PREPARATION OF RADIOACTIVE IODOCASEIN*

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We have recently prepared iodocasein containing radioactive iodine (I^{131}) for use in studies, to be described in detail elsewhere, on the metabolism of calorigenic substances in human beings. The procedure followed was, in general, that of Reineke and Turner (1, 2) except for modifications necessitated by the incorporation and handling of I^{131}. These modifications shorten the procedure somewhat and aid in obtaining conveniently a product having sufficient specific activity and biologic potency. It is the purpose of the present paper to describe the preparation and properties of the radioactive iodocasein so obtained.¹

Methods

Chemical Analyses. Tyrosine—After hydrolysis of the protein according to the method of Bhagvat and Sreeramamurthy (4), tyrosine was determined by the method of Folin and Marenzi (5).

Iodine—The method of Shahrokh (6) was used with the following changes: (1) In lieu of the transfers originally described, the entire procedure was carried out in a 100 ml. Kjeldahl flask marked at a 15 ml. level. (2) Phenol, which is added just prior to titration, was omitted, since its use led to lower iodine values. (3) Glass beads were substituted for pumice, leading to a clearer and sharper end-point.

Thyroxine—Hydrolysis with barium hydroxide and distribution of thyroxine in butanol was effected according to the procedure of Roche and Michel (7). The purified butanol extract was analyzed for iodine, as was done by Reineke and coworkers (8) in their studies of thyroxine in iodinated casein, and the resulting value was converted to thyroxine by means of the factor 1.529.

Biologic Analysis—The methods of Deanesly and Parkes (9) and of Hamilton, Albert, and Power (10) were employed.

Radiologic Analyses—0.2 ml. aliquots of solutions containing radioactive iodine were dried on copper planchettes with 0.1 ml. of silver nitrate

¹ After completion of our work, Frieden, Lipsett, and Winzel (3) also reported the use of the method of Reineke and Turner (1, 2) for the preparation of radiiodo- casein.
containing 1 mg. of silver per ml., and counted with a β-ray Geiger counter in connection with a scaling circuit (Autoscaler, Tracerlab, Inc., Boston, Massachusetts). Counts were corrected for decay and self-absorption when necessary, and expressed usually in terms of per cent of radioactivity originally involved in a given experiment or operation, by means of comparison with a simultaneously counted pilot of the sample of I\(^{131}\) used.

Reagents—Borden's Labco (vitamin-free) casein containing 4.9 per cent tyrosine was used. I\(^{131}\) was obtained from the Clinton Laboratories, Oak Ridge, Tennessee, in carrier-free solution. The solution was adjusted to pH 8.0, calcium chloride was added to precipitate oxalate, sodium iodide was added as carrier, and the solution was diluted so that 1 ml. contained 5 γ of sodium iodide and 500 microcuries of I\(^{131}\). Other reagents used were

### Table I

**Summary of Six Preparations of Radioactive Iodocasein**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Amounts used</th>
<th>Analyses of product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I(^{131})</td>
<td>Casein</td>
</tr>
<tr>
<td>I</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>J</td>
<td>186</td>
<td>1</td>
</tr>
<tr>
<td>K</td>
<td>260</td>
<td>1</td>
</tr>
<tr>
<td>L</td>
<td>150</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>6000</td>
<td>0.5</td>
</tr>
<tr>
<td>N</td>
<td>6000</td>
<td>0.5</td>
</tr>
<tr>
<td>Average...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sodium bicarbonate, 10 per cent nitric acid, 10 per cent sodium nitrite, 0.2 M acetate buffer at pH 4.6, aldehyde-free ethyl alcohol, and colloidal manganese oxides prepared according to the method of Friedemann and Kendall (11).

### EXPERIMENTAL

Sufficient sodium iodide to provide 4 atoms of iodine per mole of tyrosine plus an excess of 30 per cent to allow for loss in the liberation of iodine was dissolved in 5 ml. of water containing the desired amount of I\(^{131}\) (varying from 100 to 6000 microcuries). The solution was placed in a 50 ml. iced centrifuge tube shielded by lead bricks. Iodine was liberated in the usual manner by nitric acid and sodium nitrite. After centrifugation in
the cold, the iodine crystals were washed twice with 5 ml. of ice-cold water. To the elementary iodine was added a 2.5 per cent solution of casein in 1 per cent sodium bicarbonate containing 10 ml. of colloidal oxides of manganese per gm. of casein. The mixture was agitated in a cradle rocker at 70° for 18 to 20 hours. After incubation, the mixture was adjusted to pH 4.6 with hydrochloric acid and centrifuged. The precipitate was washed twice with acetate buffer at pH 4.6 and once with alcohol, and was dried. The entire procedure was carried out within 24 hours.

**Table II**

*Characteristics and Comparison of Iodocaseins with Desiccated Thyroid*

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Total organic I'(^{131}) per cent</th>
<th>Thyroxine</th>
<th>Per cent thyroxine I'(^{131}) of total I'(^{131})</th>
<th>Average biologic activity, (\text{m.e.d.}^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodocasein (Protamone)†</td>
<td>5.8</td>
<td>3.3</td>
<td>36</td>
<td>0.4</td>
</tr>
<tr>
<td>‡ (Preparation C)‡</td>
<td>6.2</td>
<td>2.5</td>
<td>26</td>
<td>0.5</td>
</tr>
<tr>
<td>‡ (&quot; M)§</td>
<td>5.0</td>
<td>3.0</td>
<td>38</td>
<td>0.2</td>
</tr>
<tr>
<td>‡ (&quot; N)§</td>
<td>6.3</td>
<td>3.3</td>
<td>35</td>
<td>0.3</td>
</tr>
<tr>
<td>Desiccated thyroid (strong)‖</td>
<td>1.0</td>
<td>0.4</td>
<td>27</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Median effective dose.
† Cerophyl Laboratories, Inc., Kansas City, Missouri.
‡ Non-radioactive, prepared as in the text.
§ Radioactive, cf. Table I.
‖ Parke, Davis and Company, Detroit, Michigan.

**Results**

Six preparations were made. The pertinent data are shown in Table I. Losses of radioactivity were determined at each step in the procedure. The over-all recovery of I'\(^{131}\) in the final dried product averaged 30 per cent. The incubation mixture contained 70 to 85 per cent of the original activity, indicating a 15 to 30 per cent loss during the preparation of free iodine, its addition to the casein solution, and the period of incubation at 70°. Only a small portion of this loss, however, was found by direct estimation. The combined supernatant and buffer washes of the isoelectric precipitate accounted for a loss of 46 per cent and the alcohol wash accounted for a loss of 1.5 per cent. One batch was prepared by the original method of Reineke and Turner (2) which utilizes prolonged dialysis of the incubation mixture. The yield of I'\(^{131}\) was not different from that described, indicating that the isoelectric procedure is as effective in removing occluded iodide as the dialysis method and has the advantage of consuming less time.
The average content of total organic iodine of the iodocasein was 5.5 per cent and the thyroxine content was 2.7 per cent. The proportion of thyroxine $^{131}$I to total $^{127}$I was 33 per cent, with which the radioactive analyses agreed well (37 per cent of the total $^{131}$I was thyroxine $^{131}$I). The specific activity of the compound, of course, varied with the amount of both $^{131}$I and casein used, and can be, therefore, altered to suit the particular purpose in mind. For studies on human subjects a physiologic dose of iodocasein was calculated to be 60 mg. per day on the basis of equivalence of physiologic action as compared with desiccated thyroid. Since 100 to 200 microcuries at the time of administration in man can be followed conveniently by present counting equipment, a preparation containing 2 to 3 microcuries per mg. was considered optimal for human subjects. This can be readily achieved in any reasonable amount with 12 millicuries of $^{131}$I per gm. of casein.

A comparison of the properties of two preparations of radioiodocasein of highest specific activity, a single non-radioactive preparation, a commercial brand (Protamone) prepared according to the method of Reineke and Turner (2), and desiccated thyroid is given in Table II. The total iodine and thyroxine of all iodocaseins are similar, as is their biologic activity. Desiccated thyroid, however, while containing about the same ratio of thyroxine iodine but much less total iodine, was found to be slightly less than half as active as the artificial iodoproteins.

**SUMMARY**

A procedure for the preparation of radioactive iodocasein of sufficient radioactivity and biologic activity for use in physiologic amounts in human metabolic studies is described.

**BIBLIOGRAPHY**

PREPARATION OF RADIOACTIVE IODOCASEIN
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