value. The analysis has indicated that histidine and lysine are absent from cysteic acid-keratin. The proportions of the other amino acids can be affected by the contaminants. The findings of Schroeder & Kay (1955) that the parts of feather differ slightly in composition can, of course, reflect a variation in the composition of the monomer, but are more likely to be due to different amounts of contaminants in the different parts.

SUMMARY

1. The reaction of soluble feather keratin with 1-fluoro-2:4-dinitrobenzene and with phenyl *iso*-thiocyanate indicates that the protein contains only one-tenth equivalents of *N*-terminal amino acids/mole of protein.

2. The reaction of soluble feather keratin with carboxypeptidase and with the OH⁻ ion can be interpreted as evidence for the absence of a molar proportion of α -carboxyl groups.

3. Some of the amino acids in soluble feather keratin have been estimated quantitatively.

4. It is concluded that the feather keratin monomer is an unbranched cyclic peptide.

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The Iodine-containing Proteins of Normal and Abnormal Human Thyroid Tissue

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Derrien, Michel & Roche (1948) studied the nature of the iodine-containing proteins present in the thyroid glands of hogs, cattle and horses by a method involving the fractional precipitation of the proteins from saline extracts and the determination of the iodine and nitrogen contents of the precipitates. They used either a 3.5M mixture of equimolar mono- and di-potassium phosphate (pH 6.5) or a saturated solution of ammonium sulphate (pH 6.5) as a precipitating agent. The presence of at least five different protein components which contained iodine could be demonstrated in the crude extracts, and a method of preparing a purified thyroglobulin was developed. The preparation of thyroglobulin, however, was not a homogeneous protein, but one containing three components (Derrien *et al.* 1948; Derrien, Michel, Pedersen & Roche, 1949).

Only two investigations of the iodine-containing proteins of abnormal tissue from human thyroid

glands have so far been published. Roche, Michel, Deltour & Michel (1952) concluded on the basis of solubility and other tests that the iodine-containing protein of the thyroid glands of hyperthyroid patients did not differ significantly from that of a normal control. Trunnell & Wade (1955) compared semi-quantitatively, by paper chromatography and autoradiography, the ¹³¹I-contents of the iodo-amino acid and iodide fractions of alkali-hydrolysed normal and adenomatous thyroid tissue from each of two patients who had been given sodium iodide containing ¹³¹I. Their results indicated differences in the distribution of ¹³¹I between the various fractions from the two types of tissue. In one case no labelled thyroxine or triiodothyronine appeared on the chromatograms of the hydrolysed thyroid adenoma tissue.

The present investigation is divided into three parts. The first consists of an examination of the distribution of ¹³¹I between the various fractions obtained in the course of the preparation of purified thyroglobulin by the method of Derrien *et al.* (1948).

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The second part consists of a more detailed study of the solubility properties of the iodine-containing proteins of normal and abnormal thyroid tissue which was undertaken because of the differences in distribution of ^{131}I that were observed when the saline extracts of macroscopically normal and abnormal thyroid tissue were fractionated. The abnormal thyroid tissue used in this part of the work was subsequently shown, by histological examination, to be thyroid carcinoma tissue in two cases, while in the third it was nodular tissue with fibrous degeneration. The third part of this investigation consists of an examination of the distribution of ^{131}I between the iodo-amino acids and iodide present in tryptic hydrolysates of the thyroglobulins prepared, by the method of Derrien *et al.* (1948), from normal and abnormal thyroid tissue of one patient. The abnormal tissue was subsequently found to be carcinomatous.

MATERIALS AND METHODS

Tissue from patients selected for surgical removal of abnormal thyroid glands was used in this study. In each case the iodine-containing constituents of the thyroid gland were labelled by oral administration of a dose of carrier-free Na^{131}I , generally 48 hr. before thyroidectomy. In one case a thyroid gland and metastases of thyroid origin were removed *post mortem* from a patient with carcinoma of the thyroid who had died after receiving a dose of radio-iodine for therapeutic purposes. Details of the patients and doses are presented in Table 1.

The ^{131}I -labelled glands were dissected, as far as possible, into macroscopically normal and abnormal thyroid tissue; the weights and ^{131}I -contents (by γ -ray counts) were determined (Table 1) and samples removed for the preparation of histological sections for staining and autoradiography. The separate portions of the glands were frozen with solid CO_2 , sliced and extracted with 3 ml. of 0.9% NaCl/g. overnight in the presence of toluene ($0-4^\circ$). In accordance with the method of Derrien *et al.* (1948), the solution was adjusted to 42% saturation with $(\text{NH}_4)_2\text{SO}_4$ [by adding a saturated solution of $(\text{NH}_4)_2\text{SO}_4$] and the precipitate centrifuged down and resuspended in 45% saturated $(\text{NH}_4)_2\text{SO}_4$. After adjustment of the concentration of $(\text{NH}_4)_2\text{SO}_4$ to 37% saturation by the addition of water, the suspension was allowed to stand overnight and the insoluble residue centrifuged down. The supernatant was brought to 41% saturation with $(\text{NH}_4)_2\text{SO}_4$ and the precipitate centrifuged down. The amounts of ^{131}I in the fractions were determined by liquid counting. The same procedure was applied to the thyroid metastases from the mediastinum and lungs.

The solubility curves of the iodine-containing proteins were determined by pipetting equal volumes of the saline extracts into series of centrifuge tubes, adding calculated volumes of saturated $(\text{NH}_4)_2\text{SO}_4$ (adjusted to pH 7.01 with NH_3), stirring for 50 sec. and allowing to stand overnight at room temperature (about 20°) before centrifuging. Each precipitation was carried out in duplicate, both the supernatant and precipitated ^{131}I -activities being determined after suitable dilution either in an M6 liquid counting tube (20th Century Electronics Ltd.) or in a well-type scintillation

counter. All counts were made relative to standard solutions of known ^{131}I contents and were corrected for decay during the counting and for dead-time with the M6 tube.

The analysis of the iodo-amino acid constituents of the thyroglobulin fractions [soluble at 37%, insoluble at 42% saturation with $(\text{NH}_4)_2\text{SO}_4$; see Derrien *et al.* 1948] was carried out as described by McQuillan, Stanley & Trikojus (1954). The proteins (100 mg.) were hydrolysed in this experiment in a solution (1.8 ml.) containing crystalline trypsin (Armour) (15 mg.) and crude 'trypsin' (Armour, 1:75). The pH of the solution was adjusted to 8.4 with dilute NaOH and hydrolysis allowed to proceed in the presence of toluene at 37° for 54 hr. The hydrolysate was brought to pH 1.2 with HCl and extracted successively with 2, 2 and 1 vol. of *n*-butanol saturated with dilute HCl (pH 1.25). About 90% of the total ^{131}I was extracted into the *n*-butanol phase in each case. The butanol extracts, after addition of excess of 2N-NH_3 , were evaporated to dryness *in vacuo*, and the residues dissolved in 2N-NH_3 and chromatographed on Whatman no. 3MM paper with the solvent system (BPA) of McQuillan *et al.* (1954), which consists of *n*-butanol-*n*-pentanol- 2N-NH_3 (1:1:2 by vol.). The eluates of the zones which were detected on the chromatograms by autoradiography were re-chromatographed in the BPA solvent system or in a solvent system (PPW) consisting of *n*-pentanol-propionic acid-water (20:3:15 by vol.; the latter was used after equilibrating at least 16 hr.) to obtain further resolution. Finally, the eluates were chromatographed in the appropriate solvents with added authentic non-labelled iodo-amino acids and sodium iodide for identification by the coincidence of the radioactive and non-radioactive spots. The non-radioactive iodo-amino acids were located by the method of Bowden, MacLagan & Wilkinson (1955) as modified by Fletcher & Stanley (1955).

A labelled rabbit thyroid gland was prepared by subcutaneous injection of $25\mu\text{C}$ of carrier-free ^{131}I in the form of NaI into an albino rabbit 24 hr. before thyroidectomy.

RESULTS

The distribution of ^{131}I between the various fractions that were obtained when the method of Derrien *et al.* (1948) for the preparation of purified thyroglobulin was applied to the saline extracts of normal and abnormal thyroid tissues and thyroid metastases is shown in Fig. 1. In the same figure the distributions for a labelled rabbit thyroid gland and for two hog thyroid glands (McQuillan *et al.* 1954) are included for reference. The latter were shown by histological examination to be normal thyroid glands.

The solubilities (S) of the iodine-containing proteins of normal and abnormal portions of three thyroid glands (Mr St., Mrs Wr. and Mr Ba.) are plotted in Fig. 2*a, c, e* against concentration (C) of ammonium sulphate, expressed as a percentage of saturation. Corresponding plots of $-\Delta S/\Delta C$ against C are shown in Fig. 2*b, d, f*.

In Fig. 2 the values for S in the solubility curves represent the mean values of duplicate estimations of the solubility at each concentration of ammonium

Table 1. *Details of patients who received ^{131}I*

Name	Pre-operation dose of ^{131}I (μC)	Time of administration	Time of thyroidectomy	Macroscopically normal thyroid tissue			Macroscopically abnormal thyroid tissue		
				Wt. (g.)	Activity (μC)	Label	Histology	Wt. (g.)	Activity (μC)
Mrs Pa.	150 000	18 Aug.	Died (post-mortem samples)	—	—	—	—	18.7	—
								35.2	—
								30.5	—
Miss Sc.	100	6 Dec.	8 Dec.	8.25	8.7	Sc _N [†]	Benign adenoma	11.8	1.94
Mrs Ri.	1 000	5 Jan.	6 Jan.	14.6	426	Ri _N	Normal, active	11.8	105
Mrs Ru.	500	4 Jan.	6 Jan.	8.0	58	Ru _N	Normal, fibrous	71.7	7.2
Miss To.	2 000	22 Mar.	24 Mar.	24.5	401	To _N	Normal	127	41.4
Mr St.	900	9 May	12 May	12.2	174	St _N [‡]	Normal	12.7	4.91
				2.45	22.1	St _N [‡]	Normal and some carcinoma	15.5	57.8
				5.9	58	St _N [‡]	Normal	—	—
Mrs Wr.	500	23 June	25 June	3.50	226	Wr _N [‡]	Normal and some carcinoma	17.6	186
				4.65	170	Wr _N [‡]	Normal and some carcinoma	85.9	49.7
				—	—	—	—	34.8	238
								—	—
Mr Ba.	700	18 Sept.	19 Sept.	14.3	9.83	Ba _N [‡]	Normal	50.6	25.4
				10.1	24.2	Ba _N [‡]	Normal	18.0	26.9
				9.7	10.3	Ba _N [‡]	Normal	—	—

* *thyrr.*, thyroid carcinoma; *med.*, mediastinal metastases of thyroid; *lung*, lung metastases of thyroid.

† The subscript indicates the nature of the tissue as judged by macroscopic examination. N, normal; A, abnormal.

‡ St_N[‡]: The superscript indicates the part of the gland from which tissue was taken. L, left; R, right; I, isthmus; E, extra lobe.

sulphate. The $-\Delta S/\Delta C$ values were computed from the change in S per 1% increment of C .

The quantitative examination on paper chromatograms of the iodo-amino acids present in the thyroglobulin fraction from normal and carcinomatous portions of the thyroid gland of Miss To. indicated that 31% of the ^{131}I -activity extracted into n -butanol from the enzyme digest of normal thyroglobulin was present as 3-iodotyrosine, 34% as 3,5-diiodotyrosine and 4.4% as inorganic iodide;

Fig. 1. Histograms of distribution of ^{131}I between the fractions obtained in the course of preparation of purified thyroglobulin (Derrien *et al.* 1948). 1, Insoluble residue; 2, fraction soluble in 42% saturated ammonium sulphate; 3, fraction of residue remaining from 2, insoluble in 37% saturated ammonium sulphate; 4, fraction of solution remaining from 3, soluble in 41% saturated ammonium sulphate; 5, fraction of solution remaining from 3 insoluble in 41% saturated ammonium sulphate, i.e. thyroglobulin. (a) Metastases of thyroid carcinoma from lungs (Mrs Pa.). (b) Metastases of thyroid carcinoma from mediastinum (Mrs Pa.). (c) Thyroid carcinoma (Mrs Pa.). (d) Hog thyroid (experiment R.A. 2, McQuillan *et al.* 1954). (e) Hog thyroid (experiment R.A. 6, McQuillan *et al.* 1954). (f) Rabbit thyroid. (g) Normal thyroid tissue Sc_N (Miss Sc.). (h) Abnormal thyroid tissue Sc_A (Miss Sc.). (i) Normal thyroid tissue Ri_N (Mrs Ri.). (j) Abnormal thyroid tissue Ri_A (Mrs Ri.). (k) Normal thyroid tissue Ru_N (Mrs Ru.). (l) Abnormal thyroid tissue Ru_A (Mrs Ru.). (m) Normal thyroid tissue To_N (Miss To.). (n) Abnormal thyroid tissue To_A (Miss To.).

the corresponding values for the abnormal thyroglobulin were 48, 9.1 and 33%. About 0.45% of the activity from the normal thyroglobulin was present as thyroxine and a trace as 3:5:3'-triiodothyronine, but no thyroxine or triiodothyronine could be detected in the abnormal thyroglobulin. These values have been corrected for losses during the chromatographic procedures, but should nevertheless be regarded as approximate.

DISCUSSION

Derrien *et al.* (1948) found only slight differences in the salting-out curves of hog, beef and horse thyroglobulins. Accordingly, the pattern of iodine distribution shown by the two hog thyroid glands (Fig. 1d, e) and the rabbit thyroid gland (Fig. 1f) may be taken to be the pattern for normal mammalian thyroglobulin. The macroscopically normal tissues from Miss Sc.'s gland (Fig. 1g) and from Mrs Ru.'s gland (Fig. 1k) conform to this pattern. The patterns of iodine-distribution obtained with carcinomatous thyroid tissue (Fig. 1c), lung (Fig. 1a) and mediastinal (Fig. 1b) metastases from Mrs Pa., of carcinomatous thyroid tissue from Mrs Ri. (Fig. 1j) and of carcinomatous thyroid tissue from Mrs Ru. (Fig. 1l) are appreciably different from the normal pattern; the main difference is the higher proportion of proteins still in solution at 42% saturation with ammonium sulphate. Abnormal tissue from Miss Sc.'s gland (Fig. 1h) behaved very similarly to normal tissue from the same gland (Fig. 1g). This may be explained by the fact that histological examination showed this to be a case of benign adenoma and not a carcinoma. The reason for the abnormal pattern of iodine distribution obtained with normal (Fig. 1i) as well as carcinoma (Fig. 1j) tissue from Mrs Ri.'s gland may be the difficulty of effecting a clean separation of normal and abnormal tissue on the basis of a macroscopic examination. The difference between the normal (Fig. 1m) and abnormal (Fig. 1n) iodine-distribution patterns of Miss To.'s gland consists largely of an increase in the fraction insoluble in 37% saturated ammonium sulphate and a decrease in the thyroglobulin fraction of the abnormal tissue.

In cases of Mr St. and Mrs Wr. the solubility and $-\Delta S/\Delta C$ curves (Fig. 2) show differences in the salting-out of the iodine-containing proteins from extracts of normal and abnormal thyroid tissue, the latter proving to be carcinomatous on histological examination. The differences were slight in the case of Mr Ba., whose gland exhibited a benign, nodular degeneration.

Comparison of the $-\Delta S/\Delta C$ curves for the normal iodine-containing proteins from Mr St.'s (Fig. 2b) and Mrs Wr.'s (Fig. 2d) glands show that there are some differences in solubility properties

even between the normal thyroglobulin of different glands, whereas $-\Delta S/\Delta C$ curves for the thyroglobulins of two normal portions of the same gland are virtually identical in the case of Mr St. (Fig. 2b).

There is a possibility that the peaks of the $-\Delta S/\Delta C$ curves do not represent different proteins containing iodine, but that the precipitation of normal thyroglobulin in the presence of ammonium sulphate is modified by co-precipitation, at lower salt concentrations, with proteins of the abnormal thyroid tissue which do not contain iodine. This is believed to be unlikely for the following reasons:

Roche, Michel, Michel, Deltour & Lissitzky (1951) obtained a virtually normal solubility curve for protein containing ^{131}I when total beef plasma proteins (5.25 mg. of N/ml.) were added to the saline extract of labelled beef thyroid glands (containing 0.95 mg. of N/ml.). Roche, Michel, Michel, Deltour & Lissitzky (1950) obtained a virtually normal solubility curve for protein containing ^{131}I in extracts of the thyroid glands of dogs treated with thyrotrophic hormone, although the solubility curves obtained by measurements of protein nitrogen indicated the presence of appreciable

Fig. 2. Curves showing the change with ammonium sulphate concentration (C) of solubility (S) and of $-\Delta S/\Delta C$ for the ^{131}I -labelled proteins extracted from normal and abnormal thyroid tissues by 0.9% NaCl. Saturated ammonium sulphate, pH 7.01, was used as precipitating agent at room temperature. The open circles refer to proteins from normal tissue and the full circles to proteins from abnormal tissue. (a) S curves for the ^{131}I -proteins from tissues of Mr St. St_N^L , normal tissue of left lobe; $\text{St}_N^R + \text{St}_N^I$, normal tissue of right lobe and isthmus; St_{A1}^R , sample 1 of carcinoma tissue of right lobe; St_{A2}^R , sample 2 of same. (b) $-\Delta S/\Delta C$ curves for the tissues described under (a). (c) S curves for the ^{131}I -proteins from tissues of Mrs Wr. Wr_N^L , normal tissue of left lobe; Wr_A^L , carcinoma tissue of left lobe; Wr_{A1}^R , sample 1 of carcinoma tissue of right lobe; Wr_{A2}^R , sample 2 of same. (d) $-\Delta S/\Delta C$ curves for the tissues described under (c). (e) S curves for the ^{131}I -proteins from tissues of Mr Ba. Ba_N^L , normal tissue of left lobe; Ba_A^L , abnormal tissue of left lobe; Ba_N^E , normal tissue of extra lobe. (f) $-\Delta S/\Delta C$ curves for the tissues described under (e).

amounts of proteins other than thyroglobulin. On the other hand, the solubility curve for protein containing ^{131}I in extracts of the thyroid glands of dogs treated with propylthiouracil suggested the presence of abnormal proteins that contained iodine. These results may be more readily visualized by inspecting the $-\Delta S/\Delta C$ curves (Roche *et al.* 1951). Unfortunately no $-\Delta S/\Delta C$ curves for the salting-out of the iodine-containing proteins of extracts of normal and hyperactive human thyroid glands are available, although Roche *et al.* (1952) concluded from the S curves that the ^{131}I -proteins of the two groups were identical. The differences in shape and in the position of the main peaks between the $-\Delta S/\Delta C$ curves for $\text{St}_N^R + \text{St}_N^I$ and St_N^L , on the one hand, and St_{A1}^R and St_{A2}^R (Fig. 2*b*), on the other, make it probable that there is a change in the nature of the iodine-containing proteins produced in carcinomatous thyroid tissue. This is confirmed by the curves for Wr_N^L and Wr_{A1}^R , Wr_{A2}^R and Wr_A^L (Fig. 2*d*). The difference is less marked in the case of Ba_N^L , Ba_A^L and Ba_N^E (Fig. 2*f*), possibly because the abnormality of the gland was not due to carcinoma.

The interpretation of the results of the quantitative analysis by paper chromatography, of the iodo-amino acids of thyroglobulin from normal and carcinomatous thyroid tissue of Miss To.'s gland is difficult. If the specific activities (μC of $^{131}\text{I}/\mu\text{g}$. of ^{127}I) of the 3-iodotyrosine from the normal and abnormal thyroglobulin are assumed to be the same, and the specific activities of the 3:5-diiodotyrosine from the normal and abnormal tissue are assumed to be the same, the two not necessarily being equal, then the results indicate a higher ratio of 3-iodotyrosine to 3:5-diiodotyrosine for abnormal than for normal thyroglobulin, suggesting that there is interference with the iodination mechanism leading to formation of 3:5-diiodotyrosine. If this assumption is not correct, however, the implication is that the rates of synthesis of the two compounds in normal and abnormal thyroid tissue are different.

These results are preliminary to a fuller investigation of the properties of the iodine-containing proteins with abnormal solubility which are present in carcinomatous thyroid glands. The present work was limited by the availability of appro-

priately labelled glands containing both normal and abnormal tissue in a form which could be separated.

SUMMARY

1. The method of purification of thyroglobulin by fractional precipitation with ammonium sulphate was applied to saline extracts of normal and abnormal thyroid tissues from patients given ^{131}I . Characteristic patterns were observed of the distribution of ^{131}I between the fractions obtained.

2. The solubility (S) and $-\Delta S/\Delta C$ curves for the iodine-containing proteins present in saline extracts of normal and carcinomatous thyroid tissues were found to differ.

3. A quantitative examination by paper chromatography, of the trypsin hydrolysates of the thyroglobulins isolated from normal and carcinomatous thyroid tissue of one patient indicated that the proportions of ^{131}I present as 3-iodotyrosine and 3:5-diiodotyrosine were approximately 1:1 and 5:1 respectively in the normal and abnormal thyroglobulin.

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