

## Radiochemical Break-down of Iodine-131 Labelled L-Thyroxine Preparations. A Study of the Influence of Increased Specific Activity and Time of Storage

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The radiochemical stability of  $^{131}\text{I}$  labelled L-thyroxine was investigated under a given set of conditions. The study gives information on the rate of decomposition caused by increased specific activity and time of storage in samples with specific activities from around 10 mCi/mg to 100 mCi/mg, and with a storage time up to eight weeks.

It is a recognized fact that  $^{131}\text{I}$  labelled L-thyroxine ( $\text{T}_4$ ) and similar  $^{131}\text{I}$  labelled iodoamino acids undergo a radiochemical decomposition upon storage. The mechanism of this decomposition has been investigated and discussed,<sup>1-5</sup> and it seems to be accepted that two factors, among others, are definitely influencing the formation of radioactive contaminants in such solutions, *i.e.* the initial specific activity of the amino acid and the time of storage.

The importance of using only the purest  $\text{T}_4$ - $^{131}\text{I}$  preparations available for physiological studies, and especially in studies of thyroxine binding by human serum, has been emphasized recently,<sup>6,7</sup> and it is obviously important to find measures which will contribute to minimize the formation of radiochemical impurities. This study was undertaken in order to gain more information about the effect of increased specific activity and prolonged time of storage of  $\text{T}_4$ - $^{131}\text{I}$  preparations. The other variables which might influence the rate of decomposition, were kept as uniform as possible.

A number of published chromatographic methods for identification and determination of iodoamino acids were tested experimentally in order to find the most reliable systems. Some warnings appearing in the literature were taken into consideration, for instance that even freshly purified dioxan in a system leads to artefacts,<sup>8</sup> and that a spontaneous deiodination of  $\text{T}_4$ - $^{131}\text{I}$  may occur on filter paper under special conditions.<sup>9</sup>

## EXPERIMENTAL

*Preparation and purification of  $T_4$  samples.* A series of  $T_4$ - $^{131}\text{I}$  samples with various specific activities were prepared by exchange mainly by the method of Gleason.<sup>10</sup> A procedure described elsewhere<sup>11</sup> was followed. From 0.06  $\mu\text{mole}$  to 0.6  $\mu\text{mole}$  L-thyroxine (Koch-Light Laboratories Ltd.) was mixed with 6–8 mCi of  $^{131}\text{I}$  as iodide free from reducing agents, and  $\text{I}_2$  dissolved in alcohol was added in the molar ratio  $T_4 + 0.5 \text{I}_2$ . After standing for around 5 min, a small aliquot of the reaction mixture was removed and used for chromatographic determination of the labelling yield. The yield was found to be nearly constant, the mean value for ten experiments was  $58.7 \pm 1.6\%$  as  $^{131}\text{I}$  labelled  $T_4$ . The balance of activity was present mainly in the form of iodide, in some cases together with traces of iodate.

The reaction mixture was dried and dissolved in alcoholic ammonia (ethyl alcohol and 2 N ammonium hydroxide in the ratio 30:10), and transferred to a column of 18 ml gel bed (Sephadex gel G-50). The column was equipped at the bottom with a shielded end window GM tube and a collimator. The GM tube was connected to a ratemeter and a recorder which rendered a graphical record of the separation pattern. After elution with doubly distilled water two distinct fractions were collected and measured with an ionization chamber. The first fraction comprised iodide and in some cases iodate, and the second fraction contained the purified  $T_4$ , which was diluted with water and propylene glycol until the final concentration was 0.4 mCi/ml in 50% propylene glycol in water. 2.0 ml of the sample were transferred to a separate storing vial, and the remainder of the sample was used for spectrophotometric determination of  $T_4$  content.

From 18 to 32% of the total  $^{131}\text{I}$  activity was lost during the preparation and purification procedure. An essential part of this was adsorbed to the column in the form of  $T_4$ - $^{131}\text{I}$ . The column appeared to be overloaded when around 0.4  $\mu\text{mole}$   $T_4$  or more was introduced, which in turn gave rise to less perfect separation and higher adsorption to the gel.

*Storing of samples.* Precautions were taken to ensure constant storing conditions for the samples. Pyrex bottles have been found to be most suitable for storage of  $^{131}\text{I}$  labelled  $T_4$ , as this material will minimize the adsorption of labelled  $T_4$ .<sup>12</sup> We used vials of neutral glass for storage of the samples, because this type of vials is most likely to be used for storage purposes. The vials were of a uniform shape, and were washed with a laboratory detergent before use. All samples were diluted until the final concentration was 0.4 mCi/ml. 2 ml of the dilution were transferred to the vials, and the vials were stored in separate lead containers at  $-16^\circ\text{C}$ .

*Estimation of specific activities.* The specific activities of the samples were estimated from the amount of  $T_4$  and  $^{131}\text{I}$  in the reaction mixture. After completion of the reaction the labelling yield was determined with chromatography and used as a basis for the calculation. A further control of the specific activities was performed using a semi-quantitative spectrophotometric method published by Lemmon *et al.*<sup>13</sup> 3 ml of the  $T_4$  solution were mixed with 25  $\mu\text{l}$  of 0.1 N sodium hydroxide, and the optical density was determined at 326  $m\mu$  with a Beckman spectrophotometer, model DB-G. As a blank was used 50% propylene glycol in water to which was added 25  $\mu\text{l}$  0.1 N sodium hydroxide per 3 ml solution.

The molar absorptivity (molar extinction coefficient) was found to be around  $6.0 \times 10^3$ , the reported value is  $6.05 \times 10^3$ .<sup>13</sup> The method was sufficiently sensitive for a  $T_4$  content of more than around 10  $\mu\text{g}/\text{ml}$ .

Table 1 shows the specific activities for the various samples as determined by calculation and by spectrometry.

*Chromatographic analysis.* The identification and determination of radioactive decomposition products in the  $T_4$  samples were solely performed by chromatographic methods. Paper chromatography was done on Whatman paper No. 1 ascending in *tert*-amyl alcohol saturated with 2 N ammonium hydroxide<sup>10</sup> (system 1). Thin layer chromatography was performed with three different systems. The systems are: cellulose coated glass plates and *tert*-butanol-2 N ammonium hydroxide-chloroform in the ratio 376:70:60<sup>14</sup> (system 2), silica gel coated plates and *tert*-amyl alcohol-acetone-conc. ammonium hydroxide in the ratio 25:8.7<sup>15</sup> (system 3) and silica gel plates with *tert*-butanol-acetone-10 N ammonium hydroxide in the ratio 50:25:18<sup>16</sup> (system 4). Carriers of iodoamino

Table 1. Specific activity of the various samples.

Sample	Specific activities, Cmi/mg	
	Calculated	Measured
1	9.2	11.4
2	15.7	15.5
3	28.8	30.0
4	37.2	39.4
5	54.0	About 58.0
6	101.0	—

acids were used as markers, and the markers were identified by spraying with ninhydrin reagent.

Three out of four systems showed agreeable results, both quantitatively and qualitatively. The fourth system (system 2) was valuable, because it separated  $T_4$  and 3,5,3'-triiodothyronine ( $T_3$ ) more efficiently than the others.

A chemical test for the detection of iodinated contaminants, which has been published recently,<sup>17</sup> was used occasionally in an attempt to detect possible inactive decomposition products. All these tests were, however, negative.

Table 2. Decomposition of the  $T_4$ -<sup>131</sup>I samples.

Time of storage in days	Per cent of pure $T_4$ - <sup>131</sup> I in the samples					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
0	96.3	97.3	98.5	99.3		
1		96.9	98.2			
3				98.7	96.6	98.1
5	96.0				94.3	97.2
6		96.7	97.1			
10				96.9		
12	94.5				92.3	95.1
14		95.2	96.5			
17				94.9		
19					90.8	94.8
20	93.6	94.6	96.0			
24				94.8		
26	92.7	92.8	94.5		91.0	88.4—91.5
31				92.6		
33	93.0	94.0	92.6		90.4	89.2
38				92.1		88.0
40	91.5	91.9	92.8		90.2	
45				92.0		87.2
48	91.0	90.9	91.2		88.2	
51						85.7
54						87.0

Scanning of paper strips and glass plates was performed with a Packard Radiochromatogram Scanner, Model 2701. The quantitative distribution of radioactivity among the various spots was determined by means of a scaler.

The samples were analysed regularly at least once a week over a period of six to eight weeks. 25 μl of each sample were withdrawn to separate glass tubes, and an aliquot of around 5 μl was used for each chromatographic system.

RESULTS AND DISCUSSION

The rates of decomposition of the various samples are shown in Table 2, where the percentage of <sup>131</sup>I attached to the T<sub>4</sub> is given as a function of storage time. The percentage of pure T<sub>4</sub> was calculated from the total number of counts on the glass plate or paper strip and the counts from the identified T<sub>4</sub> peak. The balance of counts were present mainly as iodide with small but variable amounts of T<sub>3</sub>, iodate, and one or possibly two unidentified radiolysis products. 3,3',5'-Triiodothyronine, which has been detected in other T<sub>4</sub>-<sup>131</sup>I

Table 3. Radioactive distribution of decomposition products.

Substance	Percentage distribution
Origin	0.2—1.0
Iodide	0.5—about 12
Iodate	0 —6.5
T <sub>3</sub>	0 —2.5
Unknown substances	0 —1.8

preparations,<sup>7</sup> was not positively identified even after a long time of storage. Table 3 shows the radioactive distribution of the decomposition products; only the minimum and maximum values from all measurements are tabulated.

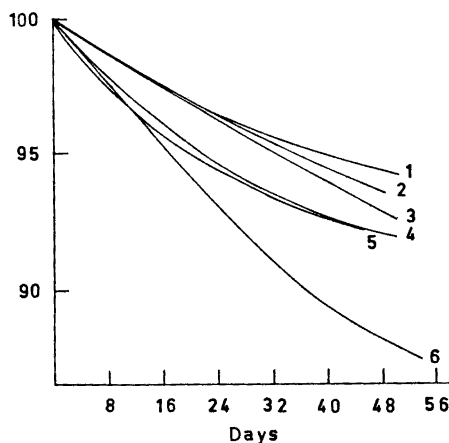


Fig. 1. Decomposition curves of T<sub>4</sub>-<sup>131</sup>I samples. The radiochemical purity at zero time is adjusted to 100 % for all the samples.

The sudden fluctuation or poor reproducibility of measured values, which appeared in sample 6 after storage for 3—4 weeks, can hardly be due to experimental errors alone. It is more likely to assume that the sample is significantly different from the others with respect to stability.

The curves from all samples are plotted together in Fig. 1. The initial radiochemical purity of all samples is adjusted to 100 %. It is seen that in general the rate of decomposition increases by increased specific activity. The curves do not have an exponential fashion, but most of them have a slight concave upward shape. In the terms of a proposed classification of decomposition effects,<sup>18</sup> it is therefore obvious that the actual decomposition in the samples is not brought about merely by primary and secondary radiation effects, but also largely by chemical effects.

In spite of the great care which was taken to ensure a uniform preparation and purification technique, the initial content of decomposition products was different in the various samples. The significance of this fact for the further decomposition progress is not clear. If the difference is caused solely by a slight variation in the purification procedure, which perhaps is the most probable explanation, the difference would not influence the further decomposition picture.

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