Methods for the Determination of Iodine-131 in Fresh Milk

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Received May 18, 1964

The investigation was carried out in order to find an improved method to determine iodine-131 in fresh milk.

(1) In order to minimize iodine loss during ignition of milk, it was recommended to add an NaOH solution to the milk in ratio of milk to the 1 N NaOH solution as 1:19.

(2) A series of experiments indicates that inorganic iodine combines with fat and protein during experimental operation by Bergh's method. Since the iodine in fat-CCl4 layer is not determined, Bergh's method is thought to be unreliable.

(3) To determine inorganic iodine, a composite system of ion-exchange and CCl4 extraction was recommended in the paper. If the assumption, about 90 percent of iodine in milk exists as inorganic form, is accepted, total iodine-131 concentration could be easily calculated from the measurement of the above.

Introduction

Iodine-131, which has a half-life of 8 days, is found in relatively high abundance in the radioactive debris of nuclear weapon detonations and in nuclear accidents. Iodine-131 is directly consumed by a cow during grazing and a part of the ingested iodine is secreted in the cow's milk. It is estimated that if an infant with a thyroid gland weighing two grams is fed 1 μc of iodine-131, about 30% will reach the thyroid gland, which will result in a radiation dose of 18 rads to the gland. Although the average consumption of fresh milk is not very high in Japan, about 0.05 l/day per person, we must bear in mind the effects of iodine-131 in milk on the bottle fed infant and children fond of milk.

To enhance biological, biochemical and dietary studies on iodine-131 in milk, a convenient and accurate determination method is required. Several papers have been presented on methods to determine iodine-131 in fresh milk, however, certain inaccuracies were found in these methods. Thus, experiments were conducted to find an improved method to determine iodine-131 in fresh milk.

Experiments and Results

1. Determination of total iodine in milk by ignition

Previously, to measure directly the total iodine content, the milk was ignited, however, treatment at high temperatures results in a loss of iodine for evaporation. In order to minimize iodine loss, an NaOH solution was added to the milk prior to ignition. Experiments were carried out to determine the most suitable conditions for ignition. Within the scope of our experiments, as shown in Table 1, it was found that the most suitable ratio of milk to the 1 N NaOH solution was 1:19.

2. Composite determination of organic and inorganic iodine in milk

According to the method reported by Dr. H. Bergh, as shown in Fig. 1, organic and inorganic iodine are determined separately. The yield in each step of this procedure was checked by adding iodine-131 to fresh milk. As shown in Fig. 2, about 20 percent of the iodine-131 is found in the coagulated protein layer but a large portion remains in the CCl4 layer without being reduced by a sulfur dioxide solution.
From thus, it is assumed that the iodine oxidized by NaNO₂, combines with casein and fat, since the coagulate protein and CCl₄ layers consist mainly of casein and fat, respectively.

![Flow sheet of iodine-131 measurement in fresh milk by Bergh](image)

To test this presumption, the transfer of ionic form iodine-131 to fat and casein was checked. After addition of iodine-131 to fresh milk, the fat and casein in the milk were extracted by ether and precipitated by trichloroacetic acid (TCA), respectively. However, as shown in Figs. 3 and 4, no activity was found in these fractions. This fact indicates that ionic iodine added to milk does not combine with fat and casein. Therefore, it seems that the remarkable appearance of iodine in the casein and fat layers separated by Bergh's method is caused by chemical reaction during the chemical process of his method, probably by the reaction of NaNO₂. To examine this assumption, the following experiments were carried out.

![Transfer of ionic iodine-131 to fat](image)

Note: Percentage in parentheses shows the yield of added iodine-131.

![Transfer of ionic iodine-131 to protein](image)

Note: Percentage in parentheses shows the yield of added iodine-131.

Two 50 ml samples of skimmed milk were placed in each 300 ml capacity separatory funnel. Unsaturated fatty acid (oleic acid), and a reagent solution of 1 μg of iodine-131 (NaI form), 20 mg of I carrier (NaI form), 5 g of NaNO₂ and 10 ml of concentrated HNO₃ were added to one sample and saturated fatty acid (palmitic acid) and the reagent solution described above, to the other. After mixing, each solution was transferred into a centrifugal tube and separated at 6,000 rpm. Each separated CCl₄ layer was allowed to settle for about 3 hours, transferred into test tube and counted with a scintillation gamma-ray counter. Also, 20 ml of a saturated sulfur dioxide solution was added to the above
CCl₄ layers for the reduction of oxidized iodine. After shaking vigorously, 1 ml of each water layer sample was placed in a test tube and the radioactivity was counted with the scintillation counter. As shown in Table 2, recovery of iodine in the final CCl₄ layer showed a higher percentage in the skimmed milk with oleic acid than that of the skimmed milk with palmitic acid. It shows that iodine in milk treated with NaNO₂ and HNO₃ combines with double bonds of oleic acid, which is representative of the unsaturated fatty acid of milk fat. This reaction is said to be halogenation.

Table 2 Transfer of iodine-131 (I₂ form) to oleic acid and palmitic acid

<table>
<thead>
<tr>
<th></th>
<th>Skim milk with oleic acid (%)</th>
<th>Skim milk with palmitic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water layer</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Coagulated protein</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>CCl₄ layer</td>
<td>79</td>
<td>73</td>
</tr>
<tr>
<td>After treatment of SO₂ saturated solution</td>
<td>CCl₄ layer 59</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Water layer</td>
<td>20</td>
</tr>
</tbody>
</table>

According to the experiment using proteins, it is assumed that the oxidized iodine treated with NaNO₂ and HNO₃ also combines with casein, therefore is not washed away with the reducing reagent.

The above series of experiments indicates that Bergh’s method is unreliable in determining iodine in milk in separate inorganic and organic forms. To ensure the individual forms of iodine in milk, it is recommended that the ordinary separation method of fat and protein, such as ether extraction of fat and TCA precipitation of protein as adopted by the authors should be used.

3. Determination of inorganic iodine

In order to measure inorganic iodine-131 in the whey of milk, fat and casein were removed by centrifugal separation and trichloroacetic acid precipitation, respectively. As shown in Fig. 5, removal of the fat and casein resulted in limiting the transfer of iodine to the organic:

![Fig. 5 Measurement of inorganic iodine-131 in whey by CCl₄ extraction.](image)

Note: Percentage in parentheses shows the yield of added iodine-131.

![Fig. 6 Elution curve of iodine-131.](image)

Note: resin; Dowex 1-×8 (20～50 mesh, NO₃⁻ form), column; 1×30 cm eluent; 2N NaNO₃ solution (pH₅), velocity; 6～10 ml/min, temperature; 18°C (room temperature)
compound layers. However, the above method is not so convenient in treating large quantities of milk. It is estimated that the composite system of ion-exchange and CCl₄ extraction provides a solution to this problem, thus the following experiments were carried out.

5 ml of Dowex 1×8 resin (20–50 mesh, NO₃ form) was charged in a column, 1 cm in diameter and 30 cm in length. 200 ml of milk containing 1 μc of iodine-131 (NaI form) and 10 mg of an I carrier (NaI form) was passed through the column at a rate of 6–10 ml per minute. After washing the resin with water, iodine held in the resin was eluted with 500 ml of 2 N NaNO₃. When the eluate reached each 50 ml point it was counted with the scintillation gamma ray counter. The elution curve is shown in Fig. 6. Inorganic iodine in milk is completely held in the NO₃ form Dowex 1×8 resin and easily eluted with 500 ml of 2 N NaNO₃ solution, that is accompanied by the higher concentration of iodine in the eluate, and effectively extracted with CCl₄ without formation of fat-CCl₄ layer and coagulated protein layer. Therefore, this composite analytical method is convenient for measurement of inorganic iodine in milk. Glascock⁵) has reported that in milk, about 90 percent of iodine is in the whey as inorganic iodine. His assumption indicates that total iodine concentration in milk could be calculated by the measurement of inorganic iodine.

Conclusion

If Glascock’s assumption is accepted, the total iodine-131 concentration could be calculated from the measurement of inorganic iodine-131 in milk. The composite system of ion-exchange and CCl₄ extraction is suitable for this purpose, however, Bergh’s method is unsuitable because of the transfer of inorganic iodine-131 to fat and protein during the chemical process.

Previously, milk was ignited to directly measure total iodine-131. In general, NaOH is added to milk in order to minimize the loss of iodine-131 during ignition. Within the scope of our experiment, the most suitable ratio of milk to 1 N NaOH was 1 : 19.

References

2) N. Yamagata: J. Radiation Res., 3, (1) 60 (1962)