AN IMPROVED METHOD OF LABELING THYROID HORMONES WITH RADIOIODINE

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The parameters of the isotopic exchange reaction between nonradioactive thyroxine or triiodothyronine dissolved in ethyl alcohol with radioiodine extracted in ether were investigated. The effect of the concentration of carrier iodine on the yield of labeling as well as the different factors leading to its formation are discussed. A radiochemical yield of 95% ^{131}I-thyroxine is obtained within 10–15 min. The method is very simple and favorable for very short-lived radioiodines.

Free thyroxine plays a dominant role in the regulation of thyroid function and hormonal effects (*1*).

A number of in vitro tests have been developed to estimate indirectly the free binding sites on the thyroxine-carrying plasma proteins (*2*). The current methods applied for labeling T_4 are based on exchange reaction (3–5), carried out either at room or at high temperature, in aqueous or in an organic medium, with a moderate yield and probable degradation of the original hormone.

In this report an improved method based on the exchange reaction in an organic solvent of low dielectric constant at its boiling point between nonradioactive T_4 or T_3 dissolved in ethanol and radioiodine extracted in ether is described.

MATERIALS AND METHODS

Thyroxine (T_4), the sodium salt of tetraiodothyronine, has four iodine atoms in the 3,5, 3′,5′ positions and over 61.5% iodine content which renders it a sensitive hormone. The d-1 salt was kindly obtained through Koch-Light Laboratories Ltd., England, and was purified by dissolving it in the minimum of absolute alcohol and recrystallized by adding few drops of glacial acetic acid (*6*). The purified salt is insoluble in 96% ethanol and has to be converted to the sodium salt by adding 0.2 ml of 0.12 *N* NaOH/10 mg T_4. The radioiodine can be used either extracted in situ with carrier iodine or injected separately after adding the required amount of carrier to the reaction mixture to minimize radiation hazards to the operator in the second case.

General procedure. In a stoppered separating funnel 0.5 ml KI solution (1 mg/ml) are added to 1.5 ml KIO_3 (2 mg/ml) plus 1–5 mCi $Na^{131}I$ plus 3 ml ether and then 0.3 ml of 1 *N* H_2SO_4 are gradually added. After gentle shaking for 3 min, the ethereal layer is carefully separated and added to a solution of thyroxine dissolved in ethanol (1 mg/ml).

The mixture is refluxed on a water bath. The reaction rate is followed by paper chromatography in an ascending technique using n-butanol:2 *N* acetic acid (1:1) as a solvent. The activity of the paper strips are read on an ECKO scintillation counter. After finishing the exchange reaction, the mixture is evaporated to dryness and the residue is dissolved in the minimum amount of 0.1 *N* NaOH. The free iodide is separated either by repeated precipitation with 2 *N* HCl or by gel filtration on a 1 gm Sephadex G-25 column. The pure labeled thyroxine is dissolved in 50% propylene glycol to reduce radiation damage. The results of different runs at optimum conditions are shown in Table 1.

Effect of water content on the yield of labeling. From the table we notice that 4% water in ethanol is ultimately necessary to obtain maximum yield. Higher water content lowers the yield possibly through a dissociative mechanism of I_2 (*7*). Similarly, longer or branched aliphatic chains give a lower yield than in ethanol probably due to a higher reaction temperature or poor solubility of T_4. The presence of 4% water in ethanol at pH 5–6 tends

Received June 5, 1973; revision accepted Oct. 15, 1973.

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TABLE 1. LABELING YIELD OF T_4 IN DIFFERENT ALIPHATIC ALCOHOLS

No	Alcohol (10 ml)	Starting pH	Extractant (3 ml)	Final pH	Temp. °C	% yield Time in min 5	10	15	30	60
1	Absolute ethanol	8.5	Ether	5.0	75	66	68	75	76	81
2	96% ethanol	8.9	Ether	5.3	75	92	94	95	95	95
3	80% ethanol	9.0	Ether	5.4	79	86	90	92	91	85
4	96% ethanol		Ether		25			80		
5	96% ethanol		Ether		55			89		
6	96% ethanol		$CHCl_3$					91		
7	96% ethanol		CCl_4					93		
8	n-butanol		Ether		118	60	66	77	71	71
9	tertiary butanol		Ether		82	67	67	70	64	57
10	96% tertiary butanol		Ether		82	65	64	76	65	50

to ionize the thyroxine molecule and thus promotes the exchange far faster than with the un-ionized one (*8,9*).

Effect of carrier iodine on the yield. The change in yield of labeled T_4 with carrier iodine (Fig. 1) implies that an optimum amount of carrier I_2 is necessary. Carrier-free ^{131}I giving the lowest yield of labeling may be due to adsorption phenomena. By increasing the carrier the yield increases to maximum and then decreases due to isotopic dilution effect. The maximum yield is attained at a molar concentration of 1 μM T_4 to 0.53 μM I_2 which proves that the reaction is primarily an exchange one.

Labeling T_3 in the same solvent and with the same ratio hormone: carrier (1:0.53) yielded a mixture of 78% labeled T_3 and 15% labeled T_4 (Fig. 2). This confirms that centers 3′, 5′ are the reactive ones and that diiodothyronines do not exchange at all (*3*). This may be due to the predominance of the negative inductive effect of the OH group on the 3′,5′ positions (*5*). This optimum condition is obtained experimentally by varying the concentration of KI and KIO_3 separately keeping all other factors constant (Fig. 3).

Effect of temperature. Labeling of T_4 at ordinary temperature (25°C) in this system can attain as

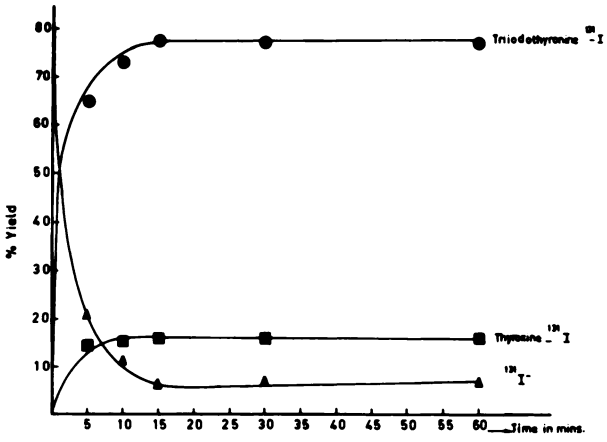

FIG. 2. Rate of exchange of T_3 in 96% ethanol-ether solvent at 75°C.

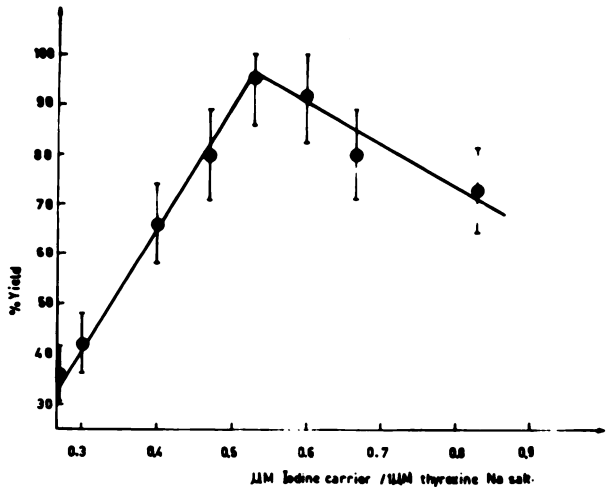

FIG. 1. Effect of concentration of carrier iodine on yield of labeling T_4 at 75°C.

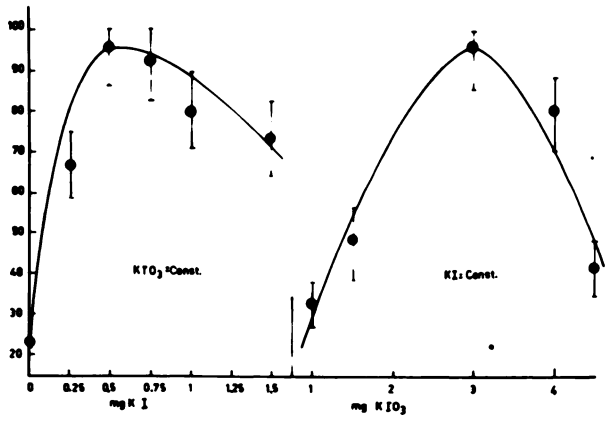

FIG. 3. Effect of KI and KIO_3 concentration on liberation of inactive I_2 in ether.

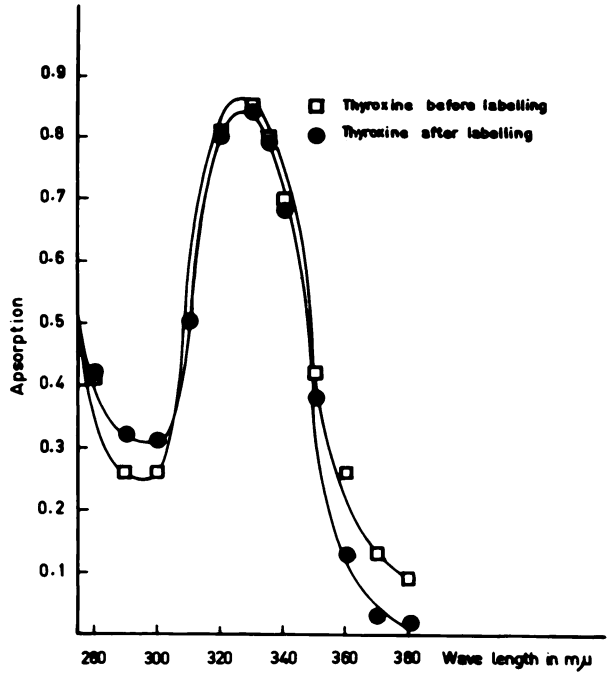

FIG. 4. Ultraviolet spectra of T_4 before and after labeling.

much as 80% within 15 min and increases to 95% at 75°C without affecting the original hormone as determined from the ultraviolet spectrum of T_4 at 330 mμ before and after labeling (Fig. 4).

DISCUSSION

On the basis of this study an isokit has been proposed for use in hospitals by which several preparations of labeled ^{131}I-thyroxine could be easily prepared.

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