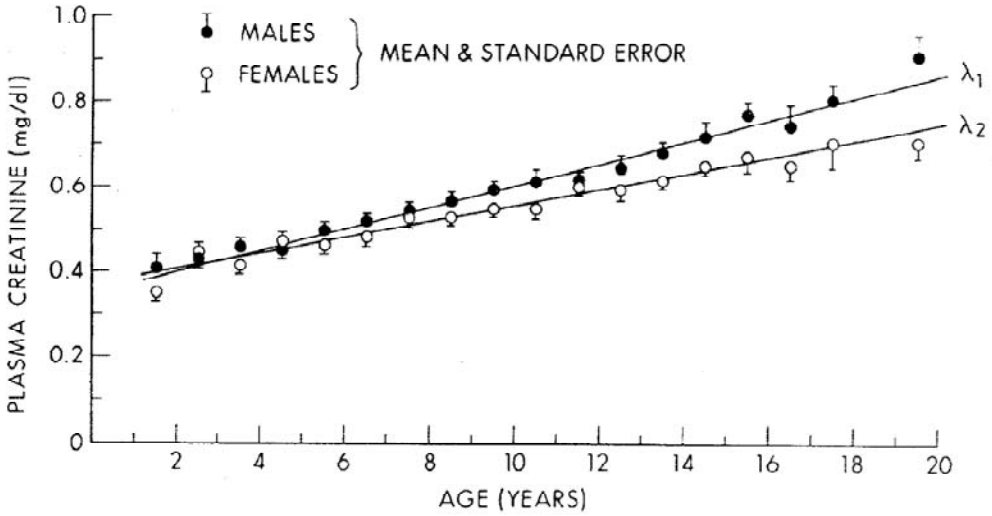

Figure 6.6 Plasma creatinine concentration in children as a function of age. Mean concentrations for each sex binned by year and fit by a linear model (solid lines). The error bars represent standard errors of the mean not standard deviations and hence cannot be used to calculate the width of the distributions. Reprinted from Schwartz GJ, Haycock GB, and Spitzer A. 1976. Plasma creatinine and urea concentration in children: normal values for age and sex. J Pediatr 88:828.

This concentration changes over the course of a pregnancy requiring that study results be interpreted within the context of the age of the fetus at the time the study is performed. One way this is accomplished is by arranging for the screening procedure to be done at the same gestational interval in every pregnant mother, such as at 16 to 18 weeks of gestation for the investigation of neural tube defects (Wald and Cuckle 1982). Then all the results can be analyzed using the same gestational age-specific reference frequency distributions.

The majority of the laboratory studies performed late in the fetal period are concerned with the determination of fetal maturity. Regardless of the fetus' gestational age, the state of maturity of the individual fetus, especially the maturity of the pulmonary epithelium, is paramount. The laboratory studies that are used in this setting are ones for which there is a known relationship between the study result and the probability of post natal difficulties arising from prematurity. The innate or therapeutically accelerated maturation of the fetus is usually followed for some period of time by serial monitoring before study results indicate that it is safe to deliver the baby.

The newborn period is marked by tumultuous physiologic changes. These changes are accompanied by rapid alterations in many laboratory study results. Childhood and adolescence is a period of growth, development, and maturation. The frequency distributions of most laboratory studies undergo changes during at least some portion of this period. The changes may be fairly abrupt, such as the increases in plasma sex hormone concentration that occur with puberty, or they may be gradual, lasting most or all of the period, as is shown in Figure 6.6 for plasma creatinine concentration (Schwartz *et al.* 1976).

Because of the profound effects of age throughout the pediatric periods, pediatric age-specific reference frequency distributions have been established for most laboratory analytes. These distributions can be found in textbooks devoted to pediatric clinical practice and pediatric laboratory medicine.

Although adulthood and old age are generally considered to be separate periods of life with distinct physiologic differences, aging actually occurs throughout adulthood (Bafitis and Sargent 1977); the changes found in old age are, in general, cumulative not distinctive. Figure 6.7 shows the aging behavior of the lungs and kidneys. The lungs complete their growth and development in late adolescence after which they immediately begin to undergo a progressive decline in functional capacity throughout adulthood. The kidneys mature a few years sooner than the lungs but, like the lungs, they begin aging in early adulthood.

The effects of age-related changes in organ function may be evident throughout adulthood or they may only become manifest late in life, often after a critical functional reserve has been lost. Creatinine clearance rate falls throughout life, for

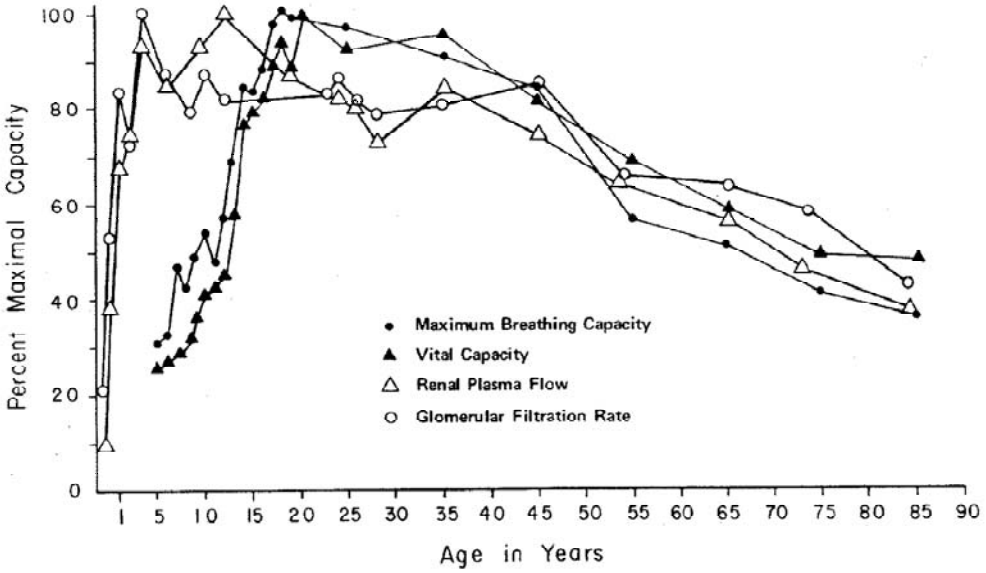

Figure 6.7 Selected measures of functional capacity of the lungs and the kidneys as a function of age. Mean maximum breathing capacity, vital capacity, renal plasma flow, and glomerular filtration rate binned according to age groups in the original reports. Reprinted from Bafitis H and Sargent F. 1977. Human physiological adaptability through the life sequence. J Gerontol 32:402.

instance, in parallel with the age-related decline in glomerular filtration rate (Rowe *et al*. 1976a, Avendano *et al*. 1987). The decline in creatinine clearance rate is not reflected in the plasma creatinine concentration, however. The concentration of creatinine remains nearly constant even into extreme old age because there is an age-related reduction in muscle mass that results in a diminution in the creatinine production rate that just matches the age-associated decline in the glomerular filtration rate (Figure 6.8).

A striking example of age-related changes appearing only later in life is the decrease in the plasma concentrations of the female sex hormones in women following menopause. The changes in the reference frequency distributions for estradiol and progesterone that occur as a result of menopause are accounted for at the Massachusetts General Hospital by listing a separate reference range for post menopausal females (Jordan *et al*. 1992). Neither the Massachusetts General Hospital nor the Johns Hopkins Hospital present adult age-based reference frequency distributions for any other analytes. This does not mean that the effects of age are not clinically significant for any of the other laboratory studies available at these hospitals. Rather, it suggests that most laboratorians and clinicians at these two institutions have not yet fully integrated an explicit recognition of the effects of aging into their routine care of adults.

BIORHYTHMS

We often think of the body as maintaining a steady state in which, for instance, the concentrations of substances in the plasma remain constant save for the effects of perturbations to the system, such as the ingestion of a meal, which temporarily upsets the balance and hence the plasma concentrations of certain substances, such as glucose. In fact, the body is in many ways not steady but rhythmic. The most obvious of these rhythms is the daily rhythm of wakefulness and sleep. This rhythm induces a circadian (*circa*, around; *dies*, day) rhythm in the secretion of a number of hormones including growth hormone and adrenocorticotropic hormone. The rhythmic secretion of adrenocorticotropic hormone in turn imposes a circadian rhythm on the secretion of and plasma concentration of cortisol. Four phases can be identified: the minimal secretory phase which occurs during the last few hours while awake and for the first few hours of sleep and during which the plasma cortisol concentration is low; the preliminary nocturnal secretory phase which occurs during the middle hours of sleep and during which the plasma cortisol concentration has a number of

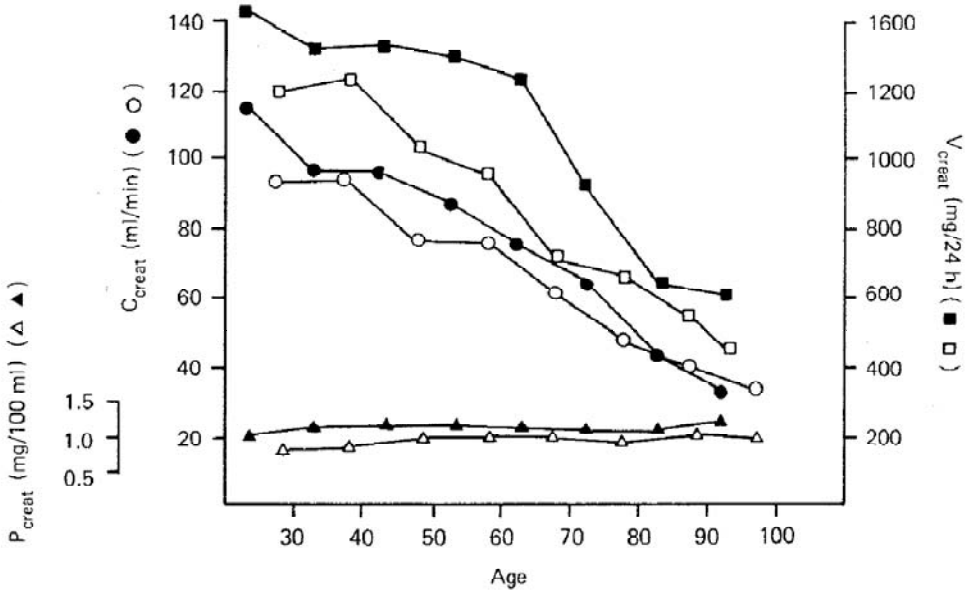

Figure 6.8 Creatinine physiology as a function of age. Mean creatinine clearance rate (C_{creat}), creatinine excretion rate (V_{creat}), and plasma creatinine concentration (P_{creat}) binned by decade. Data from males is indicated by filled symbols and that from females by empty symbols. Figure reprinted from Avendano LH and Novoa JML. 1987. Glomerular filtration and renal blood flow in the aged. In Nunez JFM and Cameron JS (eds). *Renal Function and Disease in the Elderly.* Butterworths, London. p 42.

small and medium peaks; the main secretory period which occurs during the last few hours of sleep and for the first hour after waking and during which the plasma cortisol concentration is high; and the intermittent waking secretory period which occupies most of the day and during which the plasma cortisol concentration shows occasional, irregularly spaced medium and large peaks.

At the Massachusetts General Hospital, the circadian rhythm of cortisol is handled by partitioning the reference frequency distribution for the plasma concentration of cortisol according to the time of day: for 8 am to 12 noon, the reference range is 138 to 690 nmol/L, for 12 noon to 8 pm, the reference range is 138 to 410 nmol/L, and for 8 pm to 8 am, the reference range is 0 to 276 nmol/L (Jordan *et al.* 1992). At the Johns Hopkins Hospital, the circadian rhythm is addressed by providing a single time-specific, 8 am, reference range, 220 to 500 nmol/L (Noe and Rock 1994).

The plasma concentrations of many other substances follow circadian rhythms but the contributions of the rhythmic changes to the total variability in the concentrations tend to be small. Also, the timing and magnitude of the rhythms tend to vary among individuals, as has, for example, been reported for the circadian rhythm in the plasma concentration of iron (Stengle and Schade 1957).

In addition to circadian rhythms, the body has a number of rhythms that cycle with periods greater than a day. The most important of these is, of course, the menstrual cycle. In addition to affecting the sex hormones, the menstrual cycle also affects the plasma concentrations of other steroid hormones because of alterations in the concentrations of the steroid-binding proteins in the plasma and altered competition for their binding sites. Numerous other plasma substances show long-term cyclic patterns in response to the many physiologic changes attributable to the menstrual cycle (Young 1979). In most clinical laboratories, the reference frequency distributions for the plasma sex hormone concentrations in females are partitioned according to the phase of the menstrual cycle. Taking 17-hydroxyprogesterone as an example, at the Massachusetts General Hospital, its plasma concentration reference range is listed as 0.61 to 2.42 nmol/L for the follicular phase and 2.72 to 9.20 nmol/L for the luteal phase (Jordan *et al.* 1992). At the Johns Hopkins Hospital, the plasma concentration reference ranges are 0.6 to 3.0 nmol/L for the follicular phase and 4.5 to 11.8 nmol/L for the luteal phase (Noe and Rock 1994).

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