Oxygen Consumption of Erythrocytes from Patients with Various Thyroid Conditions Related to Their Respective Serum Protein-Bound Iodine Concentrations

By Luis Angelone, David H. Watkins, and Clifford A. Angerer

It has been demonstrated for non-nucleated red cells that their respiratory metabolism is comparable to that of other normal resting tissues; that adrenalectomy causes about 73 per cent decreases in their oxygen uptake with a return to normal following appropriate therapy; that aging in vitro (storage) produces a decrease in their metabolism; that roentgen rays, within the limits of the dosages employed in vitro, give an increase in their oxygen consumption; and that the administration of whole adrenal cortical extract prior to x-irradiation appears to "protect" against, or to antagonize, the induced increase in their metabolism.

Although respiratory studies on variations in the concentration of thyroid hormone in vivo have been made on nucleated erythrocytes, e.g., whole blood of alligators and red cells of ducks, to our knowledge no experimental data exist to show the effect of variations in thyroid hormone concentration on the respiration of non-nucleated red cells of any animal. This study was undertaken, first, to determine the effect of various thyroid hormone concentrations on the respiratory metabolism of non-nucleated erythrocytes, and second, to attempt a quantitative correlation of the respiratory metabolism of these erythrocytes in terms of known concentrations of circulating thyroid hormone, as expressed by their respective serum protein-bound iodine (PBI) values.

Methods

Patients of both sexes from the Ohio State University Hospital and the Columbus State School were divided into four groups as follows: (A) patients with no indication of thyroid disturbances (euthyroid), (B) untreated hyperthyroid patients, (C) hyperthyroid patients treated with ten drops of Lugol's solution three times daily for fourteen to twenty-one days (not the same patients as used in group (B), and (D) untreated hypothyroid patients. The clinical determination of these four groups was based primarily, but not conclusively, on their serum PBI concentrations which tend to correlate with determinations of basal metabolic rates and with clinical evidences of the state of thyroid function. The serum PBI was determined as described by Connor, Curtis and Swenson, and a range of 3.7 to 6.7 μg. per cent was considered normal, i.e., euthyroid.

With heparin as an anticoagulant, 30 to 50 cc. of blood were withdrawn intravenously from each patient. No attempt was made to place the patient under basal conditions before taking a blood sample. The red cells were prepared in Krebs-phosphate-Ringer solution (50 per cent suspension) for respiratory studies according to the technic previously described. Two and one-half cc. samples of a given red cell suspension were placed into three to six respirometers for study at 37.5 C. The QO₂ (cu. mm. O₂ consumed per hour per milligram of dry wt.) of each red cell suspension was based on the mean value of the number of
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Respilometers used to represent it at any given time. All respilometers were oscillated at a frequency of 80 cycles per minute and at an amplitude of 3.9 cm.

The respiration of normal red cell suspensions was studied by the Fenn technic immediately after preparation and by the Warburg technic after overnight refrigeration (2 to 8 C.). In agreement with other workers,3, 10 no statistically significant difference between mean Qo2 values was noted, whether determinations were made either immediately after withdrawal of blood or after storage for 24 hours in vitro. Eventually the Warburg technic was used exclusively for the study not only of normal red cells but of red cells obtained from the various thyroid groups.

RESULTS

The data are summarized in table 1. Here are shown the mean values with their standard deviations (S.D.) and standard errors (S.E.) for the oxygen consumption (Qo2) and the serum PBI values of the respective red cell suspensions which were prepared from the various thyroid groups. When the mean Qo2 values are compared with the euthyroid (A) group, the untreated hyperthyroid (B) groups shows a 92 per cent increase (P < 0.04), which is considered statistically significant; whereas the iodine-treated hyperthyroid (C) and hypothyroid (D) groups show a 23 per cent increase (P < 0.50) and a 15 per cent decrease (P < 0.20) respectively, which are not statistically significant.

In figure 1 the Qo2 values of the various red cell suspensions from the indicated thyroid groups are plotted relative to the patients' respective serum PBI values in µg per cent. Fitting the best probable curve to these data, a linear relation is suggested when the natural logarithms of the various Qo2 values are related to their respective serum PBI values. Calling x the PBI and y the red cell Qo2 measurements respectively, the data are fitted by a curve of the form y = ae^bx.

<table>
<thead>
<tr>
<th>TABLE 1.—Summary of the Erythrocyte and QO2 and of the Respective Serum PBI Values for the Various Thyroid-Patient Groups Indicated</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean QO2 values (µL/100 g Hb)</td>
<td>0.013</td>
<td>0.025</td>
<td>0.016</td>
<td>0.011</td>
</tr>
<tr>
<td>% difference</td>
<td>-</td>
<td>+92</td>
<td>+23</td>
<td>-15</td>
</tr>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>S.D. (±)</td>
<td>0.003</td>
<td>0.014</td>
<td>0.012</td>
<td>0.031</td>
</tr>
<tr>
<td>S.E. (±)</td>
<td>0.001</td>
<td>0.005</td>
<td>0.004</td>
<td>0.010</td>
</tr>
<tr>
<td>P-value</td>
<td>0.04</td>
<td>0.50</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Mean PBI values (µg, %)</td>
<td>4.4</td>
<td>8.4</td>
<td>†</td>
<td>1.2</td>
</tr>
<tr>
<td>% difference</td>
<td>-</td>
<td>+91</td>
<td>†</td>
<td>-73</td>
</tr>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>5</td>
<td>†</td>
<td>10</td>
</tr>
<tr>
<td>S.D. (±)§</td>
<td>0.781</td>
<td>3.603</td>
<td>†</td>
<td>0.833</td>
</tr>
<tr>
<td>S.E. (±)§</td>
<td>0.225</td>
<td>1.606</td>
<td>†</td>
<td>0.263</td>
</tr>
<tr>
<td>P-value§</td>
<td>-</td>
<td>0.05</td>
<td>†</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* A = euthyroid, B = untreated hyperthyroid, C = iodine-treated hyperthyroid and D = untreated hypothyroid patients.
† Three to six respirometric determinations per patient.
‡ Serum PBI determinations for iodine-treated hyperthyroid patients are without meaning.
§ S.D. = standard deviation and S.E. = standard error of the indicated mean. P (probability) value is obtained from t-table, where t is the ratio of the difference between samples means to the estimated standard error of this difference.
Fig. 1.—Oxygen consumption \((Q_0)\) of erythrocyte suspensions plotted relative to their respective serum protein-bound iodine values \((\mu g. \text{ per cent})\) of various thyroid patients. The patient groups are identified as follows: \(\bigcirc\) = euthyroid, \(\bullet\) = hypothyroid, \(\bigtriangledown\) = hyperthyroid, and \(\rightarrow\) indicates the mean \(Q_0\) value (ordinate) for the iodine-treated hyperthyroid group. Of course in the last group a mean serum PBI value (abscissa) is without meaning. pH 7.4; temperature 37.5°C.

where \(a\) and \(b\) are constants to be determined and \(c\) is the base of the natural logarithms. This problem was done by fitting a straight line to the logarithms of the respective \(Q_0\) measurements by the method of least squares. The standard error of \(y\) about this curve is 6.4.* The resulting curve was then translated to its exponential form (fig. 1) where \(Q_0\) values of the red cell suspension are related to their corresponding serum PBI values in \(\mu g. \text{ per cent}\).

**DISCUSSION**

From previous observations,\(^1\text{–}^3, 9, 11, 12\) it appears that the respiration of erythrocyte suspensions is due primarily to intact cells. The negligible decrease, reported here, in oxygen consumption of non-nucleated erythrocytes after overnight cold storage has been observed by other investigators for nucleated eryth-

* We wish to thank Prof. D. Ranson Whitney, Statistical Laboratory, The Ohio State University, for his ready cooperation in fitting a curve to the data under consideration.
Because of the great variability of expressing (standardizing) values for the oxygen consumption of red cells and of preparing and concentrating red cells for respiratory studies, an absolutely quantitative comparison of these results for non-nucleated erythrocytes with those found in the literature is practically impossible. However, Ponder in a review of the subject, attempted to recalculate, and thus to standardize, the values for oxygen consumption of human erythrocytes as found in four earlier reports in terms of \( Q_{O_2} \). After making certain assumptions, these values were found to be 0.015, 0.017, 0.042 and 0.06 \( (p. 358) \). Our \( Q_{O_2} \) value of 0.013 for euthyroid patients compares favorably with the former two.

It has been adequately demonstrated that animals made either hyperthyroid or hypothyroid show an increase or decrease, respectively, in the oxygen consumption of various tissues, e.g., muscle, nerve, kidney, and liver. The erythrocytes from hyperthyroid patients show a similar significant increase (92 per cent) in respiration, whereas those from hypothyroid patients show only a tendency toward a decrease (15 per cent) in mean \( Q_{O_2} \). This latter value is not statistically significant \( (P < 0.20) \) when compared with its control (group A).

The cause of this lack of significance is explainable on the basis that this portion of the \( Q_{O_2} \)-PBI curve (fig. 1) is approaching the abscissa asymptotically. It is interesting that in this hyperthyroid (D) group of 11 patients, 5 (3 cretins) gave \( Q_{O_2} \) values comparable to the mean euthyroid value and the other 6 (3 cretins) gave values which were approximately one half that of the mean for the euthyroid group. This tends to suggest that perhaps there are two levels of activity in the so-called hypothyroid patients or, more probably, that some factor other than the thyroid is critical, e.g., an imbalance in adrenocortical function.

However, the serum PBI values in all cases, except one, were 1.6 \( \mu \)g. per cent or less; the normal values range from 3.7 to 6.7 \( \mu \)g. per cent.

Assuming that the results on the hyperthyroid (B) group are due to the amount of circulation thyroid hormone, then treatment of the hyperthyroid (C) group with iodine (Lugol’s) solution, which is known to depress thyroid hormone formation, might depress the \( Q_{O_2} \) values for erythrocytes from the latter group. It is apparent from the data that this occurs. Since iodine administration has no apparent effect on the action of the circulating thyroid hormone nor on the respiratory metabolism of isolated tissues from the rat, when added directly to the tissues in vitro, it is unlikely that the effect on the respiration of red cells of the iodine treated group is due to iodine per se. It has been reported for dogs that small daily doses of thyroxin cause variable results in the oxygen consumption of skeletal muscles from animal to animal, whereas larger doses and longer treatment result in more uniform results. Thus, the large standard deviations of the mean \( Q_{O_2} \) values in the four groups \( A = 0.003, B = 0.014, C = 0.012, D = 0.031 \) of patients with various thyroid disturbances may be due to the different functional states of the thyroid glands in question. This appears to be borne out by the large standard deviation of the mean serum PBI values for the same groups \( (0.781, 3.603, \ldots , 0.833, \) respectively).

Direct involvement of the thyroid hormone in an enzyme system has not as yet been demonstrated. Since the non-nucleated erythrocyte may utilize glu-
cose and lactic acid, apparently in conjunction with enzymes for a glycolytic system and possibly involving an active transfer of glucose across the cell membrane, it may be advantageous to use this cell for determining the nature of the action of the thyroid hormone on a cellular basis.

**Summary**

Blood was obtained from patients who were divided into four groups: (A) euthyroid, (B) untreated hyperthyroid, (C) iodine-treated hyperthyroid and (D) hypothyroid. The erythrocytes from each patient group were washed and resuspended in Krebs-phosphate solution for study by the Warburg respirometric technic. When the erythrocytes obtained from patient groups B, C and D are compared with group A (control), a +92 per cent, +23 per cent and −15 per cent change is noted, respectively, in their mean $Q_o$ values when $Q_o$ values for erythrocytes for each patient in each patient group (excluding group C) are plotted relative to their respective serum PBI concentration (µg. per cent), a curve of exponential order appears to be the curve of best probable fit for these data. The mean $Q_o$ value for the iodine-treated hyperthyroid group falls on the ordinate of this curve (fig. 1) between the euthyroid and untreated hyperthyroid groups, though the mean serum PBI value (abscissa), of course, has no meaning for this group.

**Summario in Interlingua**

Iste studio esseva interprendite (1) pro determinar le effecto de varie concentrationes de hormon thyroide super le metabolismo respiratori de erythrocytos non-nucleate e (2) pro establir in tanto que possibile un correlation quantitative inter iste metabolismo e le varie concentrationes del circulante hormon thyroide que se exprime in le correspondente valores de iodo intraproteinic del sero.

Nos obteneva specimens de sanguine ab quatro series de patientes classificabile como (a) euthyroide, (B) hyperthyroide sin tractamento, (C) hyperthyroide tractate a iodo, e (D) hypothyroide. Le erythrocytos ab cata serie de patientes esseva abluite e re-suspendite in un solution phosphatic Krebs in preparation pro studios secundo le technica respirometric de Warburg. Le datos obtenite in iste studios indicava que le valores median del consumption de $O_2$ per le erythrocytos differeva inter serie (A)—i.e. le serie de controlo—e le series (B), (C), e (D) per +92, +23, e −15 pro cento respectivemente. Solo le prime inter iste valores esseva considerate como statisticamente significative. Le valores del consumption de $O_2$ per le erythrocytos del patientes individual in cata un del series (A), (B), e (D) esseva inscribile in un sistema de coordinatas in relation al correspondente valores de iodo intraproteinic del sero. Ab isto il appare que le datos es le melio representate per un curva de ordine exponential. Le valor median del consumption de $O_2$ per le erythrocytos in le serie (C) se trova super le ordinata de iste curva inter le series (A) e (B), ben que in iste caso le valor median del iodo intraproteinic ha naturalmente nulle signification.

**REFERENCES**

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5 Unpublished Data.


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