Measurement of Iodine Concentration in Biological Material

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Iodine has been estimated in biological material by an automated combustion procedure. Advantages of the system include quick preparation of multiple samples and use of wet samples without preliminary drying.

Additional Keyphrases  AutoAnalyzer  • radioiodine  • food, tissue, and fecal iodine

Determination of iodine in biological material presents technical problems because of the large amounts of organic material present and because the concentration of iodine is often low. Many workers have used a chloric acid-digestion procedure (1), but this is tedious, and Richmond (2) found it less satisfactory for accurate measurement of fecal iodine than for determining iodine in serum or urine. Significant errors may arise from under- or overdigestion. Combustion of feces is a more convenient way of liberating iodine from organic material, and seems to be a satisfactory method for accurately determining iodine both in feces and in foodstuffs (3–5). Sodium hydroxide may be used to recover the liberated iodine (5, 6). Introduction of the Model 300 Tri-Carb Sample Oxidizer (Packard Instruments Co., Downer's Grove, Ill. 60515), designed for the preparation of samples containing tritium for liquid scintillation counting, which made use of a new automated combustion procedure (7) led us to investigate whether this equipment could conveniently be used to prepare biological samples for iodine estimation.

Methods

Samples of “Rabbit Chow” (Ralston Purina of Canada, Ltd.) and of rat thyroid glands weighing 6 to 21 mg were wrapped without previous drying in Whatman No. 1 filter paper. In order that recovery of iodine could be calculated for each sample, 0.2 μCi of ¹³¹I in 10 μl of de-ionized water was first added to each filter paper and the paper dried. Before combustion of the sample, radioactivity was determined in a well-type scintillation counter. The sample was then placed in the electrically heated platinum combustion basket in the preheated combustion chamber of the Tri-Carb sample oxidizer, and combusted. Combustion rate was controlled by the rate of oxygen flow through the chamber. Water and other products of combustion, having passed through a cooling chamber, were collected in a glass vial. After combustion was complete an automatic cycle was initiated and a preselected volume of 0.1N NaOH was automatically dispensed through the cooling system into the vial. The time required for the cycle was 30 s plus the time required to burn the sample. The volume of NaOH used ranged from 2 to 10 ml, depending on the expected iodide concentration of the sample. The precise volume in each vial was measured. To avoid any “memory” effect, three or four blanks were run between each sample. Twenty samples could be processed per hour in this way. Radioactivity in the vial was

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Received Aug. 17, 1970; accepted July 14, 1971.
determined and recovery of $^{131}$I was calculated for each sample. Iodine was estimated by use of the AutoAnalyzer (8) (Technicon Instruments Corp., Tarrytown, N.Y. 10591), and the iodine concentration was calculated as follows:

$$I \text{ per sample (µg/g)} = \frac{I \text{ per vial (µg/100 ml) \times vol in vial (ml)}}{\text{Recovery } ^{131}\text{I} (\%) \times \text{wt sample (g)}}$$

**Results**

**Recovery of $^{131}$I**

In 100 samples combusted, recovery ranged from 30% to 64%, with a mean of 46%. To determine whether recovery of $^{131}$I as Na$^{131}$I added to the wrapping paper reflected the actual recovery of iodine from the sample, we injected six rats with 10 µCi of $^{131}$I. Eighteen hours later the thyroids were excised and feces collected. The thyroid and fecal specimens were wrapped in the filter paper to which had been added 0.2 µCi of $^{131}$I. Both $^{125}$I and $^{131}$I were measured before the samples were combusted. Recoveries of $^{131}$I ranged from 10.2% to 75.4%, probably related to variations in the rate of combustion. The recovery of $^{131}$I applied to the filter paper correlated well with the recovery of $^{131}$I from the endogenously labeled thyroid glands and feces ($P < 0.001$) (Figure 1).

Thus, recovery of inorganic $^{131}$I added to the wrapping paper was a measure of recovery of organic iodine compounds from the sample.

**Form of Recovered Iodine**

To determine the form of the recovered iodine in the vial, we injected 20 µCi of $^{131}$I into a rat and 18 h later the thyroid was excised and combusted. No $^{131}$I was added to the wrapping paper in this case. The material recovered in the vial was chromatographed. The chromatographic systems used were ascending paper chromatography in n-butanol:pyridine:1.5N NH$_4$OH (2:1:2, by vol) and in n-butanol saturated with 1.5N NH$_4$OH (9). Only iodide was demonstrated, no iodate or organic iodine compounds. Similar results were obtained when $^{131}$I-labeled thyroxine was combusted.

**Recovery of Added Iodide**

Six samples of Purina Rabbit Chow—to which had been added 0.238, 0.238, 0.594, 0.594, 1.188, and 1.188 µg of iodide as potassium iodide—were combusted. Recovery of $^{131}$I ranged from 30% to 40%. There was good agreement (Figure 2) between the iodide concentration measured in the vial sample and the iodide concentration predicted from knowledge of the iodide content of the diet, the amount of stable iodine added, and the percentage recovery of $^{131}$I ($P < 0.001$).

**Reproducibility of Estimations**

Five samples Rabbit Purina Chow were analyzed to test the reproducibility of the method. The results, Table 1, show agreement.

CLINICAL CHEMISTRY, Vol. 17, No. 10, 1971 1021
Table 1. Iodine Estimated in Purina Rabbit Chow

<table>
<thead>
<tr>
<th>Diet</th>
<th>Wt, mg</th>
<th>Vol in vial, ml</th>
<th>I, μg/100 ml</th>
<th>131I Recovery, %</th>
<th>I, μg/g diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>515</td>
<td>3.5</td>
<td>5.7</td>
<td>53.4</td>
<td>0.64</td>
</tr>
<tr>
<td>2</td>
<td>535</td>
<td>3.5</td>
<td>3.1</td>
<td>34.9</td>
<td>0.58</td>
</tr>
<tr>
<td>3</td>
<td>583</td>
<td>3.5</td>
<td>3.0</td>
<td>30.8</td>
<td>0.58</td>
</tr>
<tr>
<td>4</td>
<td>587</td>
<td>3.4</td>
<td>4.2</td>
<td>40.7</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>580</td>
<td>3.7</td>
<td>3.4</td>
<td>38.6</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Mean 0.593
SE 0.015

Influence of Oxygen Flow on Recovery of 131I

To determine whether variation in the flow of oxygen during combustion produced changes in iodine recovery, we wrapped wads of filter paper weighing 470–480 mg in Whatman No. 1 filter paper to which 131I had been added and combusted them at six flow rates from 0.5 liters/min to 4 liters/min. At a flow rate of 0.5 liters/min, combustion was incomplete. The data for flow rates greater than 1 liter/min are shown in Table 2. At 1 liter/min, 131I recovery was 27%, rising linearly to 78% at a flow rate of 3 liters/min; with further increase in oxygen flow, recovery decreased to 45%.

Discussion

Measurement of the concentration of iodine in biological material—such as diet, feces, and thyroid—is of practical importance. The iodine content of the diet, for example, is reflected not only in the incidence of iodine-deficiency goiter but also in the range of thyroid radioiodine uptake measurements for the population. The technique described above for the preparation of biological samples for iodide estimation has inherent advantages. It combines the advantages of a closed combustion system with automation. When used in conjunction with an AutoAnalyzer for iodine determinations, large numbers of samples can be processed and results can be available the same day. The fact that samples need not be previously dried is convenient and saves considerable time. The volume of NaOH used and the weight of the sample can be adjusted to suit the expected iodide concentration of the sample; e.g., where the iodide concentration is high a larger volume of NaOH and a smaller weight of sample can be used.

A disadvantage of the technique is the variable recovery between samples. It is necessary therefore to measure and correct for the recovery for each sample by adding 131I. This can most conveniently be added to the wrapping paper, and the recovery of inorganic iodide from the paper is a measure of the recovery of organic and inorganic iodine compounds from the sample. Optimal oxygen flow rate appears to be approximately 3 liters/min.

We are grateful to Mr. J. Macaulay of the Packard Instrument Company and Miss Anne Williamson for technical assistance, and to Mrs. Irma Reicholds for the iodine estimations.

This work was supported by grants from the Medical Research Council of Canada (MA-3723 and MT-584), from the USPHS (AMO-4121-10), and from the Wellcome Trust.

References


Table 2. Effect of Oxygen Flow Rate on Recovery of 131I from Sample

<table>
<thead>
<tr>
<th>Oxygen flow, liters/min</th>
<th>Before</th>
<th>After</th>
<th>Vol, ml</th>
<th>Total cpm</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>92,210</td>
<td>7,523</td>
<td>3.63</td>
<td>27,308</td>
<td>29.62</td>
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<tr>
<td>1.5</td>
<td>79,272</td>
<td>5,321</td>
<td>3.63</td>
<td>19,315</td>
<td>24.37</td>
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<tr>
<td>1.5</td>
<td>72,335</td>
<td>5,565</td>
<td>3.73</td>
<td>20,757</td>
<td>28.70</td>
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<tr>
<td>2.</td>
<td>69,521</td>
<td>6,298</td>
<td>3.52</td>
<td>22,169</td>
<td>31.89</td>
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<tr>
<td>2.5</td>
<td>74,812</td>
<td>10,982</td>
<td>3.72</td>
<td>40,853</td>
<td>54.61</td>
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<tr>
<td>2.5</td>
<td>151,158</td>
<td>22,864</td>
<td>3.73</td>
<td>85,283</td>
<td>56.42</td>
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<tr>
<td>3.</td>
<td>80,378</td>
<td>18,380</td>
<td>3.53</td>
<td>64,881</td>
<td>80.72</td>
</tr>
<tr>
<td>3.5</td>
<td>49,758</td>
<td>10,632</td>
<td>3.53</td>
<td>37,531</td>
<td>75.43</td>
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<td>4.</td>
<td>50,122</td>
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<td>3.63</td>
<td>26,651</td>
<td>53.17</td>
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<tr>
<td>4.5</td>
<td>78,891</td>
<td>8,270</td>
<td>3.61</td>
<td>29,855</td>
<td>37.84</td>
</tr>
</tbody>
</table>
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