CONCENTRATION OF SERUM PROTEIN-BOUND IODINE IN NORMAL MEN 1, 2

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During recent years the determination of the protein-bound iodine (PBI) in the serum or plasma has been used with increasing frequency as a diagnostic aid. Various limits have been suggested for the serum PBI concentration in euthyroidism. The range most frequently recorded in the literature is from 4 to 8 gamma per cent of PBI. These values are not fiducial expressions of full statistical analyses of adequate numbers of persons; they are based on observations made by several groups of workers on limited numbers of persons judged to be euthyroid but otherwise not fully characterized (Table I). The present paper reports the results, including statistical analysis, from 402 young and middle-aged men who are clinically "normal" in all respects and who represent active urban "white collar" workers in the upper Midwest.

1 Abridgement of material contained in a thesis submitted by Robert G. Tucker to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

2 The subjects studied in the Laboratory of Physiological Hygiene were available in connection with a long-range research project sponsored by the U. S. Public Health Service on the recommendation of the Cardiovascular Study Section.

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METHODS

Serum PBI concentration was determined by the method of Barker (7) modified as follows: (1) In the distillation of iodine, sodium arsenite solution was substituted for sodium sulfite solution as the alkaline reducing substance in the trap. This modification was suggested by Dr. Barker in a personal communication. To 20 ml. of 5 N NaOH, 1.5 grams of As2O3 are added, and the resulting solution is diluted with distilled water to 100 ml. One-half ml. of this solution is used in the trap in place of 0.5 ml. of sodium sulfite solution. Aeration is no longer necessary. The cooled distillate and washings can be diluted to 25 ml., mixed, and the iodide content determined directly by cerate colorimetry. (2) Phosphorous acid was found to be the reagent which was most apt to cause difficulty. An aqueous 50 per cent solution of phosphorous acid (Fisher Scientific Company) was filtered through an asbestos mat and boiled with 2 ml. of Superoxol for one hour at approximately constant volume. This effectively removed traces of iodine and organic matter. (3) Blood serum was used for analysis rather than blood plasma. (4) Blank values were determined from completely inorganic samples.

The PBI method was studied and standardized with great care before any of the material reported here was analyzed. Known amounts of iodine as sodium iodide were added to serum in repeated recovery experiments which demonstrated apparent complete recovery of iodine with an occasional high value. For example, in the last series of such tests, 0.1 y of iodine was added to each of four aliquots of pooled serum which had on repeated analysis an average of 4.75 y of PBI per 100 ml.; the apparent recoveries of added iodine were 100, 100, 100 and 130 per cent. Three samples of serum were exchanged with the Department of Biochemistry of the Mayo Clinic with the results shown in Table II; both of the laboratories used the same analytical procedure.

Finally, the reproducibility of single measurements was checked. In a series of 12 aliquots, with a mean value

**TABLE I**

<table>
<thead>
<tr>
<th>Authors</th>
<th>N</th>
<th>Mean</th>
<th>S. D.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salter, Bassett, and Sappington (1)</td>
<td>21</td>
<td>5.7*</td>
<td>1.0*</td>
<td>4.0-8.0</td>
</tr>
<tr>
<td>Talbot and associates (2)</td>
<td>11</td>
<td>7.0</td>
<td></td>
<td>6.0-8.4</td>
</tr>
<tr>
<td>Perry and Cosgrove (3)</td>
<td>34</td>
<td>5.9</td>
<td>1.3</td>
<td>4.0-9.3</td>
</tr>
<tr>
<td>Conner and associates (4)</td>
<td>48</td>
<td>4.8</td>
<td></td>
<td>4.0-6.0</td>
</tr>
<tr>
<td>Kydd, Man, and Peters (5)</td>
<td>83</td>
<td>5.3†</td>
<td>1.0†</td>
<td>3.8-8.5</td>
</tr>
<tr>
<td>Starr and associates (6)</td>
<td>100</td>
<td>5.6</td>
<td>0.9†</td>
<td>4.0-8.5</td>
</tr>
</tbody>
</table>

* Calculated from Figure 2.
† Calculated from Figure 1.

**TABLE II**

<table>
<thead>
<tr>
<th>Collaborative check samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values for micrograms of iodine per 100 ml. of serum.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test</th>
<th>Mayo Clinic</th>
<th>U. of Minn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td>Total I</td>
<td>3.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Human serum</td>
<td>PBI</td>
<td>5.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Human serum (exophthalmic goiter)</td>
<td>PBI</td>
<td>16.2</td>
<td>16.4</td>
</tr>
</tbody>
</table>
of 4.77 $\gamma$ per 100 ml. of serum, the standard deviation was ± 0.60. This suggests that in nine out of 10 cases a single measurement of PBI will be within ± 1.0 $\gamma$ per 100 ml. of the true (mean) value. It seems possible, however, that the error of measurement is not absolute but is related to the concentration of PBI in the sample. If the error were strictly proportional to the total concentration, we might estimate that in nine out of 10 cases a single PBI measurement will be within ± 21 per cent of the true (mean) value of the serum examined. From trials with high PBI sera, however, this appears to be an overestimate of the error and we conclude that for ordinary clinical work the reliability of a single measurement can be taken to be within 1 $\gamma$ per cent.

The serum total cholesterol determination was made by application of the Liebermann-Burchard reaction to a Bloor extract. This procedure as used here at constant temperature, with cholesterol standards for every batch, closely checks with the Schoenheimer-Sperry digitonin method which was, in fact, applied in parallel from time to time (8). The basal oxygen consumption was determined with the Sanborn apparatus by the standard technique used by Boothby, Berkson, and Dunn (9). The normal weight for each subject was estimated according to his height and age by referring to the tables published from the Medico-Actuarial Investigations of 1912; these are sometimes called Davenport's tables and are, in essence, the tables almost universally used in the United States. The per cent of normal weight was calculated according to the formula: $\frac{\text{actual weight}}{\text{normal weight}} \times 100 = \text{per cent of normal weight}$.

**MATERIAL**

The normal men used in the present work have been studied rather intensively in other respects (8, 10). They are of northern European ancestry (chiefly descended from stock from the British Isles) and are actively and successfully engaged in non-manual pursuits in the metropolitan communities of Minneapolis and St. Paul. Included here were 130 younger men (18 to 25 years of age) students in the University of Minnesota and 272 middle-aged (45 to 56 years of age) business and professional men. Each man was subjected to a physical examination by a competent internist and was carefully questioned as to medical history, fatigue, "nervousness," etc. The absence of complaints, tremor or abnormal heart rate and the presence of normal basal metabolic rate, all contributed to the considered judgment that these men were euthyroid. This judgment was sustained by repeated examinations in three successive years. All of the men came to the laboratory in the morning without breakfast, and all data refer essentially to the basal state.

**RESULTS**

The descriptive statistics for the sample of normal men are given in Table III. The sample of normal serum PBI value did not form a normal frequency distribution curve (Figure 1). By calculation of the beta coefficients the distribution was found to be significantly skewed and peaked so that the standard deviation is an inappropriate

![Frequency Distribution of Serum PBI Values in Normal Men](image-url)
and for the group of young men and the group of middle-aged men.

No significant correlation was observed between age and the serum PBI within the limited ranges of either the young group or the middle-aged group. However, the mean for the young group of normal men was significantly higher (above the 1 per cent level), as shown by calculating Snedecor’s F ratio, in serum PBI than the mean for the middle-aged group.

A slight but significant positive correlation was demonstrated for the combined groups between the serum PBI and the basal metabolic rate (r = +0.113). This was accepted with reservations, because it was shown that the relation approaches curvilinearity. No correlation was observed between the serum PBI and the basal metabolic rate within the young group or within the middle-aged group of men. Therefore, in normal men variations in the basal oxygen consumption are due largely to factors other than the circulating PBI.

In the middle-aged group of normal men a slight but significant, positive, and linear correlation (r = +0.163) was observed between the serum PBI and the serum cholesterol concentration; no such relation was found in the group of normal young men. For the gross sample of normal men a curvilinear relationship was demonstrated between the serum PBI and the serum cholesterol.

No relationship was demonstrated between the serum PBI concentration and the per cent of normal body weight in normal young men, in normal middle-aged men, or in the gross sample.

**DISCUSSION**

The serum PBI data for the sample of men reported here constitute the largest sample of normal men which has been studied with modern analytical techniques. The sample is by no means random. The subjects were selected with respect to sex, race, age, economic status, geographic habitat and physical condition. Nevertheless, the frequency distribution of the 402 serum PBI values is remarkably similar to the sample described by Kydd, Man, and Peters (5). Our mean value of 5.8 gamma per cent of PBI is significantly higher than the mean of 5.3 gamma per cent calculated from Kydd’s data. However, by using a Chi
square test for goodness of fit, and by making the
two distributions comparable by expressing them
in units of absolute deviation from their respective
absolute means, the obtained Chi square value
of 1.83 associated with seven degrees of freedom
indicates that a discrepancy as large as that ob-
served could occur through chance alone 97 times
out of 100. From this very close relationship we
infer that there is no statistical difference between
the shape of the two frequency distribution curves,
and that the distribution of PBI values in the com-
mon population from which they were drawn is
non-Gaussian. The two samples would be identi-
cal in all respects except for the apparent presence
of a systematic analytical difference which could
be explained, possibly, as a result of slight differ-
ences in method or of a difference in the age com-
position of the samples.

With respect to age our results are in agreement
with those of Kountz, Chieffi, and Kirk (11) who
reported, "a gradual decrease in the organic se-
rum iodine concentration . . . with advancing
years." The resemblance to the basal metabolic
rate trend with age may be noted, but the age
trend in serum PBI concentration is considerably
less pronounced. The lack of correlation be-
 tween the serum PBI concentration and the basal
oxygen consumption merely emphasizes that the
one is not closely dependent on the other in this
normal range.

Our data on cholesterol are somewhat difficult
to interpret. However, we are in substantial
agreement with Peters and Man (12) who found
no important relationship between the precipitable
iodine and the cholesterol in the serum in euthy-
roidism. There is no denying the fact that a very
slight relation does exist between the serum PBI
and the cholesterol level in the middle-aged men,
however.

Williams (13) "concluded that an alteration
of thyroid function does not have much etiologic
significance in obesity." This view is confirmed
by the present study. In 33 obese men of the
Minnesota study (those whose body weight ex-
ceeded 126 per cent of the normal mean standard
weight), only three had serum PBI values of less
than 4.0 gamma per cent. This suggests that in
otherwise normal men obesity is due, for the
greater part, to factors other than the activity of the
thyroid gland.

SUMMARY

From the study of the protein-bound iodine
(PBI) in the serum of 402 normal men in Min-
nesota, the following conclusions are drawn:

1. The frequency distribution of serum PBI
values in normal men is significantly peaked and
skewed.

2. Standards for serum PBI values have been
established for the 95 and for 99 per cent of the
population for which these normal men represent
a sample.

3. Normal middle-aged men (45 to 56 years of
age) are significantly lower in serum PBI than
normal young men (18 to 25 years of age).

4. In normal men a small but significant, posi-
tive correlation \( r = +0.113 \) exists between the
serum PBI and the basal oxygen consumption.
Differences in the basal metabolic rate of normal
men are dependent largely on factors other than the
circulating PBI.

5. In normal middle-aged men (45 to 56 years
of age) a positive, linear correlation \( r = +0.163 \)
exists between the serum PBI and the serum total
cholesterol concentration; no such relation ob-
tains in normal young men (18 to 25 years of
age). For both young and middle-aged normal
men studied collectively, a curvilinear relation
exists between the serum PBI and the serum
cholesterol.

6. In normal young men and in normal middle-
aged men, no relationship exists between the se-
rum PBI and the relative obesity of the individual.

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