Influence of Demographic Factors on Serum Concentrations of Seven Chemical Constituents in Healthy Human Subjects

David M. Goldberg, Alan J. Handyside, and David A. Winfield

Serum from 519 subjects attending a screening clinic and from the laboratory staff was used to study the influence of age, sex, body weight, social class, blood pressure, and smoking habit upon the concentration in serum of urea, uric acid, cholesterol, inorganic phosphate, total protein, calcium, and magnesium. Values for urea, uric acid, calcium, and magnesium were significantly higher in males. Age was a positive determinant of urea and cholesterol in both sexes, and of magnesium in females only. The correlation of uric acid with age was positive in females and negative in males, but in both sexes the correlation between uric acid and weight was strongly positive. Regression equations were developed to express the demographic and nondemographic contributions to the individual’s blood chemistry values. From these, a set of demographically-corrected reference values were derived. When applied to a selected group of female hospital outpatients, these seemed to discriminate more accurately between subjects with and without disease than did conventional parametric limits.

Additional Keyphrases: influence of age, sex, weight, social class, blood pressure, and smoking on serum urea, uric acid, cholesterol, P₄, total protein, calcium, magnesium

Many attempts have been made to study the demographic factors influencing the concentration of certain chemical constituents in the blood plasma or serum of healthy human subjects. These studies have usually been restricted to a single constituent but, even so, much useful information has accrued. It has been shown, for example, that uric acid (1-7) and cholesterol (8-11) vary with the age, weight, race, smoking habits, and social class of the individual. Such extensive information is available for few other constituents, and only within recent years have investigators attempted to study the concentrations of a wide range of chemical constituents in a single group of healthy subjects and to determine their relationship to age and sex (12-15). Such investigations have produced conflicting conclusions in many instances; the demographic variables studied have been few; the mathematical treatment of these relationships has been unsophisticated; and their influence has not been expressed in a practically useful format.

With the wider availability of automated techniques and the growing interest in well-population screening, it is becoming easier and at the same time more necessary to identify these demographic factors influencing the biochemical variables measured in screening programs and, if possible, to quantify these influences with the object of interpreting more precisely the significance of borderline values. We describe here an attempt to do so, using computer-assisted techniques to analyze the data so obtained, and to develop refined criteria as alternatives to the “normal range” concept that could prove more accurate and useful in health screening.

Methods

Satisfactory venous blood samples were obtained from subjects attending a well-population screening clinic held on two successive days between 2:00 p.m. and 6:00 p.m. (79% of the total case material), and healthy members of the hospital staff examined between 11:00 a.m. and 12:00 noon (21% of the total case material). The former were asked not to engage in heavy exercise and to have only a light snack before attending; the latter took only a light breakfast on the day blood was drawn. Venipuncture was carried out with the subject seated, conditions being typical of those pertaining at hospital outpatient clinics. All subjects were Caucasians of British birth. The screening clinic, based on the West Derbyshire village of Baslow, drew upon a predominantly rural population of whom 40% had been resident in the area for over 30 years and approximately 20% for less than 10 years; most of the latter were commuters of Social Class 1 or 2. The hospital staff were residents of, and in virtually all cases born in, the city of Sheffield.
A full account of the procedures employed at the screening clinic has been presented (16, 17). They included routine urine testing, hematological examination, chest roentgenogram, electrocardiogram, pulmonary function tests, psychologic assessment (18), cervical cytology, and full clinical examination including thyroid assessment and breast palpation. Blood pressure, age, height, weight, were recorded in all subjects, and their smoking habit and social status were assessed. The percentage of ideal weight was derived from tables of the Metropolitan Life Insurance Company (19).

Serum was separated by centrifugation not more than two hours after the blood was drawn and seven determinations were carried out as described in Table 1. All hemolized and lipemic samples were rejected. Information on each subject was transferred to punch cards. A preliminary inspection excluded all subjects showing evidence of clinical disease, or whose body weight was more than 25% in excess of the ideal, or for whom the results of any of the screening tests were abnormal. No assumptions were made with respect to the biochemical estimations, all of which were accepted if there were no other grounds for exclusion.

By machine-sorting, the subjects were divided by sex, and subdivided into classes based on intervals of age and weight, histograms then being constructed to display the data. Analysis continued, using a multiple product moment correlation program written in FORTRAN IV and run on the ICL 1907 digital computer. This provided the mean and standard deviation of each variable, and the value of the correlation coefficient associating each biochemical and demographic parameter with every other parameter in an X by X matrix. Males and females were analyzed independently. The significance of the difference between the sexes in the means for each variable and the significance of the correlation coefficients were calculated manually, standard tables being used in the assessment (20).

The data were next analyzed by using a multiple linear regression program written in “Mercury Auto-code,” in which the value of each biochemical constituent was expressed in terms of a constant term (C), and the following four independent variables: age, diastolic blood pressure, body weight, and social class. Output also included the variance of each coefficient for the independent variables and their covariance with other coefficients in the same equation. Further tests, conducted manually because of limitations of the program, utilized items of output to calculate the multiple regression coefficient (R) for the equation and its significance, the 95% confidence limits for the equation (D), and the significance of the coefficients associated with each independent variable in the equation. A more detailed account of the methods used has been presented elsewhere (17).

Results (Figures 1 and 2)

A total of 519 subjects satisfied all criteria. Their age distribution is shown in Table 2 and their demographic composition in Table 3.

Males were significantly heavier than females (P < 0.001) and smoked more (P < 0.001). Their weight tended to decrease with age (r = -0.166; P < 0.05) but was uninfluenced by smoking. Their blood pressure was not related to age or weight but tended to decrease as smoking increased (r = -0.168; P < 0.05).

In females, weight tended to increase with age (r = 0.134; P < 0.05) and with social class (r = 0.186; P < 0.05). Blood pressure was strongly influenced by age (r = 0.396; P < 0.001) and by weight (r = 0.250; P < 0.001), less so by social class (r = 0.154; P <

Table 1. Methods and Instrumentation Used in Biochemical Investigations

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Chemistry</th>
<th>Instrumentation</th>
<th>Reference to method</th>
<th>Within-batch SD at upper normal limit, mg/liter</th>
<th>Rate per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>diacetyl monoxime</td>
<td>AutoAnalyzer Mark I</td>
<td>Technicon Handbook N-10a</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>Uric acid</td>
<td>phosphotungstate + cyanide</td>
<td>AutoAnalyzer Mark I</td>
<td>Technicon Handbook N-13b</td>
<td>1.3</td>
<td>40</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>ferric chloride</td>
<td>AutoAnalyzer Mark I</td>
<td>Technicon Handbook N-24a</td>
<td>87</td>
<td>40</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>ammonium molybdate + ANSA</td>
<td>AutoAnalyzer Mark I</td>
<td>Technicon Handbook N-26a</td>
<td>1.7</td>
<td>40</td>
</tr>
<tr>
<td>Total protein</td>
<td>bluret</td>
<td>Technicon Mark II</td>
<td>Sampler plus Beckman DB</td>
<td>21</td>
<td>1.5g</td>
</tr>
</tbody>
</table>

Calcium atomic absorption spectroscopy EEL–Atomic Absorption Spectrophotometer plus Technicon Mark II Sampler plus Beckman Recorder EEL Atomic Absorption Handbook 2.1 120

Magnesium atomic absorption spectroscopy EEL Atomic Absorption Handbook 0.7 120

* g/liter.
Table 2. Age Distribution of Population Studied

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>20-29</td>
<td>44</td>
<td>37</td>
<td>81</td>
</tr>
<tr>
<td>30-39</td>
<td>42</td>
<td>43</td>
<td>85</td>
</tr>
<tr>
<td>40-49</td>
<td>59</td>
<td>61</td>
<td>120</td>
</tr>
<tr>
<td>50-59</td>
<td>52</td>
<td>81</td>
<td>133</td>
</tr>
<tr>
<td>60-69</td>
<td>36</td>
<td>33</td>
<td>69</td>
</tr>
<tr>
<td>&gt;70</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 3. Demographic Features of Population Studied (Mean ± SD)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social class 1-5</td>
<td>48.7 ± 12.8</td>
<td>48.0 ± 12.8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>93 ± 14</td>
<td>92 ± 16</td>
</tr>
<tr>
<td>Smoking habit (no. cigarettes per day)</td>
<td>7.5 ± 8.3</td>
<td>3.8 ± 6.7</td>
</tr>
<tr>
<td>Weight (lbs)</td>
<td>170 ± 23</td>
<td>143 ± 23</td>
</tr>
<tr>
<td>Percent overweight</td>
<td>1.9 ± 12.4</td>
<td>2.2 ± 15.4</td>
</tr>
</tbody>
</table>

Table 4. Biochemical Values in Population Studied (Mean ± SD)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Males</th>
<th>Females</th>
<th>Student's t&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>387 ± 86</td>
<td>356 ± 96</td>
<td>3.48</td>
</tr>
<tr>
<td>Uric acid</td>
<td>59 ± 12</td>
<td>49 ± 10</td>
<td>9.54</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2300 ± 430</td>
<td>2370 ± 510</td>
<td>...</td>
</tr>
<tr>
<td>Magnesium</td>
<td>21 ± 2</td>
<td>20 ± 2</td>
<td>5.17</td>
</tr>
<tr>
<td>Calcium</td>
<td>87 ± 7</td>
<td>95 ± 5</td>
<td>3.52</td>
</tr>
<tr>
<td>Phosphate</td>
<td>32 ± 5</td>
<td>32 ± 5</td>
<td>...</td>
</tr>
<tr>
<td>Total protein</td>
<td>72 ± 13</td>
<td>74 ± 13</td>
<td>...</td>
</tr>
</tbody>
</table>

<sup>a</sup> Tests for significance of difference between values for males and females. In all instances where t is given, P < 0.001.

Table 5. Product Moment Correlation Coefficients for Biochemical Constituents with Age and Weight (Significant Results Only)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Males</th>
<th>Females</th>
<th>Age</th>
<th>Weight</th>
<th>Age</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>0.239&lt;sup&gt;a&lt;/sup&gt;</td>
<td>...</td>
<td>0.293&lt;sup&gt;b&lt;/sup&gt;</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>-0.182&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.341&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.301&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.394&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.172&lt;sup&gt;c&lt;/sup&gt;</td>
<td>...</td>
<td>0.363&lt;sup&gt;b&lt;/sup&gt;</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>...</td>
<td>...</td>
<td>0.172&lt;sup&gt;a&lt;/sup&gt;</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01.
<sup>b</sup> P < 0.001.
<sup>c</sup> P < 0.05.

Values for the various biochemical tests are summarized in Table 4. Urea, uric acid, calcium, and magnesium were significantly higher in males. As demonstrated in Table 5, age was positively correlated with urea, uric acid, cholesterol, and magnesium among females. Urea and cholesterol were positively correlated with age in males, but this association was less striking than among females; in contrast to females, there was a significant negative correlation between uric acid and age in males. In both sexes total body weight and serum uric acid concentration were very significantly correlated.

Neither age nor weight showed any significant association with the serum concentration of calcium, inorganic phosphate, or total protein in either sex. Except for a highly significant correlation between diastolic blood pressure and uric acid concentration in females (r = 0.243; P < 0.001), the remaining demographic factors studied did not correlate with any of the serum constituents. There was no association between the biochemical constituents except for calcium and magnesium, which were highly correlated.

Fig. 1. Relationship between age and serum concentration of three serum constituents among males (broken line) and females (solid line). The horizontal bars represent the mean value for each constituent at each class interval.

Fig. 2. Relationship between serum uric acid concentration and total body weight. The horizontal bars represent the mean value for each constituent at each class interval. The highest class interval for females and the lowest for males includes all female and male subjects, respectively, above and below the stated limits.
Table 6. Regression Equations Relating Biochemical Value (in mg/liter) to Age (A), Social Class (SC), Diastolic Blood Pressure (BP), and Weight (W) \(^a\)

| Constituent | Males | | | | | Females | | | | |
|-------------|-------|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C(\pm D) | k(\pm SD) | C/k | d/k | C(\pm D) | k(\pm SD) | C/k | d/k | | | | | |
| Urea | 328 \pm 164 | 387 \pm 86 | 85 | 15 | 243 \pm 180 | 356 \pm 96 | 68 | 32 | | | | | | |
| Uric acid | 42 \pm 20 | 59 \pm 12 | 71 | 29 | 11.6 \pm 18.7 | 49 \pm 11 | 24 | 76 | | | | | | |
| Cholesterol | 2020 \pm 810 | 2300 \pm 430 | 88 | 12 | 1820 \pm 930 | 2370 \pm 510 | 77 | 23 | | | | | | |
| Magnesium | 19.5 \pm 4.1 | 21 \pm 2 | 93 | 7 | 19.1 \pm 3.5 | 20 \pm 2 | 95 | 5 | | | | | | |
| Calcium | 98 \pm 12 | 97 \pm 7 | 101 | | | 97 \pm 11 | 95 \pm 5 | 102 | | | | | | |

\(^a\) Equations given only for constituents where value for multiple regression coefficient (R) was significant at P < 0.05.

The expression on the left-hand side of Equation 3 was generated for each subject for each of the equations listed in Table 6, and in all instances 95% of the subjects lay within the range C \(\pm\) D defined for that equation.

The contribution of the demographic component (d) and of the constant term (C) to each biochemical parameter was expressed as a percentage of the mean (\(x\)) for that parameter (Table 7). Clearly, demographic factors influence the values of all biochemical parameters more strongly among women than among men. Viewed in another way, it seems that among men, biologic variability not accountable in the demographic terms considered in this study represents the main source of inter- and intra-individual variation in the concentration of chemical constituents in blood serum; clearly, the term C \(\pm\) D also includes analytic variance, but there is no reason to suppose that this would influence results from both sexes other than uniformly.

From Equation 3 above, we established a series of reference values for males and females independently corrected for the demographic composition of each
subject by use of the equations listed in Table 6. To test the usefulness of this scheme, a trial was made among female subjects referred for hospital outpa-
tient investigation. These subjects were Caucasians of British birth living in the city of Sheffield with no history of hospital admission for acute or chronic ill-
ness during the preceding 10 years. They were atten-
ting various specialist clinics for the first time at the request of their general practitioners. Some were being referred for specialist management of diseases newly-diagnosed by their practitioners; others were referred as problem cases in which the presence or the nature of organic disease could not be unequivocally identified by the practitioner. They were typi-
cal of subjects referred for the first time to the out-
patient departments of a British Teaching Hospital, and differed from the screening clinic population in having an urban industrial background. The ages of the subjects tended to be higher and their social class lower than those of the screening clinic. Al-
though patients were randomly selected, the criteria for selection were so strict that several thousand had to be reviewed to obtain the case material described in Table 8.

Comparison was restricted to urea, uric acid, and cholesterol among females in whom organic disease was firmly excluded or a final diagnosis reached after not more than 12 months investigation. Patients were selected on the basis of having a value between 100–110% of the mean +2 SD conventional normal limit, and therefore classified as abnormal, or a value between 90–100% of this limit, and therefore classified as normal. They were reclassified on the basis of computer-assisted analysis and calculation of the function:

\[
\text{Biochemical value} - d
\]

If this was greater than \(C + D\) for that constituent, the patient was classified as abnormal, and if less, the classification was normal. To avoid bias, no pa-

tient was included in respect of more than one bio-
chemical result. The other biochemical constituents, where estimated, were <90% or >110% of the mean +2 SD limit for that constituent established earlier in this study (Table 4).

A relatively small number of subjects fulfilled these criteria, and the results (Table 8) can only be regarded as tentative. Correction for demographic variability did seem to improve the accuracy of each test in predicting underlying disease, and the condi-
tion in question was usually one accepted as having a high incidence of the particular biochemical abnor-
mality (e.g., urea and uric acid in renal disease; urea and cholesterol in ischemic heart disease, hyperten-
sion, diabetes, and vascular disease; cholesterol alone in hypothyroidism; and uric acid alone in gout and blood dyscrasias).

### Discussion

The first requisite in a study of this nature is to ensure that only individuals free of disease are se-
lected. The second is to obtain a wide and represen-
tative segment of the population concerned. A third requisite also seems desirable: that the population should be ethnically homogeneous to prevent racial differences from blurring the influence of demo-
graphic factors. It is doubtful whether these criteria have been so completely fulfilled in any previous work as in the present study, either in respect of single or multiple chemical constituents, although the patients examined by Keating et al. (13) were subjected to almost as thorough a set of screening tests as our own. Those of Roberts (12) and of Werner et al. (14) were screened only by questionnaire, while the basis for selection in the report by Reed et al. (15) was not clearly defined; moreover, seasonal and geographic factors were not controlled. Our subjects were examined over the shortest practicable period consistent with uniformity concerning diet and exercise; the material comprised a high propor-
tion of the population of a typical English dormitory
village including professional, agricultural, and industrial personnel. The biochemical tests were completed by automated methods in the shortest possible time, thereby minimizing analytic variation. The statistical treatment of our data was designed to test for linear relationships throughout the range of observations, rather than the comparison over strictly defined class intervals adopted by the earlier authors (12-15) because this approach was better suited to the construction of the demographically-corrected reference values we had in mind from the inception of this work. It nevertheless seems worthwhile comparing our findings with those in the literature.

Urea. As in the present study, higher values for males and increasing concentration with age in both sexes have previously been reported (13-15), but Roberts (12) noted this increase with age in females only. A dependence upon exercise has already been observed (22), but this factor was unlikely to influence our data. We were unable to confirm a report that urea concentration is lower in smokers than in nonsmokers of both sexes and is negatively correlated with the amount smoked (8).

Uric acid. Our observation that the concentration of uric acid in serum is higher in males than females has been reported previously (1, 15, 23, 24). Findings on the effect of age on the serum uric acid in both sexes have not been so uniform. Roberts (12) noted a slight increase with age for males but no effect for females; Reed et al. (15) found a statistically significant increase with age only in females. Werner et al. (14) found in males that after an initial sharp rise at puberty the serum uric acid concentration continued to rise until the third decade and then remained constant; in females they reported a rise at puberty and a second gradual increase after the fourth decade. A similar pattern in females was described by Mikkelsen et al. (1), who suggested that estrogens and progesterones may have a urate-depressing effect.

In accordance with our results, others have found serum uric acid and body weight to be strongly correlated in both sexes (2, 24, 25). This relationship was, however, not obtained in two racial groups and led to the proposal that environmental factors can modify the weight-dependence of serum uric acid concentration (7, 26, 27).

There have been reports that uric acid increases with social class (4), decreases with social class in English males only (6), and decreases with social class in American females only (6). A relationship with drive has also been claimed (3), and smoking reportedly lowers serum uric acid concentrations in males (5, 28). We were unable to confirm any of these findings, and instead noted a significant association between uric acid concentration and blood pressure in females. Our findings with respect to age are also at variance with those of others; although an increase with age was observed in females, uric acid decreased with age in males. These results seem reconcile on the speculative assumption that weight exercises the dominant influence in both sexes. Weight increased with age in our female subjects and decreased with age in our males, and blood pressure was significantly related to weight in our females only; thus, the differential effects of age and blood pressure on uric acid in both sexes can be explained by their interaction with weight.

Cholesterol. Schilling et al. (9) found that, until the age of 23 years, values for serum cholesterol were not significantly different between males and females. Thereafter serum cholesterol increased in both sexes, more quickly in males than in females, until the age of 48 years. Above 48 years cholesterol tended to plateau in males and eventually decreased after 58 years, whereas in females the serum cholesterol continued to rise until 65 years of age. Thus, in persons older than 50 years, cholesterol was higher for women than men. Similar observations were reported by other authors (8, 11, 14). Reed et al. (15) demonstrated an increase in serum cholesterol with age for both sexes, but only the increase in females was statistically significant. Their results did not show a statistically proven sex-related difference, but suggested that serum cholesterol concentrations are greater for women than for men after 50 years. Lewis et al. (8) reported a weak association between cholesterol and body weight in certain age groups, whereas no such association was observed by Schilling et al. (9). More recently, claims have been made that in males serum cholesterol correlates with percent overweight (13) and with Quetelet's index of weight divided by square of height (28). There is disagreement concerning the effect of smoking on serum cholesterol (13, 28, 29) and concerning the relationship between serum cholesterol and blood pressure measurements (8, 29).

Our findings demonstrated a significant increase of cholesterol with age in both sexes, but we were unable to detect a significant sex-difference for the mean values, nor any association with smoking and blood pressure in either sex.

Calcium. Roberts (12) found serum calcium concentrations to be higher in males than in females. He also found a significant decline with increasing age in females, although males were unaffected. The results of Keating et al. (13) were contrary to those of Roberts; they found that in males, but not females, serum calcium significantly decreased with increasing age. Werner et al. (14) reported similar findings in males to those of Keating's group, but in females they found an increase between the fourth and sixth decades, with a decrease after the sixth decade. Thus up to the age of 49 years the serum calcium concentration was greater in males than females, but after this age there was no sex-related difference. The findings of Reed et al. (15) were similar to Werner's group, the only difference being in the 50 years-and-older groups, where females had a slightly higher
upper limit than males, and this tended to increase with advancing age. We found significantly higher values in males, and were unable to detect any significant influence exerted by age.

Magnesium. Keating et al. (13) found no significant sex-related difference in magnesium values, and for both males and females the results for serum magnesium were independent of age. Roberts (12) had earlier found no relationship between age and serum magnesium in males, but he did find a significant regression equation for females, magnesium increasing with age as in our own study. The fact that males had significantly higher values for magnesium as well as calcium in our series is not surprising in view of the strong association between the two constituents that we observed in both sexes.

Inorganic phosphate. Greenberg et al. (30) found that in males values for serum inorganic phosphate decrease progressively for the first three decades of life, and then change no further except for a slight increase after 70 years of age. In women the decrease was accentuated during the earlier decades, with a minimum at 45 years of age followed by a progressive rise. Keating et al. (13) found similar results in females, but observed in males a progressive decrease with age up to the eighth decade. Werner et al. (14) found that serum inorganic phosphate fell more markedly in the first two decades in both sexes, but they also noted a progressive rise after 40 years of age in females. Hamilton et al. (31) had earlier reported a marked decrease in the first two decades, but unlike the previously mentioned workers found no significant change in the serum inorganic phosphate throughout the remainder of life in either men or women. Finally, Reed et al. (15), who only tested serum from people older than 20 years, found no obvious effect of age or sex on the concentration of inorganic phosphate in serum, in line with our own observations.

Total protein. Two groups agree that values for total protein in males were higher than in females, that these values decrease with increasing age in both sexes, and that there is no significant sex-related difference in the rates of decrease (12, 13). Werner et al. (14) also found decreasing values with age from the second decade in females, but in males the total protein progressively rose until the fourth decade, and then began to fall. The data of Reed et al. (15) showed that there was no significant effect of age on the concentration of total protein in serum, and that the 2.5 percentile limit for males was higher than that for females in every age group, although the means were not significantly different; our findings agree with both their conclusions.

This study has yielded positive conclusions concerning demographic factors influencing the serum concentration of chemical constituents in healthy subjects and has demonstrated the feasibility of quantitating these influences in a manner that has useful practical applications. Our findings in many instances substantiate those of previous workers, but in other instances there is substantial disagreement. The literature already reviewed contains extensive areas of controversy, and undoubtedly many of these can be attributed to racial and environmental factors interacting with demographic factors and modifying the relationships in different populations. Other reports, not previously mentioned, support this view (32-34). It therefore becomes mandatory for those intending to exploit and develop the concepts outlined in this report to establish the relevant equations applicable to their own population. Elsewhere, we describe in greater detail the resources required to do so, in the belief that these guidelines will prove useful to those undertaking such a study (17).

Addendum

Since submission of this manuscript, a detailed study of the age-dependence of serum biochemical constituents has been published (35). The population was drawn from patients referred to a private clinic in the City of London and was heavily biased toward Social Classes 1 and 2. No attempt was made to exclude patients on grounds of health or obesity, nor were precautions taken to ensure that subjects were in the postabsorptive state. Despite these differences, the conclusions of these authors are in good agreement with our own in most instances. They did not determine magnesium, but found that all other constituents included in our study obeyed a gaussian distribution with the exception of cholesterol, for which a nonparametric technique indicated a 97.5 percentile limit 2% higher than their mean +2 SD. Our data for males and females separately were tested for gaussian distribution in the following ways and with the following results:

(a) Visual inspection. This is arbitrary and subjective.
(b) Nonparametric graphical derivation of the 2.5- and 97.5-percentile limits (36). The ranges obtained were in good agreement with those calculated on a gaussian basis.
(c) Calculation of the actual number of subjects exceeding the mean + 2 SD limit and falling below the mean - 2 SD limit approximated 5% of the total for each constituent.
(d) Estimates of skewness and kurtosis failed to yield significantly positive values of g1 or g2 (37) for any of the constituents in either sex.

We therefore assume that all of these constituents follow a gaussian distribution for each sex in our population, which was ethnically homogeneous and excluded all subjects with latent disease and obesity.

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References


