into a slurry of 200–300 g of finely crushed dry-ice in 200–300 ml of ether with vigorous shaking. Yield 24.6 g (86 %) of dry substance, m.p. 110–111° (95–97°) [8] (decomp.), which should be kept in a cool place.

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Small Scale Preparation of ^{131}I Labelled 3,5,3′-Tri-iodo-L-thyronine and L-Thyroxine

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Labelling of 3,5,3′-triiodo-L-thyronine (T_3) with iodine-131 has been described by Gleason,[1] who found that iodine exchanges rapidly with thyroxine as well as with triiodothyronine when the pH conditions are favourable. He also claimed that the exchange reaction is reversible, and that only the prime position, *i.e.* the *ortho* to the hydroxy group, are concerned in the exchange mechanism.

Many investigators have reported methods for the separation of T_3 in the nanogram range from other iodoamino acids and inorganic iodide. Several of these methods have been tried in this laboratory, but they were found less suitable for preparative purposes in a microgram scale.

A simple procedure has now been developed, which is useful for the preparation of 1–2 mCi ^{131}I labelled T_3 with a radiochemical purity of 96–98 % at the time of preparation. The separation is performed on a Sephadex gel column with distilled water as elution medium. Iodide and iodate ions are removed in advance by washing of the dry reaction mixture with water. The eluate from the column containing the purified T_3 is free from chemical impurities excepting traces of ammonia and ethyl alcohol. These two components may easily be removed by gentle evaporation.

The same preparation and purification procedure may be used for the production of around 2 mCi ^{131}I labelled L-thyroxine (T_4), as it is seen from Table 1. Still more

Table 1. Separation pattern from a column of Sephadex gel G-50.

Fraction	T_3 in %	T_4 in %	I^- in %	Activity in mCi
18.5–19.0 ml	82		18	0.20
19.0–19.5 »	97.6		2.4	0.73
19.5–20.0 »	98.0		2.0	0.58
20.0–20.5 »	98.4		1.6	0.64
20.5–21.0 »	99.0		1.0	0.50
21.0–21.5 »	95.0	5.0		0.46
21.5–22.5 »	35.0	65.0		0.14
22.5–23.0 »	6.0	94.0		0.50
23.0–24.0 »	2.6	97.0	0.4	0.84
24.0–25.0 »	1.0	98.0	1.0	0.56

T_4 may be obtained if an excess of I_2 is added to the reaction mixture before purification. In that case the yield of T_3 is of course accordingly less. No further studies of this effect has been performed, however, since the direct labelling of T_4, as pointed out by Gleason,[1] is considered to be more efficient.

The appearance of radioactive contaminants in stored solutions of ^{131}I labelled T_3 and T_4 has been investigated previously,[2-4] and it has been claimed that this phenomenon is mainly due to radiation decomposition.[5] It has further been suggested that the rate of decomposition is dependent on the specific activity and the radioactive concentration of the solution. On the basis of this information a series of

samples with various specific activities was prepared in order to find an acceptable compromise with regard to specific activity and the rate of decomposition. The samples were stored and analyzed regularly for four to five weeks. The results are tabulated in Table 2.

Table 2. Decomposition of T_3 samples with various specific activities.

Time of storage	Per cent impurities in the various samples				
	5 mCi/mg	17 mCi/mg	22.2 mCi/mg	26.4 mCi/mg	30.6 mCi/mg
1 days	2.0	3.9	2.1	1.8	3.0
7 »	3.4	8.2	3.4	2.9	15.4
14 »	3.3	6.6	4.5	5.0	23.7
21 »	3.0	7.5	8.2	5.7	23.2
28 »	2.8	18.0	6.2	6.5	24.0
35 »	3.0	17.0			26.5

All analytical work of the iodoamino acids has been carried out using thin layer chromatography (TLC), which has become a well established method for this purpose.[6-10] Non-radioactive carriers of T_3, T_4, and 3,5-diiodo-L-thyronine were used as markers. These compounds are easily located with a ninhydrin spraying reagent.

Experimental. 6.4 mg of 3,5,3'-triiodo-L-thyronine (Koch-Light Laboratories Ltd.) were dissolved in 2.5 ml alcoholic ammonia (ethyl alcohol and 2 N ammonium hydroxide in the ratio 30:10). 0.1 ml of this solution was mixed with about 10 mCi ^{131}I in a small glass tube. The ^{131}I solution must be free from reducing agents. 80 μl I_2 solution (5.1 mg I_2 in 100 ml alcohol) were then added. This ratio of T_3 and ^{131}I will normally give a specific activity of around 25 mCi per mg of T_3. The distribution of radioactivity in the reaction mixture, as determined with TLC, has varied between 60 and 70 % in the form of T_3 and T_4, the balance of activity was present mainly as iodide. This result agrees well with the values reported by Gleason.[1]

The T_3 is purified in two steps. The reaction mixture is first left for around 10 min after preparation, and then dried by means of an air stream. 3 ml of doubly distilled water is carefully added. The supernatant is immediately decanted off, and the washing procedure repeated once. The purified iodoamino acids are then air dried again, and finally diluted with 0.1 ml alcohol and 2 N ammonia (30:10). Inspections of the supernatant and the purified iodoamino acids have shown that approximately 25 % of the labelled iodoamino acids and more than 95 % of the total iodide and iodate are found in the supernatant.

A column of 18 g gel bed (Sephadex gel G-50 fine in doubly distilled water) is prepared. The length of the column is about 900 mm. After slow elution with doubly distilled water the various fractions are counted for radioactivity and analyzed by TLC. A typical separation pattern is seen from Table 1.

The purified T_3 fractions are diluted with water and propylene glycol until the final concentration is about 0.2 mCi per ml of 50 % propylene glycol.

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