5. A paper-chromatographic technique is given for the separation and detection of the reduced and oxidized forms of ergothioneine, cysteine and glutathione.

6. Mixed disulphides of ergothioneine with cysteine and glutathione have been prepared.

The greater part of the work described in this paper was carried out in America but it was completed in England, and one of us (H.H.) wishes to thank Mr R. Boardman for technical assistance in the final stages and Burroughs Wellcome Ltd. for a generous gift of ergothioneine.

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The Purity of Thyroglobulin Isolated from Normal and Carcinomatous Thyroid Tissue on one Patient by Fractional Salting-out with Ammonium Sulphate

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Thyroglobulin was prepared (Stanley, 1956) by the method of Derrien, Michel & Roche (1948) from normal and carcinomatous thyroid tissue of a patient (Mrs Ru.) with a follicular carcinoma of the thyroid gland. The patient had received a tracer dose of radio-iodide 2 days before thyroidectomy. Differences in the distribution of ^{131}I between the fractions obtained during the preparation of pure thyroglobulin have been reported and discussed (Stanley, 1956). The homogeneity and question of identity of the thyroglobulins (Ru_N and Ru_A) from normal and carcinomatous thyroid tissue have now been investigated by electrophoresis and by an immunological test.

MATERIAL AND METHODS

The preparation of Ru_N and Ru_A was described previously (Stanley, 1956). Solutions [1% (w/v); 2.5 ml.] of the two proteins in 0.05M-veronal buffer (pH 8.6, I 0.05) were subjected to electrophoresis in the 2 ml. cell of a Perkin-

Elmer apparatus, model 38, after dialysis against the buffer at 0–1° for at least 16 hr. This analysis was repeated with 0.4% solutions of both proteins.

The solutions of Ru_N and Ru_A were recovered from the electrophoresis cells and used for immunological analysis by the method of Ouchterlony (1948). Antibodies against Ru_A were prepared in a rabbit.

RESULTS

Figs. 1 and 2 represent the electrophoretic schlieren patterns obtained with 1% (w/v) solutions of Ru_N and Ru_A respectively, at the times indicated on the figures. Both proteins contain a single electrophoretic component, but differ significantly in mobility. The mobilities in the descending (μ_D) and ascending (μ_A) limbs were as follows: Ru_N : μ_D 5.38×10^{-5} , μ_A 5.85×10^{-5} cm.²v⁻¹sec.⁻¹; Ru_A : μ_D 4.97×10^{-5} , μ_A 5.44×10^{-5} cm.²v⁻¹sec.⁻¹. The difference in mobility was confirmed in a second run with 0.4% solutions of the proteins, when the following mobilities were determined: Ru_N : μ_D 5.53×10^{-5} , μ_A 5.71×10^{-5} cm.²v⁻¹sec.⁻¹; Ru_A : μ_D 4.91×10^{-5} , μ_A 5.09×10^{-5} cm.²v⁻¹sec.⁻¹.

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A contact print of the cell in which a gel-diffusion test of anti-Ru_A serum was run against Ru_N and Ru_A is reproduced in Fig. 3. No antigenic component occurs in Ru_A which is not also present, in roughly the same proportion, in Ru_N. This test indicates, moreover, the presence of one major and two, possibly three, minor antigenic components in each protein.

DISCUSSION

Thyroglobulin prepared by the method of Derrien *et al.* (1948) has been shown by the same workers to be microheterogeneous in spite of the fact that it is isolated by fractional salting-out within narrow limits of ammonium sulphate concentration. This microheterogeneity is indicated by breaks in the solubility curve, which suggest the presence of three components. This microheterogeneity has also been found with thyroglobulin prepared by the same method from human thyroid glands (Roche,

Michel, Deltour & Michel, 1952). Derrien, Michel, Pedersen & Roche (1949) demonstrated that thyroglobulin from porcine glands is electrophoretically homogeneous.

In the current investigation thyroglobulin from normal and from carcinomatous thyroid tissue of one patient was shown to be immunologically heterogeneous, each protein containing three antigenic components. Moreover the Ouchterlony gel-diffusion test enabled us to conclude that no antigenic components were present in Ru_A which were not also present in Ru_N.

Electrophoretic analysis suggested that Ru_N and Ru_A were homogeneous and had mobilities of the same order as those determined by Derrien *et al.* (1949) for porcine thyroglobulin in phosphate buffer (pH 7.68, *I* 0.2). The difference in mobility determined for each protein at 1 and 0.4 % (w/v) concentration may be due to a difference in viscosity between the more and less concentrated solutions, but the difference between the mobilities

Fig. 1. Electrophoretic schlieren patterns of thyroglobulin (1%, w/v) in 0.05M-veronal buffer (pH 8.6, *I* 0.05). This sample of thyroglobulin (Ru_N) was prepared by the method of Derrien *et al.* (1948) from the normal thyroid tissue of a patient with a follicular carcinoma of the thyroid gland. Times at which the photographs were taken are: (a) 60 min.; (b) 120 min.; (c) 165 min.

Fig. 2. Electrophoretic schlieren patterns of thyroglobulin (1%, w/v) in 0.05M-veronal buffer (pH 8.6, *I* 0.05). This sample of thyroglobulin (Ru_A) was prepared by the method of Derrien *et al.* (1948) from the abnormal thyroid tissue of a patient with a follicular carcinoma of the thyroid gland. Times at which the photographs were taken are: (a) 60 min.; (b) 120 min.; (c) 180 min.

Fig. 3. Gel-diffusion precipitin pattern obtained by the method of Ouchterlony (1948). Solutions (about 1% in 0.05M-veronal buffer, pH 8.6) of thyroglobulin from normal (Ru_N , 0.1 ml.) and abnormal (Ru_A , 0.1 ml.) thyroid tissue of a patient with a follicular carcinoma of the thyroid gland and an anti-serum prepared against Ru_A in a rabbit, were placed in the positions marked Ru_N , Ru_A and anti- Ru_A respectively and allowed to diffuse together. At least three precipitin bands formed; the fourth and lowest band, visible also under Ru_A to the naked eye, was too diffuse to show in the photograph. The white streak is an artifact.

of Ru_N and Ru_A at the same concentration appears to be due to a structural difference which is not reflected in the antigenic properties of the proteins.

SUMMARY

1. Thyroglobulins prepared from normal and carcinomatous thyroid tissue of one patient migrated as single components during electrophoresis in 0.05M-veronal buffer (pH 8.6, I 0.05) at concentrations of 1 and 0.4% (w/v). They differed significantly in mobility.

2. Analysis by the gel-diffusion method of Ouchterlony revealed the presence of three, possibly four, antigenic components in each thyroglobulin but no qualitative difference between the two thyroglobulins.

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Biosynthesis of Intestinal Mucins

1. SURVEY OF INCORPORATION OF [35 S]SULPHATE BY ISOLATED GASTROINTESTINAL TISSUES

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When [35 S]sulphate is injected into mammals most of it is eliminated in the urine and faeces (Dziewiatkowski, 1949). The comparatively small amount of isotope which is retained is incorporated by a number of tissues, notably the epithelium of the gastrointestinal tract and cartilaginous tissue (Lewison, Levi, Jones, Jones & Silberstein, 1951; Dziewiatkowski, 1953; Bélanger, 1954). By the use of radioautographic techniques it has been shown (Bélanger, 1953; Boström, 1953; Davies &

Young, 1954; Jennings & Florey, 1956) that in the gastrointestinal tract the various mucin-secreting cells are the principal sites of localization and that here inorganic sulphate is incorporated into sulphated amino polysaccharides (Crevier & Bélanger, 1954; Kent, Whitehouse, Jennings & Florey, 1956).

In order to study in greater detail the biosynthesis of such polymeric substances, which are major organic components of epithelial mucin, a survey has now been made of the utilization of [35 S]sulphate *in vitro* by surviving slices and segments of various types of gastrointestinal

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