Concentrations of Serum Protein Fractions in White Women: Effects of Age, Weight, Smoking, Tonsillectomy, and Other Factors

John Wingerd and Ernest E. Sponzilli

We measured the concentrations of protein in electrophoretic fractions (albumin and $\alpha_1$-, $\alpha_2$-, $\beta$, and $\gamma$-globulins) of serum from 9547 white women to determine their relations to age, weight, smoking and other factors. Albumin concentration decreases with age, beta globulin concentration increases. Increasing weight is associated with a decrease in albumin, but with increases in all the globulins. Cigarette smoking is associated with highly significant differences in all the protein fractions. Surprisingly, very significantly lower concentrations of $\beta$- and $\gamma$-globulins were also found in women who reported a history of tonsillectomy. Height, amenorrhea, education, and alcohol and coffee consumption have some small but statistically significant effects upon the various fractions. Diurnal variation was also slight.

Additional Keyphrases: alcohol, coffee consumption - height - education - amenorrhea - factors correlating with protein concentration - variation, source of - normal values

In 1968 a longitudinal survey, the Contraceptive Drug Study (1), was instituted to follow the health status of a population of users and nonusers of oral contraceptives.

In the course of assessing the effects of oral contraceptives and of pregnancy on serum proteins in this population (2), several other factors were observed to be associated with differences in the electrophoretic fractions. These findings are the subject of this paper.

Materials and Methods of Analysis

Subjects. The subjects of the Contraceptive Drug Study are members of the Kaiser Foundation Health Plan in Walnut Creek, California. The characteristics of this predominantly white, middle-class suburban population have been described in detail elsewhere (1).

As part of periodic health checkups, these women undergo extensive testing in the Automated Multitest Laboratory at the Kaiser-Permanente Medical Center in Walnut Creek. Included is a lengthy questionnaire relating to both personal and medical history.

Tests. Protein electrophoresis was performed on cellulose acetate membranes with the Beckman Microzone cell. Protein was stained with a Ponceau-S fixative dye solution and read in an analytical densitometer. Total protein was determined by a modified biuret method (Autochemist, AGA, Sweden). Individual protein fractions were derived by applying the electrophoresis results, recorded as percentages, to the concentrations of total protein.

Exclusions. Since the effects of pregnancy and of oral contraceptive use on serum protein concentrations have already been reported (2), pregnant or postpartum women and those taking oral contraceptives or other estrogenic hormones were excluded from this analysis. The small number of nonwhites appeared to have somewhat above-normal $\gamma$-globulin concentrations; because these women were few in number they were also excluded, as were women younger than 20 or older than 54 years. A comparison of serum protein values with questionnaire responses disclosed that protein concentrations were somewhat different in diabetics (i.e., in women who claimed to be diabetic or who had taken insulin or other hypoglycemic agents in the past) and in those women who had taken cortisone within the past year. The differences were very slight, but to reduce heterogeneity these women were also excluded. Some cases with incomplete information were unusable, and finally, a few protein measurements seemed so aberrant that they were presumed to be errors of measurement or recording. In all, 5838 cases were excluded from the total of 15 093 tested during 1970 to 1972, leaving 9255 for analysis. Details of the exclusions are as follows:

| Oral contraceptive users | 3377 |
| Other hormone users       | 1401 |
| Pregnant or postpartum    | 16   |
| Nonwhite                  | 227  |
| Age < 20 years            | 325  |
| Age > 54 years            | 91   |

The Kaiser-Permanente Contraceptive Drug Study, 1515 Newell Ave., Walnut Creek, Calif. 94596.
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Table 1A. Percentage of Women with Selected Characteristics, by Age

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>20-24</th>
<th>25-29</th>
<th>30-34</th>
<th>35-39</th>
<th>40-44</th>
<th>45-49</th>
<th>50-54</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of T&amp;A</td>
<td>48.8</td>
<td>60.5</td>
<td>61.1</td>
<td>62.2</td>
<td>60.2</td>
<td>62.9</td>
<td>58.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Amenorrheic</td>
<td>0.1</td>
<td>2.1</td>
<td>5.5</td>
<td>8.3</td>
<td>12.3</td>
<td>25.7</td>
<td>65.5</td>
<td>13.8</td>
</tr>
<tr>
<td>Current smokers</td>
<td>34.9</td>
<td>34.1</td>
<td>34.9</td>
<td>33.3</td>
<td>33.7</td>
<td>32.4</td>
<td>31.6</td>
<td>33.6</td>
</tr>
<tr>
<td>Coffee use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6+ cups/day</td>
<td>7.8</td>
<td>16.2</td>
<td>23.0</td>
<td>28.1</td>
<td>30.2</td>
<td>32.0</td>
<td>25.1</td>
<td>24.2</td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondrinkers</td>
<td>29.2</td>
<td>23.4</td>
<td>22.9</td>
<td>25.1</td>
<td>25.5</td>
<td>29.4</td>
<td>35.6</td>
<td>26.3</td>
</tr>
<tr>
<td>3+ drinks/day</td>
<td>0.5</td>
<td>0.9</td>
<td>3.2</td>
<td>3.8</td>
<td>5.3</td>
<td>5.9</td>
<td>6.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;High school</td>
<td>5.6</td>
<td>5.3</td>
<td>8.3</td>
<td>10.6</td>
<td>11.6</td>
<td>12.2</td>
<td>16.9</td>
<td>9.8</td>
</tr>
<tr>
<td>College graduate</td>
<td>9.8</td>
<td>22.5</td>
<td>23.8</td>
<td>24.2</td>
<td>23.0</td>
<td>20.2</td>
<td>17.5</td>
<td>21.3</td>
</tr>
</tbody>
</table>

Unknown height, weight, or hour of examination 60
Incomplete questionnaire 37
Diabetic 170
Taking cortisone 115
Outliers 19

Total 5838

The responses to almost 200 items from the questionnaire were surveyed to discover possible relations to serum protein concentration. Most of these showed no associations whatever, while a few were found to be associated at such low levels of significance (generally taken to be $P > 0.001$) that they could well have appeared by chance in such a survey. It may be of interest to mention that among the factors that proved to be irrelevant were marital status, number of pregnancies, religion, history of allergy or of operations other than tonsillectomy, ABO blood type, and hours since last food ingestion.

Age, height, and weight proved to be related to differences in some or all of the protein fractions; the time of day that blood was drawn also showed a small effect.

Of the responses to the questionnaire, highly significant associations were found in connection with drinking (both alcohol and coffee) and smoking habits, with history of tonsillectomy and adenoidecotomy (T&A), with educational level, and with amenorrhea. This last factor was defined by a positive response to the question, "If you are no longer having menstrual periods, at what age did you stop?" No difference according to age was found in the relations of this factor to serum protein concentration, hence no attempt was made to distinguish the postmenopausal women from those otherwise amenorrheic.

As shown in Table 1A, age was related to some but not all of the other factors. The percentage of women with history of tonsillectomy and adenoidecotomy showed little change according to age; the same was true of the percentage of current smokers. However, the proportions of women who drank six or more cups of coffee a day or three or more alcoholic drinks a day increased markedly with age. The proportion of women in the lowest education category also increased with age.

Other correlations were present as well, e.g., smokers tended to drink more coffee and alcohol than did non-
smokers, and also tended to be less well educated (Table 1B).

To sort out the net effects of each factor, we used multiple regression in the analysis. In this analysis relative weight, rather than actual weight, was used as the measure of adiposity. This is the ratio of actual weight to the mean height-and-age-specific weight for the Contraceptive Drug Study population. Thus a woman who is 15% above the mean for her height and age will have a relative weight of 1.15. This measure is convenient for analysis because it is correlated neither with height nor with age.

In the multiple regressions age, height, relative weight, and the time of day that blood was drawn were entered into the computations as continuous variables, with a quadratic term where required, and the questionnaire responses were treated as categorical, 0–1 variables.

While all of the variables included showed highly significant ($P < .0001$) associations with one or more of the protein fractions, some of the effects were, in absolute or percentage terms, quite small, and owed their significance levels to the large sample size. They have been included nonetheless because of their correlations with the other independent variables. For example, inclusion of amenorrhea, a minor factor, serves to refine the estimates of age, and inclusion of alcohol and coffee drinking clarifies the effects associated with smoking.

Mean serum protein levels concentrations also varied with date of examination. This variation did not appear to represent a seasonal effect, but rather an irregular drift, probably due to changes in technique or reagents (3). To decrease the variance from this source and to avoid possible confounding with the other variables, a regression adjustment for the "nuisance variable" of month of examination was included in the computations, although this precaution proved to affect the estimates for the other factors negligibly.

Results
The means and standard deviations (in g/liter) for the five protein fractions ($n = 9255$) were as follows:

<table>
<thead>
<tr>
<th>Protein Fraction</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>41.02</td>
<td>3.05</td>
</tr>
<tr>
<td>$\alpha_1$-Globulin</td>
<td>2.40</td>
<td>0.56</td>
</tr>
<tr>
<td>$\alpha_2$-Globulin</td>
<td>6.96</td>
<td>1.19</td>
</tr>
<tr>
<td>$\beta$-Globulin</td>
<td>9.32</td>
<td>1.35</td>
</tr>
<tr>
<td>$\gamma$-Globulin</td>
<td>12.56</td>
<td>2.39</td>
</tr>
</tbody>
</table>

Table 2 shows the regression coefficients with their $t$-values (the coefficients divided by their standard errors). The coefficients of the continuous variables (age, height, relative weight, and time of day) represent mean change per unit specified, after adjustment for all other factors in the regression, while the coefficients for the categorical variables are the adjusted mean differences.
Table 2. Regression Coefficients (in mg/dl), with t-Values, of Serum Protein Fractions in Relation to Other Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Albumin</th>
<th>Globulines</th>
<th>Globulines</th>
<th>Globulines</th>
<th>Globulines</th>
<th>Globulines</th>
<th>Globulines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coeff t</td>
<td>Coeff t</td>
<td>Coeff t</td>
<td>Coeff t</td>
<td>Coeff t</td>
<td>Coeff t</td>
<td>Coeff t</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>-6.9 -17.2</td>
<td>-0.8 -1.0</td>
<td>-0.59 -3.7</td>
<td>1.47 8.1</td>
<td>-4 -1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age²</td>
<td>-</td>
<td>0.03 3.4</td>
<td>0.09 5.7</td>
<td>0.10 5.6</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (per cm)</td>
<td>-0.6 -1.2</td>
<td>-0.2 -1.7</td>
<td>-1.6 -7.8</td>
<td>-0.8 -3.4</td>
<td>1.3 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative weight (per 10% inc)</td>
<td>-25.2 -13.7</td>
<td>1.7 5.0</td>
<td>13.3 18.8</td>
<td>17.4 21.8</td>
<td>9.3 6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of day (per hour)</td>
<td>9.0 3.7</td>
<td>1.1 2.4</td>
<td>6.4 6.8</td>
<td>1.7 1.6</td>
<td>-3.3 -1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>0.0</td>
<td>-0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>-0.0 -0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee consumption</td>
<td>0.0</td>
<td>-0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>-0.0 -0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>0.0</td>
<td>-0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>-0.0 -0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.0</td>
<td>-0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>-0.0 -0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of tonsillectomy</td>
<td>0.0</td>
<td>-0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>0.0 -0.0</td>
<td>-0.0 -0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance levels (two-sided):</td>
<td>2.0 p &lt;.05</td>
<td>2.3 p &lt;.01</td>
<td>3.2 p &lt;.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
between the protein values of the stated categories and those of the comparison groups shown in the table with regression coefficients of 0.

Age

The mean values by age for each of the fractions, adjusted for the effects of the other factors, are shown in Figures 1 and 2. The greatest age-related effect was found for albumin, which decreased by an average of 69 mg/liter per year (Table 2).

A significant increase, most noticeable after age 40, was found in the β-globulin fraction, while α₂-globulin was increased only in the youngest age group.

Height and Weight

Height was found to be of minor importance in accounting for normal variation in serum protein levels, except for α₂-globulin, which decreased very significantly (P < .0001) with increasing height. The associations of the remaining fractions with height were obviously trivial (Table 2), even though some were technically significant with these large numbers.

Relative weight showed much stronger associations with all the protein fractions, albumin decreasing with increasing weight and all the globulins increasing. As can be seen in Figures 1 and 2, these relations were essentially linear.

Time of Day

Appreciable diurnal variation was evident only in the albumin and α₂ fractions, both of which showed significant increases from 0900 to 1500 hours, the times during which blood was drawn.

Smoking and Drinking

Highly significant differences in all the protein fractions were found in connection with cigarette smoking, the effects in general increasing with the amount smoked. Past smokers showed γ-globulin values that were significantly below those of women who had never smoked, but no difference between never and past smokers was seen in any of the other fractions.

Coffee consumption of six or more cups a day was associated with reductions only in the β- and γ-globulin fractions, while differences according to alcohol consumption were found in all fractions, the most significant being increases in albumin and β-globulin, and a decrease in γ-globulin. For each fraction, the estimates of the effects were greater for heavy drinkers (three or more alcoholic drinks a day) than for the much larger group claiming to drink two or less drinks a day.

Education

Only in α₂- and γ-globulin were there differences according to educational level. Both fractions decreased significantly with increasing education.

Tonsillectomy

History of tonsillectomy/adenoidectomy was associated with highly significant reductions in β- and γ-globulins, but no associations were found in the other fractions.

The similarity between the effects of smoking and of tonsillectomy/adenoidectomy on these two fractions suggested the investigation of a possible interaction, i.e., that women with already low values due to one factor might not be subject to further reductions from the other. Figure 3 shows the means of β- and γ-globulins...
by both smoking habit and history of tonsillectomy/adenoidectomy. Here it can be seen that there is some indication, particularly in β-globulin, that the effect of history of tonsillectomy/adenoidectomy decreases as the amount of smoking increases (or, conversely, that the effect of smoking is less in the tonsillectomy/adenoidectomy group), but the trend is slight.

Discussion

The differences in serum protein concentrations related to the factors reported here are, except for γ-globulin, considerably less than those associated with pregnancy or with the use of oral contraceptives.1 None of the variables shown in Table 2 appears important enough to affect the definition of “normal range.” Nevertheless, even if the relations brought into relief by this large study sample are not of direct clinical utility, they are of interest in showing that serum protein concentrations depend on a variety of factors.

Because more than 50 distinct proteins have been identified in serum, each electrophoretic fraction is a complex of heterogeneous proteins, many of which overlap adjacent fractions. Under these circumstances, differences in concentration of serum protein fractions occurring in association with the factors identified in this study are net results, reflecting possible changes in many components. An exception, of course, is albumin, and to a degree the γ-globulin fraction is another in that it is at least functionally homogeneous, 90% of the fraction being composed of immunoglobulins.

Any of the results found may be due in part to differences in plasma volume (no direct measure of this was available); even though hemodilution or hemoconcentration may be present and superimposed upon the other effects, they alone cannot account for the nonuniform changes observed among the various fractions. Even diurnal variation, the most likely candidate for such an explanation, cannot be accounted for by assuming changes in plasma volume only, because the concentrations of the fractions are not all changed in the same proportion.

All results have been reported here in terms of protein concentrations, but it should be kept in mind that only total protein was measured directly. Differences in the individual fractions may represent changes in dye-binding capacity as well as in amount of protein, hence conclusions should be drawn cautiously. In this connection, however, it should be pointed out that in Table 2 the change in total protein associated with each factor can be obtained simply by adding the coefficients of the individual fractions. This sum is independent of the electrophoretic procedure.

Age

Most of the reported age-related variation in serum protein concentration has been found outside the age range of this study, notably in the early months of postnatal life. Within the age range 20–54 years, age-related variations found in this population were small, the greatest being a decrease in albumin of about 1.5% per decade and an increase, though not a linear one, in β-globulin. It should be noted that both these age effects would tend to be obscured if oral contraceptives were not taken into account, either by adjustment or, as in the present case, by exclusion.

Height and Weight

The finding that α2-globulin concentration decreases with increasing stature is difficult to interpret, given that it is the most heterogeneous of all the protein fractions. Presumably the association is with one of the major components of the fraction, because the effect is so clearly apparent in the total.

With regard to relative weight, a plausible explanation for the increased alpha and beta fractions is that the concentration of lipoproteins should be expected to increase with weight. The effects on albumin or γ-globulin are more difficult to rationalize. Conceivably they even may be artifacts (3), if increasing weight—and hence higher serum lipid concentrations—were to cause slight systematic changes in the electrophoretic mobilities or staining characteristics of the various proteins.

Coffee and Alcohol Consumption

It is difficult to find a reasonable explanation for the lower values for β- and γ-globulin observed in women who were drinking six or more cups of coffee a day. The differences, however, are significant at such levels that they are unlikely to be chance findings.

In view of the well-known association of alcohol consumption with liver pathology and this in turn with reduced albumin synthesis, the finding that albumin is lowest among nondrinkers is unexpected. However, in...
a group of women seeking routine health checkups, few alcoholics are likely to be encountered and the effects due to these may well be obscured by other factors, behavioral or physiological, associated with differences in drinking habits.

Cigarette Smoking

The relation of serum proteins to cigarette smoking has apparently not been investigated in large populations, except for the study of Dales et al. (4), who found that in white women, smoking was associated with significantly lower concentrations of albumin and of total globulin.

It is clear from Table 2 that the effects of smoking cannot be explained by differences in blood volume: two of the fractions, α1- and α2-globulin, show higher values among smokers, while the remaining fractions are lower.

The presence of the other factors in the regression equations ensures, at least as far as is possible with these data, that the estimates shown for smoking are net effects, and are not accounted for by correlations with the other variables. In any case most of these other factors had such low correlations with amount of smoking that adjustments for them had little effect. Even alcohol and coffee consumption, the two factors most closely related to smoking habit, made an appreciable difference only in the γ-globulin regression. Here their inclusion decreased the estimate for the heaviest smoking group, for example, from −1.61 g/liter to the −1.43 g/liter shown in Table 2.

The similarity of the effects in both alpha fractions suggests that possibly a single component, overlapping the two, is affected. On the other hand, both the profusion of chemical components in cigarette smoke and the diversity of globulins which migrate to the alpha zone make it seem equally likely that several different proteins may be involved.

In the gamma fraction, the magnitude of the reductions implies that the immunoglobulins, which constitute the bulk of this fraction, must be affected. Further, the deficiency is likely to be at least in part in IgG, the largest immunoglobulin component; changes confined to the quantitatively minor components IgM and IgA would have to be very great to account for as much as a 10% decrease in the total gamma fraction.

The lower concentrations of β-globulin among smokers are also consistent with the conclusion that the immunoglobulins are involved, because they are known to migrate in part to the beta zone. The possibility remains, however, that the effects seen in the beta fraction may be partly attributable to changes in other components.

The present data provide no clue as to the mechanism of the observed reductions, and still less as to their pathological implications, if any. However, it has already been suggested by Holt et al. (5) that smoking may result in impaired response and immunosurveillance and that this may contribute to the increased prevalence of chronic bronchitis and carcinoma among smokers. If cigarette smoking does act in such a fashion, then lower concentrations of immunoglobulins would be a likely result. Some animal (6, 7) and human studies (8) support this hypothesis.

History of Tonsillectomy

That measurably lower β- and γ-globulin concentrations levels should be present in women with a history of tonsillectomy/adenoidectomy seems so unlikely that were it not for the extraordinary significance levels (P < .0001) the findings would be attributed to chance sampling variation. The relation clearly seems to be real, however. Analysis of an independent sample, namely the oral contraceptive users excluded from the present study, yielded estimates (−270 mg/liter for beta and −500 mg/liter for gamma) almost identical to those shown in Table 2.

Because only the beta and gamma fractions are involved, and because tonsillar tissue is primarily lymphoid in nature, it seems probable that the results are attributable to differences in the immunoglobulins.

Though these findings can scarcely be due to sampling error, they may well be the result of selection. Women with low immunoglobulin concentrations may have been more susceptible to tonsillitis as children, and thus have been selected for T&A.

Another possibility is that the association shown here may be indirect, i.e., that it may be mediated by some other factor correlated with immunoglobulin concentration and with history of tonsillectomy/adenoidectomy. In the present data, only educational level was correlated with history of tonsillectomy/adenoidectomy. Of the women who had not finished high school, 43.6% reported history of tonsillectomy/adenoidectomy, while for high school graduates the percentage was 59.8%, and for college graduates, 68.2%. Adjustment for this factor (Table 2) had small effect upon the difference in γ-globulin concentration associated with history of tonsillectomy/adenoidectomy, reducing it only from −530 to −500 mg/liter. Thus, while it is possible that unknown factors, socioeconomic or otherwise, may account for the differences observed, in order to do so they would have to be very strongly correlated both with history of tonsillectomy/adenoidectomy and with immunoglobulin concentration.

In any case the finding, although unexpected, is not implausible. In animal studies (9, 10), fetal development of tonsils was shown to be similar to that of thymus, and in addition, tonsils transplanted into thymectomized animals acted in a fashion that was similar to thymus implants. Therefore, if tonsils, as is so for the thymus, were the source of a particular set of T-cell lymphocytes, then their absence could conceivably deprive B-cell lymphocytes of activation leading to immunoglobulin release.

There appear to be no previous studies of the effects of tonsillectomy/adenoidectomy upon the electrophoretic fractions, and few of the effects upon serum immunoglobulins. Veltri et al. (11) measured serum immunoglobulin before and after tonsillectomy/adenoi-
dectomy in a group of 17 children, and found that serum IgG was significantly decreased six months after the operation; the decrease, however, was from abnormally high to within normal limits. In another study (12) serum immunoglobulins were measured before and immediately after tonsillectomy in a group of 80 children, with results apparently inconsistent both with the data reported here and with the mechanism proposed above: serum IgG and IgM concentrations increased significantly in the first 60 days after the operation, while IgA showed a reduction at 7 days, followed by a return to initial values by 60 days.

The data of the present study have demonstrated, as was the case with cigarette smoking, only reduction in quantity, not necessarily impairment of function. Further, the reduction has been shown only for circulating, and not for secretory antibody. In view of these limitations, speculation as to the pathological implications of the findings would be premature.

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References
1. Ramcharan, S., Ed., The Walnut Creek Contraceptive Drug