GOITRE DUE TO DYSHORMONOGENESIS

WITH PARTICULAR REFERENCE TO SPORADIC GOITROUS CRETINISM

AND

SPORADIC FAMILIAL GOITRE IN YOUNG PATIENTS

By

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CHAPTER I

INTRODUCTION

My interest in goitre in general and sporadic goitrous cretinism in particular was aroused by an invitation from Dr. James H. Hutchison, Royal Hospital for Sick Children, Glasgow, in 1951, to cooperate with him in the investigation of four children, members of a tinker family, who were hypothyroid and had goitres. I had recently started to use radioiodine (^{131}I) as a diagnostic aid in thyroid disease, and it seemed that it might prove a useful tool in elucidating the pathogenesis of this unusual condition.

At the start of my studies in 1951 little was known or indeed had been written about sporadic goitrous cretinism. The classical type of endemic cretinism with an enlarged thyroid gland and the common type of sporadic cretinism, in which thyroid tissue is absent or rudimentary, were, of course, well recognized. It was known, too, that goitre might occur in an occasional case of sporadic cretinism because of the action in utero, or after birth, of a goitrogenic substance such as thiouracil. From time to time hypothyroid children with enlarged thyroids were seen who could not be fitted into one of these well defined groups. Nineteenth century writers recognized these uncommon

cases of sporadic goitrous cretinism but their occurrence aroused little interest.

Fagge (1871) was aware that endemic cretinism only occurred where goitre was highly endemic. In other parts of the world, including the United Kingdom, there are some areas where goitre is more common than in others, and in which it is said to be endemic, but in them endemic cretinism was apparently then as now almost unknown. Any record of goitrous cretinism occurring outside a highly endemic area is, therefore, of unusual interest. In practice it appears well-nigh impossible to distinguish between these rare cases of so-called endemic cretinism and the infrequent cases of sporadic goitrous cretinism.

As far as can be ascertained the only instance in England of an "endemic" of cretinism occurred in the village of Chiselborough in Somerset in the first half of last century (Norris 1848). Among the population of 540, there were 4 complete cretins, 17 semi-idiots and 5 deaf-mutes. The majority of the community were dull-witted and were goitrous. Norris noted that intermarriage existed to a greater extent in this parish than in others. He was reluctant to accept that the constant practice of intermarriage alone accounted for the malady,

though others were obviously of this opinion. His impression was that the disease was due to a combination of circumstances in which he included the effects of a water supply of inferior quality and impure air. He went on to opine that "circumstances concurring thus to render the parish so uninviting to strangers, the poor inhabitants were under the necessity of "putting up" with each other, and thus perhaps unwittingly completing the unfortunate circumstances which may have produced such miserable results". In 1871 Fagge recorded that the cretins in Chiselborough had almost died out. The factors credited with the eradication of the condition were improved sanitary measures, better food, better education, and greater contact with the outside world, together with fewer intermarriages. In 1896 Parker stated, on the advice of Dr. Hugh Norris, that only one solitary cretin of about 50 years of age then survived. He considered that goitrous cretinism was obsolescent because of improved sanitation and the opening up of secluded valleys. He thought that it occurred "in the worst breeding grounds of goitre (namely, in valleys where the right conditions for goitre happen to exist) and which are sufficiently secluded and benighted to induce frequent intermarriage among goitrous families".

The other early references to cases of goitre associated with cretinism in Britain are scanty, but they are of interest because they show that the condition was uncommon even at a time when endemic goitre was being freely sought for, and they show that the cases reported were scattered widely over the country.

Reid (1836) referred to cases which were to be found in the Isle of Arran. Guggenbuhl was reported in the London Medical Gazette of 1851 to have made a tour through England and to have found cretinism in several of the counties including Somerset, Lancashire, and Derbyshire. According to Blackie (1855) cretinism had once existed on the Fifeshire coast. In 1855 a case was reported in York. Railton (1891) described two cretinous brothers in Manchester, each of whom had a palpable thyroid gland. Stevenson (1896) referred to a family in a goitre-stricken village in the North of England, near Penrith, in which the mother had a goitre, one of the children was a cretin, and another a deaf-mute. Beach (1896) was able to collect records of one hundred and sixteen cases of cretinism. In ninety-one of them it was noted whether the thyroid gland was present or not; he found that it was not felt in seventy-three, was palpable in eleven, and was enlarged in only seven.

In a survey of sporadic cretinism in the United States of America Osler (1897) found a low but definite incidence of goitre. An enlarged thyroid was present in seven out of sixty sporadic cretins. In one family of five children three were sporadic cretins with enlarged goitres. Osler was probably the first physician to recognize the familial tendency in sporadic goitrous cretinism and to comment upon the occurrence of consanguinity of the parents. His observations are all the more noteworthy because nothing was added to our knowledge of this condition, nor indeed was much attention devoted to it until Hamilton et al. (1943) used radioactive iodine (^{131}I) in its investigation and so introduced a technique which has greatly furthered our understanding of its cause and pathogenesis.

Since 1943 much greater interest has been shown in sporadic goitrous cretinism and it is from the biochemical and genetic studies made in the patients seen since 1943 that knowledge has developed about the aetiology of this condition. The literature between 1943 and 1957 has been carefully scanned and in all records of one hundred and thirty-four patients have been found. These case reports have come from the United States of America (Hamilton et al. 1943, Lerman et al. 1946, Hurxthal and Musulin 1946, Stanbury and Hedge 1950, Stanbury 1951, Silverman and

Wilkins 1953, Wilkins et al. 1954, Sexton and Mack 1954, Stanbury et al. 1955a, Whitelaw et al. 1956, Levy et al. 1956, Hayles et al. 1956, Schultz et al. 1957, Di George and Paschkis 1957, Werner et al. 1957, Pickering et al. 1957, Kundstadter et al. 1957, Frierson et al. 1957), from the United Kingdom (Murray et al. 1948, Braid 1951, Hubble 1953, McGirr and Hutchison 1953, Hutchison and McGirr 1954, Jackson 1954, Burrell and Gairdner 1955, McGirr et al. 1956), from Europe (Horst and Harnack 1953, Bernheim and Berger 1954, Uzan 1955, Stanbury et al.1955b, Stanbury et al.1956 a & b, Debré et al. 1956, Lelong et al.1956), from the Middle East (Zondek et al. 1955, 1956), and from New Zealand (Logan 1956).

In none of the reports of sporadic goitrous cretinism cited has the condition been due to iodine deficiency, or to the ingestion of goitrogenic substances. In goitrous cretinism the hypothyroid state is not due to a simple lack of thyroid tissue as it is in the common type of sporadic cretinism. It is due to an inability on the part of the thyroid gland to meet the body's requirements, even when considerable enlargement of the gland has occurred. Studies with radioactive iodine (^{131}I) have shown that the thyroid glands of these patients are commonly unusually avid for iodine, although there is little if any production of effective thyroid hormone. Evidence from

biochemical and genetic studies has been gradually accumulated to support the belief that this type of cretinism is due to intrinsic defects in the synthesis of the thyroid hormone and that these defects are genetically determined.

Consideration of the sequence of reactions by which the thyroid hormone is synthesized reveals that there is ample opportunity for defects in synthesis to occur. A short account of current knowledge about the biosynthesis of thyroid hormone is given in Chapter 2 (Stanbury and McGirr 1957).

When I started my investigations in 1951 only one defect in thyroid hormone synthesis had been defined in sporadic goitrous cretinism. It had been shown by Stanbury and Hedge (1950) that the thyroid glands of three siblings with goitrous cretinism could trap but could not utilize iodide. Their glands were unable to change iodide to iodine, and in consequence iodination of tyrosine could not occur. Presumably the enzyme peroxidase was lacking (Stanbury et al. 1955a).

The first part of this thesis is concerned with the investigation of sporadic goitrous cretinism in tinkers in Scotland. From the initial studies to the final determination of the responsible enzyme defect, namely impaired dehalogenase activity, the investigation extended over the period 1951 to 1958. It was necessary to adopt and develop new techniques to elucidate the cause of the hypothyroidism and the goitres in these patients. The

necessity for these developments is best appreciated when the findings are reported and discussed chronologically in relation to the techniques available to me at the time they were obtained. Accordingly I have adopted this method of presenting the material in so far as it is consistent with clarity. Some repetition has proved unavoidable, but it has been kept to the minimum.

The dehalogenase defect was but one of several defects that were defined during this period by different investigators in sporadic goitrous cretinism. The delineation of several specific biochemical abnormalities in sporadic goitrous cretinism and the evidence for their genetic basis made me wonder whether similar defects might not also explain some cases of sporadic goitre. Reference to the literature on goitre soon revealed that it had long been believed that hereditary predisposition was a factor in the aetiology of endemic goitre and cretinism (Hutchinson 1855, McCarrison 1917, Pfaundler 1924, Davenport 1932). It was apparent, too, that defects in hormone synthesis had been suspected. Brain (1927) postulated in patients with sporadic goitre inherited defects of iodine utilization of varying severity. Gates (1946) suggested that the inherited element in goitre might be based on the lack of the enzyme which controls the transformation of

tyrosine to thyroxine.

As a first step in the examination of the hypothesis that disturbances of hormone synthesis similar to those found in sporadic goitrous cretinism, but of lesser degree, might be responsible for some cases of sporadic goitre, I decided to study adolescent or young adult patients who presented with goitres. For my purposes the ideal goitre would be one which was large enough to make thyroidectomy desirable. In the second part of the thesis I present my findings in 10 such patients and consider their significance in relation to current knowledge of the aetiology of sporadic goitre.

CHAPTER 2

BIOSYNTHESIS OF THE THYROID HORMONE

Iodide is absorbed through the gastro-intestinal tract and is rapidly distributed through the extracellular fluid. The thyroid gland and the kidneys are the chief organs which compete for the plasma iodide. The mechanism of the accumulation or trapping of iodide by the thyroid is unknown (fig.1). It is oxygen-dependent (Slingerland 1955) and is likely to be under enzyme-control. The ratio of thyroid to plasma iodide may vary from twenty to one in the resting gland to many hundreds to one in the hyperplastic gland. It is governed by the thyrotrophic hormone of the anterior pituitary, and it is dependent upon the quantity of iodine stored within the gland. The trapping of iodide is inhibited by a variety of anions such as thiocyanate and perchlorate. These substances also discharge any iodide present in the gland, but they do not affect iodine which has been formed into iodinated tyrosines and thyronines.

The iodide is almost immediately and completely oxidized to elemental or free iodine (fig.2). The enzyme peroxidase is probably responsible for the removal of an electron from the

iodide (De Robertis and Grasso 1946). The oxidation of the iodide is inhibited by a number of drugs, particularly those containing the thiocarbonamide group, for example, by thiouracil. They achieve their well-known therapeutic effect by greatly diminishing if not actually preventing the further metabolism of iodide.

The iodine liberated by the oxidation of iodide reacts with tyrosine in thyroglobulin (fig.3). Iodination of tyrosine is easily and quickly accomplished in vitro under suitable conditions (Pitt-Rivers 1956), but a tyrosine iodinase, which has been described by Fawcett and Kirkwood (1953) may accelerate the process in vivo. It is not clear whether the iodination occurs within the thyroid cells or whether it occurs in the colloid at the cell surface. Intact cells are almost certainly required to ensure that the final stages in the synthesis are achieved efficiently.

Studies of the rate of incorporation of ^{131}I into the various amino acids found in the thyroid suggest a sequential build-up of monoiodotyrosine, diiodotyrosine and thyroxine (Taurog et al. 1949, Chaikoff and Taurog 1948).

Monoiodotyrosine and diiodotyrosine may be stored in the thyroglobulin of the colloid, or they may condense to form

iodinated thyronines, of which thyroxine and triiodothyronine are the best known (Kendall 1915, Harington 1926, Gross and Pitt-Rivers 1953) (fig.4). The available evidence favours the hypothesis that thyroxine is produced by the coupling of two molecules of diiodotyrosine and extrusion of an alanine group. No good explanation of the coupling reaction has been forthcoming since it was first suggested by Harington and Barger (1927). Enzymic control is likely but convincing proof of the existence of such an enzyme is as yet lacking. Coupling may require the presence of free iodine within the gland. There is indirect evidence that anti-thyroid drugs such as thiouracil may inhibit it (Pitt-Rivers 1948).

The mechanism and site of synthesis of triiodothyronine are even more uncertain. Roche and Michel (1955) consider that it is most probably produced by the condensation of one molecule of monoiodotyrosine and one molecule of diiodotyrosine. Alternatively it may be formed by the partial deiodination of thyroxine either in the thyroid gland itself or in other tissues of the body.

Thyroxine and triiodothyronine are usually stored in the thyroglobulin of the thyroid colloid and are not secreted directly

into the circulation. Their release is governed by the thyrotrophic hormone. It is inhibited by iodide. The thyroglobulin is digested by proteases and peptidases (fig. 5). Thyroxine and triiodothyronine are released and are transferred across the epithelial cells to the blood directly or through the lymphatics (Dobyns and Hirsch 1956). Monoiodotyrosine and diiodotyrosine are also released from storage but they do not usually reach the circulation in detectable amounts. Their iodine is rapidly removed by the enzyme dehalogenase, which is present in the thyroid gland as well as many other tissues (Roche et al. 1952 and 1953). The iodine is retained within the gland and is reutilized.

PART I

SPORADIC GOITROUS CRETINISM

CHAPTER 3

SPORADIC GOITROUS CRETINISM

The term cretinism is usually reserved for hypothyroidism present at or appearing soon after birth. After the first year hypothyroidism in childhood is usually referred to as juvenile myxoedema. Such distinction between cretinism and juvenile myxoedema is artificial in the group of patients considered in this section. Most of the patients belonged to one family group and indeed all might have had a common ancestry. Because their hypothyroidism appears to be due to an inborn error of metabolism and the basic genetic abnormality must therefore have been present from the moment of conception, as well as for the sake of brevity, they are all classified as cretins, despite the evidence that the ages of onset of recognizable features varied from a few months to a few years after birth.

Clinical Material

In 1951 4 children were referred for investigation (fig.6). They were members of one family, and had presented with the unusual combination of hypothyroidism and thyroid enlargement, unrelated to iodine deficiency or the action of any known goitrogen. The diagnosis of sporadic goitrous cretinism was made (McGirr and Hutchison 1953). Between 1951 and 1958 16 patients, all of whom were of itinerant tinker stock leading a nomadic existence in Scotland, were studied. For descriptive purposes these patients are divided into 3 groups (Table I).

Group I comprises cases 1-12 of which 5 were males and 7 females. Their ages ranged from 2 years 5 months to 24 years at the time of their initial investigations. They were all members of one family group. A family tree giving such information as is known about them is shown in fig.7. It includes a thirteenth case (fig.7, IV.14) which was not available for study. This patient, a male, was a brother of cases 6, 7 and 10. The family tree shows that these 13 patients came from 5 closely interrelated families. Two of the families each had 4 affected members, one had 3, and 2 had one each. The parents of the affected persons were

normal to outward appearances. This family group of tinkers moves around Kintyre, Argyll, and the Isle of Islay. From time to time they travel in other parts of Scotland, and occasionally migrate to England.

Group II comprises cases 13 and 14, both female children of a common mother but having different fathers. They were aged $6\frac{1}{2}$ and $3\frac{1}{2}$ years respectively. There was a third case in this family, a male who was a full brother of case 14. The mother was of tinker stock from Perthshire and Angus.

Group III comprises cases 15 and 16, a male and a female sibling, aged 6 years 9 months and 5 years 5 months respectively. These children were referred from a home for deprived children in Renfrewshire. The parents were itinerant tinkers who could not be traced.

Clinical Features

Group I cases: In all of the 12 patients in Group I except in case 8, the myxoedematous changes were readily observed. There was a striking family likeness but in addition there were the coarse features of myxoedema (fig.8), as well as scanty eyebrows, dry lustreless hair and dry skin. All of the patients were dwarfed (fig.9) and mentally retarded. Nevertheless, in

none of the patients were the signs of hypothyroidism as gross as they would have been in untreated sporadic or endemic cretins of comparable ages. In case 8 (fig.10) the evidence of hypothyroidism was not obtrusive, although easily obtained by careful clinical observation. Case 10 had been seen in her home district by Dr. Hutchison 2 years prior to her hospital admission. She was obviously hypothyroid then. Subsequently she had been taking an unknown amount of dry thyroid prescribed for her cretinous brother and sister and had become thyrotoxic. Forty days after thyroid medication was stopped she was obviously myxoedematous again.

In each of the 12 patients there was a very obvious goitre when they were first seen (fig.11). The enlargement in case 10 was considerable, sufficient to make thyroidectomy cosmetically desirable, but the patient, after requesting operation, changed her mind and refused operation. Thyroidectomy was performed elsewhere on case 2 approximately 7 years after her initial investigations.

In case 3 there was in addition to myxoedema a mild spastic paraplegia, for which tendon lengthening had been performed.

The ages of the patients at the time of onset of hypothyroidism or goitre could rarely be determined with accuracy. It would appear that they were all hypothyroid by the time they reached school age. Abnormal development was noted in cases 5 and 11 before the age of 1 year, and in case 12 during her second year. In one patient only, case 9, was there definite evidence that the goitre preceded the hypothyroidism. He was admitted to hospital at 4 weeks because of a swelling of the neck (fig.12). The swelling was due to moderate soft lumpy enlargement of both lobes and isthmus of the thyroid gland. At that time he was a well nourished infant and no cretinous features were apparent. When he was readmitted to hospital at 2 years 5 months because of severe constipation he was myxoedematous (fig.13).

Radiography of the skeleton demonstrated delayed ossification in every patient in this group with the exception of case 10, who had been taking dry thyroid. The cardio-thoracic ratio exceeded 60 per cent in the teleradiograph of the heart in cases 1, 2, 3 and 5. Low voltage electrocardiographic tracings were obtained in cases 1 to 7, associated with

flat P and T waves in cases 2, 3 and 4, with inverted T1 and T2 waves in case 1, and with an inverted T3 wave in case 5. Plasma cholesterol values varied from 120 to 500 mg. per 100 ml. In the only 2 patients in whom it was possible to have the serum protein-bound iodine (^{127}I) estimated, namely cases 10 and 12, it was 1.8 µg.(40 days after thyroid was stopped and myxoedema had developed) and 3.0 µg.per 100 ml. respectively. The basal metabolic rate was evaluated only in the 3 oldest patients. In cases 6 and 7 who were hypothyroid it was 8 and 42 per cent below standard (Aub and du Bois) respectively. Case 10, who was thyrotoxic because of exogenous thyroid, had a basal metabolic rate 34 per cent above standard on admission. Serum from case 11 was sent to Miss Judith Brown, B.Sc., Clinical Endocrine Research Unit, Medical Research Council, Edinburgh University, for assay of the serum thyrotrophin (TSH) level. Miss Brown reported that duplicate estimates gave a very high serum TSH level.

Group II cases: The diagnosis in these patients (cases 13 and 14) was made by Professor J.L. Henderson of Dundee. They had received treatment with dry thyroid

for 6 and 8 months respectively before they were referred for study in Glasgow. The thyroid had been stopped for 2 months. In contrast with the patients in Group I these children, when first seen in Dundee, were typical untreated cretins, with coarse facies, large tongues, supraclavicular pads, pot-bellies, severe dwarfism and mental deficiency (fig.14). Case 14 had an umbilical hernia. In each patient there was an obvious but not very large goitre. As far as could be ascertained failure of normal development had been noted before the age of 2 years. Ossification had been markedly delayed in both patients before treatment had been instituted. The diagnosis of cretinism was made in the third affected child of this family by Professor Henderson at 3 months.

Group III cases: The early histories of these children (cases 15 and 16) were not available. They had received dry thyroid for approximately 2 years until 3 months prior to their admission to hospital for investigation. Both patients were undersized. The residual stigmata of hypothyroidism were more marked in case 15 (fig.15) than in case 16. The boy was still mentally dull; his skin

was dry and eyebrows scanty; his abdomen was large and protuberant. The girl was mentally retarded, but not markedly so; her skin was moist. Each had a large soft goitre. Ossification was still delayed in both cases. Protein-bound iodine (^{127}I) was 3.0 and 4.2 μg. per 100 ml. respectively.

Response to Treatment

All the patients showed a striking response while they were in hospital to treatment with dry thyroid by mouth in doses of 1 to 2 grains daily. The signs of hypothyroidism disappeared. They were vastly improved both mentally and physically. Growth was stimulated and bone age advanced. The satisfactory response to oral treatment with dry thyroid removed any suspicion that their condition might have been due to tissue insensitivity to thyroid hormone (figs. 6 and 16).

Case 16 made the most satisfactory recovery. She developed normally both physically and mentally, and at school she caught up with children of her own age. During treatment with thyroid the thyroid gland diminished in size, but in none of the 7 patients I have been able to follow did the goitre

completely disappear. Unfortunately regular treatment was exceptional after the patients left hospital. Usually it was so irregular that over a period of years alteration in the size of the thyroid gland was rarely striking, and in one patient, case 2, thyroidectomy was performed 7 years after her initial investigations because her goitre was increasing in size. A substantial amount of thyroid tissue, approximately 100 g., was removed.

CHAPTER 4

RADIOACTIVE IODINE (^{131}I) STUDIES

Between 1951 and 1958 various ^{131}I tests were performed. Chronologically they fall into 4 phases.

Phase I tests.

These were the only tests used between 1951 and 1954. They were also used with improved equipment in 1955 and later. The earlier results compare sufficiently well with the later results for them all to be considered together.

Phase I tests consisted of the measurement of

1. Thyroid uptake of ^{131}I, and the effect on the ^{131}I content of the thyroid of potassium thiocyanate.

2. Urinary excretion of ^{131}I.

3. Blasma or serum ^{131}I as (a) total ^{131}I ($T^{131}I$)
   (b) protein-bound ^{131}I ($PB^{131}I$)
   (c) thyroxine-like ^{131}I ($BE^{131}I$)

4. Analysis by chromatography of the chemical basis of plasma ^{131}I (Dr. J. Gross and Mrs. R.Pitt-Rivers).

Phase II tests.

These tests were used in 1955 and 1956. They consisted of analysis by radio-chromatography of extracts of serum and urine after an oral dose of ^{131}I. The position of the radioactive compounds on the chromatograms was recognized by the blackening produced in non-screen X-ray film to which they were attached. The chemical identity of some of these radioactive compounds was established by reference to the position of known marker substances on the chromatograms.

Phase III tests.

These tests were used in 1957 and 1958. They consisted of the chromatographic analysis of urinary ^{131}I after an oral dose of radioactive (^{131}I) monoiodotyrosine ($M^{131}IT$).

Phase IV tests.

These tests were used in one patient only of this group in 1958. They consisted of

1. Demonstration of impaired dehalogenase activity of thyroid tissue by incubating fresh slices of

thyroid tissue <u>in vitro</u> with $M^{131}IT$.

2. Chromatographic analysis of the ^{131}I containing compounds present in serum and urine and thyroid gland.

3. Electrophoresis of thyroid proteins.

CHAPTER 5

PHASE I TESTS

Methods

(1) Thyroid uptake of ^{131}I

The accumulation and retention of ^{131}I in the thyroid gland was measured at intervals varying from 1 to 72 hours, after an oral dose of carrier-free ^{131}I given as sodium iodide at least 2 hours after a meal.

Between 1951 and 1954 the measurements were made by a lead shielded Geiger-Müller counter (Type G.E.C. G.M.4) which was placed 30 cm. from the manubrial notch. To diminish the effect of body radiation the counter was protected from the trunk by a lead shield 0.5 cm.thick. The counter was connected through a quench and amplifier unit (type 1014) to a standard Dynatron power unit and scaler (type 200). Five minute counting periods were used so that for a tracer dose of 50 μc of ^{131}I the method was accurate within the limits of $\pm$ 6 per cent of the dose.

From 1955 onwards an EKCO (type 509) scintillation counter was used with standard counting equipment, such as an EKCO automatic scaler (type N 530) or a modified

PANAX scaler (type 100c). The crystal was shielded by a lead cone which was constructed so that at a distance of 25 cm., its optimum working distance, it viewed an area of 12 cm. diameter. The method was accurate within the limits $\pm$ 2 per cent for a dose of 25 μc counted for 2 minutes.

A standard, consisting of the same dose of ^{131}I as the patient had received was counted on each occasion that the ^{131}I was measured in the patient's thyroid. It was made up in a constant volume of water in a glass container, which was placed at the same distance from the counter as the anterior surface of the patient's neck. To correct for back scatter of radiation from the vertebral column behind the thyroid the factor 0.82 has been used in these patients. This factor is the mean of the factors determined in 250 patients by measuring the dose in air and on the anterior surface of the patient's neck. The earlier results were originally published uncorrected for back scatter. The same correction factor has now been applied to all the cases.

In cases 1-4 the effect of an oral dose of 2 g. potassium thiocyanate on the amount of ^{131}I in the thyroid gland was observed. It was given in cases 1 and 2, 4 hours, in case 3, 24 hours, and in case 4, 48 hours after the dose of ^{131}I. The retention of ^{131}I in the thyroid was measured at 15 minute intervals during the succeeding 1 hour.

(2) Urinary excretion of ^{131}I.

The urine was collected in divided periods over 48 hours in 12 of the 16 cases. For cases 1, 5-8, 12, 15, 16, the periods were 0-6, 6-12, 12-18, 18-24, and 24-48 hours after the dose, and for cases 2-4 and 10 the periods were 0-6, 6-24 and 24-48 hours.

Between 1951 and 1954 samples of urine of approximately 10 ml. volume were counted for 5 minutes in the beta liquid counter designed by Veall (1948). A dilution of the standard ^{131}I solution was kept for estimation of the dose. The amount of ^{131}I excreted in each period was calculated and the result was expressed as a percentage of the dose administered.

In 1955 and 1956 the radioactivity of 10 ml. samples of urine from each period was estimated in an EKCO liquid

scintillation counter with a pillar crystal, using annular plastic cups. In 1957 and later a well-type crystal was used to measure the radioactivity in 5 ml. samples.

(3) Plasma or serum ^{131}I.

Samples of blood were taken off at various times, usually at 48 hours, and the plasma (anticoagulant either oxalate or heparin) or serum was separated by centrifuging. Between 1951 and 1954 plasma samples were used; in 1955 and later serum was used. The total radioactivity present in 5-10 ml. samples was determined and the result expressed as per cent of dose per litre.

Between 1951 and 1957 radioactivity of the protein-bound iodine in the plasma or serum was determined by trichloracetic acid precipitation (Sheline and Clark 1950, Goodwin et al. 1951): 5-10 ml. samples were used and the proteins precipitated by 10 per cent trichloracetic acid; the precipitate was washed twice with 2 per cent trichloracetic acid, redissolved in 2N-NaOH and the volume made up to a suitable volume for counting. After 1957 the serum protein-bound iodine was measured by the resin extraction method of Zieve et al.(1956).

Samples of plasma or serum from cases 1-3, 8, 9, 13-15, were examined for thyroxine-like iodine by a modification of the method of Taurog and Chaikoff (1948). The iodine was extracted by three washings of N-butyl alcohol from 3 to 10 ml. samples after they had been acidified to pH2 with N-H_2SO4 or HCl. The extract was treated twice with a solution of 5 per cent Na_2CO_3 in 4 N-NaOH, and the mixture was allowed to separate. The alcohol layer was separated and concentrated in vacuo to a suitable volume for counting.

Between 1951 and 1954 the radioactivity of plasma or serum samples was measured in the beta liquid counter designed by Veall (1948). A dilution of the standard ^{131}I solution was kept for estimation of the dose. The amount of ^{131}I present was expressed as a percentage of the dose per litre. Counts for at least 10 minutes were made on the total iodine samples ($T^{131}I$) and 20 minutes on the protein-bound iodine samples ($PB^{131}I$) and the thyroxine-like iodine samples ($BE^{131}I$). With a tracer dose of 50 µc, volume of 10 ml., and counting time of 10 minutes the method was accurate to $\pm$ 0.15 per cent of the dose/litre.

In 1955 and 1956 the radioactivity was estimated in an EKCO liquid scintillation counter (type N.550) with a pillar crystal. With a tracer dose of 25 µc, volume of 10 ml., and counting time of 5 minutes, the method was accurate to $\pm$ 0.10 per cent of the dose/litre.

In 1957 the pillar crystal was replaced by a well-type crystal, and an additional lead shield constructed to reduce the background. With a tracer dose of 25 μc, volume of 5 ml. and counting time of 100 seconds, the method was accurate to ± 0.07 per cent of the dose/litre.

(4) Chromatography of plasma radioactivity.

In 1952 samples of plasma from cases 5, 6 and 7, who received 200 μc of ^{131}I were submitted to Dr.J. Gross and Mrs. R. Pitt-Rivers at the National Institute for Medical Research, Mill Hill, London, for analysis by chromatography of the radioactivity in a butanol extract of the plasma (Gross and Pitt-Rivers 1952).

Results

The uptake by the thyroid gland, the excretion by the kidney, and the amount in the plasma of radioactive iodine are shown in Tables II-VI and figs. 17 a, b and c, and 18. It will be seen not only that the thyroid glands accumulated ^{131}I from the blood stream, but also that most of them did so with abnormal rapidity and in excessive amounts. The glands did not retain the ^{131}I, but released it into the

circulation, some of it in a chemical form which could be precipitated by trichloracetic acid and extracted by butyl alcohol.

Dr. Gross and Mrs. Pitt-Rivers reported that radioactivity was present in butanol extracts of plasma samples withdrawn from cases 6 and 7 at 24 and 48 hours, but the amount was insufficient to give an autoradiograph allowing identification of its origin. Radioactivity was present in the butanol extract from case 5 which was examined on a kieselguhr column. The radioactivity was not elutable with 20 per cent chloroform in butanol equilibrated with 0.05 per cent NaOH but was removed from the column with water. It was neither thyroxine nor triiodothyronine.

Discussion

(1) Accumulation of ^{131}I by the thyroid gland (Tables II and III, figs. 17 a b & c).

In general the uptake of ^{131}I by the thyroid gland suggests hyperthyroidism. Direct comparison with the results of other workers is difficult owing to considerable variations in techniques used. By any standards, however, it is obvious that

the thyroid glands of cases 1-4, 9, 10, 12-15, in which serial measurements were made, accumulated ^{131}I more rapidly than normal, and that the greatest uptake was well above the normal range.

In primary myxoedema the ^{131}I accumulated in the neck is usually negligible, and only on rare occasions does it exceed 20 per cent of the ingested dose (Murray and McGirr 1959). None of the tinker patients failed to exceed this value. Indeed in 10 out of the 13 patients in whom appropriate observations were made the ^{131}I content of the thyroid gland was greater than 40 per cent at 4 hours or earlier. Such a value is in excess of normal and is characteristic of thyrotoxicosis (Goodwin et al.1951) though it is also found in some patients with non-toxic goitres. In contrast the 24 hour uptake of ^{131}I by the thyroid gland only exceeded 50 per cent, the upper limit of normal, in 2 of 14 patients at their initial tests. The 24 hour uptake was well below the 4 hour (or earlier) uptake in 8 out of 12 patients in whom both early and late estimates were made. Usually the peak uptake of ^{131}I in normal adults does not occur until approximately 24 hours

after the oral ingestion of ^{131}I, and there is little change thereafter for several days. It is apparent that in the majority of the tinker patients there was not only an usually rapid uptake of ^{131}I, there was also an unusually rapid loss of ^{131}I from the thyroid gland.

Cases 1-3 were reinvestigated in 1955 after intermittent thyroid therapy. In case 2, who had been taking her thyroid fairly regularly in the year before her second test and who at that time showed good progress, the thyroid gland, though still markedly enlarged, was less active, presumably due to the suppressive effect of the exogenous thyroid. Cases 1 and 3 had only very irregular treatment, and the results of their second tests were very similar to those obtained in the first test.

In the 4 patients given it, an oral dose of 2 g. potassium thiocyanate had no immediate effect on the ^{131}I content of the thyroid glands (Table III, and fig. 18). The thiocyanate ion acts by blocking the iodide trapping mechanism and inhibiting the uptake of ^{131}I by the thyroid gland; it also discharges any iodide present in the gland; iodine which has been formed into iodinated tyrosines and thyronines is not discharged in this way. The failure of potassium thiocyanate

to discharge any ^{131}I from the thyroid indicated that the thyroid glands were able to convert the iodide which they accumulated into an organic form. It suggested that the ^{131}I which was being lost from the thyroid glands was likely to be in an organic form. It clearly distinguished these patients from those described by Stanbury and Hedge (1950) and Stanbury (1951). They found that the ^{131}I which was so rapidly accumulated by the thyroid glands of their patients was even more rapidly discharged by potassium thiocyanate, a fact taken to indicate that in their patients the thyroid gland was unable to combine inorganic iodide into a protein-complex.

(2) Urinary excretion of ^{131}I (Table IV).

It proved extremely difficult with this group of patients to get accurate urine collections. Their unreliability consequently detracts from the value of the urinary ^{131}I excretion studies. None the less the results obtained are of interest, if for no other reason than that they corrected the impression of hyperthyroidism given by the uptake studies.

In contrast with the uptake of ^{131}I by the thyroid gland, in no case did the amount of ^{131}I excreted in the urine suggest

hyperthyroidism. For example, in none of the cases did the amount of ^{131}I excreted in 0-24 hours fall below 20 per cent of the dose (Goodwin et al. 1951) or in 6-24 hours 5 per cent of the dose(Mason and Oliver 1949). The amounts excreted in 0-24 hours by 9 patients who had not received any recent thyroid medication (cases 3-8, 12, 15-16) were within the ranges found in normal and hypothyroid patients, namely 20-65 per cent (Goodwin et al 1951). Of these same 9 patients so studied 7 excreted more than 25 per cent of the dose in 6-24 hours. Mason and Oliver (1949) considered that an excretion of more than 25 per cent in this period was characteristic of hypothyroidism, and my experience in Glasgow supports their finding. In the period 24-48 hours amounts in excess of normal such as occur in primary myxoedema were common. For example in 8 of these 9 cases which had not received any recent thyroid medication over 10 per cent of the dose was excreted in this period and in the remaining case (case 8) the amount was a borderline value of 9.8 per cent of the dose.

The pattern of the renal excretion of ^{131}I was unusual. After a relatively slow start during the first 6 hours the urinary excretion of ^{131}I continued in considerable amounts. The

T factor*, which I have derived from the urinary excretion values and which is a useful index of thyroid activity, was less than 1 and thus indicative of hypothyroidism in 8 of 9 patients who had not received any recent thyroid medication. In contrast the total radioactivity excreted in the period 0-48 hours only exceeded 69 per cent and therefore suggested hypothyroidism in 2 of these 9 cases.

* The T factor is derived from the ^{131}I urine excretion values thus:

$$T = \frac{100}{\text{Per cent of dose of } ^{131}I \text{ excreted 6-24 hours}} \times \frac{\text{Per cent of dose of } ^{131}I \text{ excreted 0-6 hours}}{\text{Per cent of dose of } ^{131}I \text{ excreted 0-48 hours}}$$

after the manner of Fraser et al.1953, who used the time periods 0-8, 8-24 and 24-48 hours. It was designed to pick out the thyrotoxic rather than the hypothyroid cases. With my periods of collection the ranges of values are:

Less than 1	hypothyroid
1 - 1.5	equivocal
1.5- 7.5	euthyroid
7.5- 10	equivocal
Over 10	hyperthyroid

Large amounts of ^{131}I were excreted in the urine and the T values were low (less than 1) in cases 1, 4, 12, 15 and 16 which also had thyroid glands that both accumulated and released ^{131}I with unusual rapidity. These findings contrast with the small amounts of ^{131}I excreted in the urine, and the high T values (greater than 10) found in thyrotoxic subjects, even in those in whom there is a considerable decrease of thyroidal activity by 24 hours. These observations suggest that the organic iodine compound released into the circulation is different in thyrotoxicosis and sporadic goitrous cretinism. In the latter condition it is probably not being metabolized but is being excreted. Hence considerable radioactivity is excreted after the first 6 hours and the excretion curve cannot be analysed by the method Keating et al. (1947) used in normal, hypothyroid and thyrotoxic patients as substances other than iodide are probably present in the urine.

(3) Plasma or serum studies (Table V).

Measurement of the plasma or serum ^{131}I 48 hours after its ingestion revealed protein-bound ^{131}I levels of 0.40 or more per cent of the dose per litre in 9 out of 15 patients. In 8 of these 9 patients both early and late estimates had been

made of the ^{131}I content of the thyroid gland. Seven (cases 3, 4, 9, 12-14 and 15) showed an unusually rapid loss of ^{131}I from the thyroid. At the time of the earlier tests in 1951 and 1952 a value of $PB^{131}I$ of 0.40 per cent per litre or more was taken to indicate a state of thyrotoxicosis (Goodwin et al.1951). It was assumed that the $PB^{131}I$ precipitated by trichloracetic acid was essentially a measure of the thyroxine content of the plasma. In thyrotoxicosis the conversion factor (the percentage of the total radioactivity present in a protein-bound form) exceeds 50 per cent. The conversion factor was in excess of 50 per cent in 5 of these 9 patients.

At first it was difficult to reconcile the presence of apparently substantial amounts of $PB^{131}I$ with the hypothyroid state of the patients. The first explanation that suggested itself was that the $PB^{131}I$ was not thyroxine. The exact chemical composition of the compounds included with the protein-bound iodine fraction was apparently unknown, but it appeared that some diiodotyrosine might be precipitated with the proteins (Man et al.1951). Rall (1950) had reported a case of myxoedema in which 27 per cent of the plasma radioactivity was in the iodotyrosine fraction. The likelihood that

iodotyrosines were the basis of the radioactivity in the $PB^{131}I$ had therefore to be carefully considered.

Accordingly, the plasma of some of the patients (cases 1-3, 8, 9, 13-15) was examined by the butanol-extraction method of Taurog and Chaikoff (1948). In this method any inorganic iodide and iodotyrosine present in the butanol extract is removed by re-extraction with alkali. Thyroxine remains in the butanol extract. In three patients (cases 13-15) studied in 1952-1954 there was probably a difference between the $PB^{131}I$ and the $BE^{131}I$ values but the difference, namely 0.13, 0.15, and 0.21 was not enough to justify any definite conclusion, and the question was left open. In two patients (cases 3 and 9) studied in 1955 with more accurate counting methods differences of 0.64 and 0.97 were found. On the basis of these results it seemed likely that some of the $PB^{131}I$ might well be monoiodotyrosine and/or diiodotyrosine.

The chemical basis of the remainder of the activity in the $PB^{131}I$ was also unknown. The thyroxine-like ^{131}I ($BE^{131}I$) was appreciable, namely 0.48, 0.36, 0.25 and 0.40 per cent of the dose in cases 9, 13, 14 and 15 respectively. At first on clinical grounds it seemed unlikely that it was thyroxine,

for despite its presence the patients had presented with the clinical picture of hypothyroidism, and by their clinical improvement on treatment showed that they were responsive to dry thyroid given orally. There was no evidence of tissue insensitivity to thyroid hormone. The chromatographic studies of Dr. Gross and Mrs. Pitt-Rivers in case 5, which failed to detect the presence of thyroxine or triiodothyronine in the plasma, seemed to support the opinion that thyroid hormone was not being released into the circulation. Unfortunately the $BE^{131}I$ had not been estimated in this patient, so that there was in fact no evidence whether or not there was any thyroxine-like ^{131}I.

Some explanation of the remainder of the $PB^{131}I$ was sought, though it was appreciated that the conclusion could only be tentative. If the residual $PB^{131}I$ was in fact formed by thyroid hormone it was a most unexpected finding at the time of the earlier studies in 1951-1953 (McGirr and Hutchison 1953). The formation of $PB^{131}I$ in myxoedema was first reported by McConahey et al. (1949), who tentatively suggested that it might have been produced by extrathyroidal organic binding of iodine. Riggs (1952), discussing their finding, considered a more likely alternative explanation was

the possibility that scanty but very active foci of functional thyroid tissue, under intense stimulation with thyrotrophin, and with no storage capacity for organically bound iodine, should secrete into the blood stream "very limited" amounts of $PB^{131}I$. Blom and Terpstra (1953) reported the presence of appreciable amounts of $PB^{131}I$ namely 0.43 per cent and 0.19 per cent of the dose per litre at 48 hours, in 2 patients with clear-cut hypothyroidism following subtotal thyroidectomy for thyrotoxicosis. The protein-bound ^{127}I in the serum of each of these patients was 1.3 µg. per 100 ml. The total output of hormone was apparently inadequate. In explanation of their findings they envisaged the very rapid production and secretion of inadequate amounts of hormone with an unusually large number of its molecules labelled with ^{131}I. They suggested that these findings were possible because of the lack of a diluting pool of organic iodine in the gland remnants. A similar explanation is now accepted for the misleadingly high values of $PB^{131}I$ which are a commonplace in patients, previously thyrotoxic, who have been made euthyroid or hypothyroid by thyroidectomy or by ^{131}I therapy.

The low $PB^{127}I$, namely 3.0 µg per 100 ml., and elevated $BE^{131}I$, namely 0.40 per cent of the dose per litre, in case 15 (fig. 17c) are in keeping with a low organic iodine pool. The situation of course differs in that in case 15 we are concerned with a patient with a goitre and not scanty thyroid tissue.

The alternative explanation that the residual $PB^{131}I$ might be due to some organic ^{131}I compound other than thyroxine must also be considered. At the time of the earlier observations in 1951-1953 Robbins et al.(1953) reported the presence of such a compound in the serum of 2 patients with thyroid carcinoma who had received a therapeutic dose of ^{131}I. It was precipitable with the plasma proteins, but unlike thyroxine, it was not soluble in butanol. A similar but not necessarily identical substance was later reported in the serum of patients with Hashimoto's thyroiditis (Owen and McConahey 1956), congenital familial goitre (Stanbury and McGirr 1957) and congenital goitrous hypothyroidism (Di George and Paschkis 1957, De Groot et al. 1958). While it was not possible to identify the chemical basis

of the $PB^{131}I$ in the tinker patients by simple fractionation, it was possible to distinguish it from the $PB^{131}I$ of this miscellaneous group of conditions. In the tinker patients practically all of the $PB^{131}I$ was butanol-extractable. In them the discrepancy was between the $BE^{131}I$ (the thyroxine-like or non-alkali re-extractable fraction of the total butanol-extractable ^{131}I) and the $PB^{131}I$. To clarify this distinction a comparison is made in Table VI of the relative proportions of the various iodine fractions in the serum of one of the tinker patients (case 2,1958) and of a patient with a congenital goitre, who had an abnormal iodinated substance in her serum which was precipitable with the proteins but was not butanol-extractable (case VIII, Chapter 19).

At their most conservative evaluation the plasma and serum studies confirmed the conclusions reached from the studies of thyroid uptake that the thyroid glands could take up inorganic iodide from the blood and link it to protein, forming an organic iodine compound. In addition they showed that the glands could release iodine in an organic form into the circulation. The problem of the chemical basis of the $PB^{131}I$ had to await the development of

chromatographic techniques before it could be answered.

Conclusions

The ^{131}I tests demonstrated the unusual avidity of the thyroid gland for iodide which was to prove a feature common to all the sporadic goitrous cretins in whom radioactive iodine studies were reported (Stanbury and McGirr 1957). They showed that the hypothyroid state developed despite an enlarged thyroid gland which was able and eager to trap iodide, to link it to protein, and to release it freely into the circulation in organic combination. There was suggestive evidence that a hormone precursor was being released from the thyroid gland into the circulation, and that, as it was not being metabolized usefully, it was excreted in the urine. The chemical identity of the protein-bound radioactivity in the blood of these patients was not identified, but it seemed worthy of consideration that an iodotyrosine might be included. Lack of thyroid hormone probably arose from a defect in the synthesis of the thyroid hormone. The defect was clearly different from that described by Stanbury and Hedge (1950). As there

was good evidence that the various stages in hormone synthesis were under enzyme control, it seemed probable that enzymatic or other biochemical defects might occur spontaneously at various levels in hormone synthesis, just as they can readily be produced by drugs such as potassium thiocyanate and methyl thiouracil (McGirr and Hutchison 1953, Hutchison and McGirr 1954).

CHAPTER 6

PHASE II TESTS

It was apparent in 1953 and 1954 that the simple ^{131}I tests were in themselves insufficient to define the defect in synthesis which was the cause of the hypothyroidism and goitres in this group of patients. Determination of the chemical basis of the radioactivity in the blood by chromatography seemed a hopeful method of providing the answer.

Until such time as suitable cases were available for study consideration was given to the likelihood that some of the blood radioactivity was due to monoiodotyrosine and diiodotyrosine. After administration of ^{131}I very little (Benua and Dobyns 1955), if any (Rosenberg 1951, Dingledine et al. 1955) free monoiodotyrosine or diiodotyrosine had been found in the serum. Costa et al. (1953) had identified diiodotyrosine in 3 patients with endemic cretinism, and in 1955 Stanbury et al. (1955 b) identified monoiodotyrosine and diiodotyrosine in the blood of a patient who was a sporadic goitrous cretin. Roche et al.(1953) had

suggested that monoiodotyrosine and diiodotyrosine do not normally appear in the blood, when they are released from storage in the colloid, because they are deiodinated by the enzyme dehalogenase, and the iodine thus conserved is reutilized in the production of hormone.

Late in 1955 and early in 1956, when I had acquired some experience with chromatography, I had the opportunity to investigate 2 hypothyroid goitrous members of the main tinker family-group (cases 9 and 10).

Case 9. A male infant, aged 4 weeks, was first admitted to hospital on July 21, 1953. He had a large goitre but no signs of hypothyroidism (fig. 12). On November 19, 1955, he was readmitted to hospital because of severe constipation. He then appeared obviously hypothyroid and still had a goitre (fig. 13). Mentally he was dull and apathetic. Moderate anaemia was present; Hb was 9.2 g.per 100 ml. and red cells 4,110,000 per c. mm. Radiography showed 6 months' delay in ossification. His response to thyroid medication, started on December 16, 1955, was remarkable. Within 2-4 weeks his face was no longer myxoedematous, and he was beginning to repeat single words and had become lively.

Case 10. A woman, aged 24 years, was admitted to hospital on March 11, 1956. She had had an enlarged thyroid for many years but did not know at what age it had developed. She had been examined by Dr. J.H. Hutchison two years previously when she was obviously hypothyroid. During the past year she had been taking an unknown amount of dry thyroid prescribed for her cretinous brother and sister. At the time of her admission she had lost her hypothyroid appearance and was thyrotoxic. The thyroid was diffusely enlarged to a considerable degree. Her disposition was happy, but she was simple and illiterate. Basal metabolic rate was 34 per cent above standard. During the 40 days she was in hospital without thyroid medication all signs of thyrotoxicosis disappeared, and she again became obviously myxoedematous. The amount of protein-bound iodine (^{127}I) in her serum was estimated as 1.8 μg. per 100 ml. Thyroidectomy was planned for cosmetic reasons when she left hospital abruptly to join the annual migration of her family. She was restarted on dry thyroid gr.$\frac{1}{2}$ twice daily.

Methods

The accumulation and retention of ^{131}I in the thyroid gland and the radioactivity of samples of blood serum and urine were measured by the methods outlined for Phase I tests. In addition serum and urine samples were examined by chromatography (Gross 1954, Pitt-Rivers, personal communication).

Serum and urine samples for chromatographic analysis were extracted with 3 washes of twice their volume of N-butanol after samples had been brought to pH2 with N-H_2SO_4. The volume of samples varied from 2.5 to 10 ml. Volumes of urine greater than 10 ml. did not give good chromatograms with well-defined spots of radioactivity.

The butanol extracts were evaporated to dryness, and the residue was taken up in one drop of methanol-ammonia (4 drops of methanol and 1 drop of ammonia), and placed on Whatman No.1 filter paper. Ascending chromatograms were prepared; one-way chromatograms were run for approximately 25 cm. in butanol acetic acid solvent[1]; two-way chromatograms were run firstly in butanol phosphate buffer solvent[2] and then in butanol dioxane ammonia solvent[3]. A selection of appropriate markers from iodide (I^-), monoiodotyrosine (MIT),

diiodotyrosine (DIT), thyroxine (T4) and triiodothyronine (T3) were run with the extracts. Experience taught that it was essential not to overload the chromatograms by attempting to run too many markers with the extracts. If this experience was ignored poorly defined spots of radioactivity resulted. The markers were stained as follows:

for iodide	1 per cent palladium chloride spray
for iodotyrosines and iodothyronines	0.2 per cent ninhydrin spray.

The one-way strips and the two-way chromatograms were applied to non-screen X-ray film for periods of from 10 to 28 days and autoradiographs were prepared.

1. Butanol acetic acid solvent; butanol saturated with a mixture consisting of 1 part glacial acetic acid and 5 parts of water. A beaker containing butanol and a beaker containing mixture of glacial acetic acid and water placed on bottom of chromatography tank.

2. Butanol phosphate buffer solvent; butanol saturated with M/5 phosphate buffer of pH 7.2: A beaker containing butanol and a beaker containing phosphate buffer were placed on the bottom of the chromatography tank.

3. Butanol dioxane ammonia solvent: mixture of 4 parts of butanol and 1 part of dioxane saturated with 2 N-NH_4OH. A beaker containing butanol and a beaker containing ammonia were placed on the bottom of chromatography tank.

Results

Case 9 (Tables II, IV and V; figs. 19-22).

On December 12th., 1955, he was given by mouth 100 µc carrier-free ^{131}I. An uptake by the thyroid of 50 per cent of the ingested dose at 4 hours had fallen by 24 hours to 24 per cent (fig.19). The total serum radioactivity after 48 hours was 2.22 per cent of the dose per litre; 1.45 per cent was $PB^{131}I$ and 0.48 per cent was $BE^{131}I$. Urine collections were incomplete and their total radioactivity was not estimated.

Despite the relatively large tracer dose of ^{131}I radioactivity in butanol extracts of the serum was too low for identification. This failure was in part due to inexperience with the techniques and to difficulty in getting adequate serum samples.

In the autoradiographs prepared from the 0-6 hour (fig.20) and 6-48 hour (fig.21) two-way urinary chromatograms iodide (I^-) monoiodotyrosines (MIT) and diiodotyrosine (DIT) were identified. There was an unidentified spot (U1) of radioactivity near the origin and there was a second unidentified spot (U2) approximating to the expected site of thyroxine, which had not been applied as a marker. There was also an unidentified spot of radioactivity near the origin of the one-way chromatograms run in butanol acetic acid, and there was a second unidentified zone of radioactivity over-riding the thyroxine zone (fig.22).

Case 10. (Tables II, IV, and V; figs. 23-30).

On March 19th., 1956, she was given by mouth 50 µc of carrier-free ^{131}I. An uptake by the thyroid gland of 75 per cent of the dose at 4 hours and 24 hours had fallen

to 56 per cent in 72 hours (fig.23). The total serum ^{131}I after 48 hours was 0.25 per cent of the dose per litre; 0.12 per cent was PB^{131}I. The urine excretion figures were 4.8 per cent of the dose in the period 0-6 hours, 19.2 per cent in the period 6-24 hours and 6.7 per cent in the period 24-48 hours. The T factor was 0.8

On March 23rd., 1956, in anticipation of thyroidectomy she was given by mouth 1 mc of ^{131}I. Samples of blood were withdrawn at 6, 24, 48 and 72 hours, and urine was collected in divided periods.

In the serum chromatograms (figs. 24-26) iodide (I^-), monoiodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T4) were identified. Iodide, monoiodotyrosine and diiodotyrosine zones of radioactivity were more pronounced in 6 hour serum (figs. 24 and 25). By 48 hours the thyroxine-triiodothyronine zone (T4T3) showed the greatest activity (fig. 26).

In the urinary chromatograms (figs. 27-30) iodide (I^-) monoiodotyrosine (MIT) and diiodotyrosine (DIT) were identified. As in case 9 there was an unidentified spot (U1) near the origin of the two-way urinary chromatograms (figs.27 and 28), and there were also several other unidentified spots

(U2 and U3 in the 0-6 hour urine, and U2, U3 and U4 in the 6-24 hour urine) approximating to and beyond the expected sites of thyroxine and triiodothyronine which had not however been applied as markers. There was a zone of blackening at the solvent front, which might have been due to overloading the paper. There was unidentified radioactivity near the origin of the one-way chromatograms run in butanol acetic acid, and in this solvent there were at least 2 marked spots of radioactivity in and beyond the thyroxine-triiodothyronine zone (figs.29 and 30). Unfortunately the urinary chromatograms were over-exposed and the intense blackening that resulted detracts from their appearance. Radioactivity persisted in the thyroxine zone in the butanol acetic acid chromatograms when the urine or eluate from the unknown zones in this region was heated with N-HCl for 1 hour at 60°C.

Discussion

The results of the uptake, urinary and serum ^{131}I tests are similar to those of other affected members of the tinker

family-group. They have been considered with the tests in Phase I of the investigation.

The results of the chromatographic analysis of the serum and urine of cases 9 and 10 established beyond reasonable doubt that monoiodotyrosine and diiodotyrosine, as well as some thyroxine, were present in the serum of one of these patients (case 10), and that monoiodotyrosine and diiodotyrosine were excreted in the urine of both. The identification of monoiodotyrosine and diiodotyrosine in the serum of one of them and in the urine of both confirmed the contention that the rapid loss of radioactivity from the thyroid glands in many of this group of patients was due to the release of an organic iodine compound which was not the thyroid hormone, but was rapidly excreted in the urine.

It also suggested that the defect in synthesis in this group of patients was similar to the defect described by Stanbury et al. (1955 b, 1956 a & b) in a 27-year-old male, who had a congenital goitre and was hypothyroid, in his brother, and in an unrelated 12-year-old girl, who were also goitrous cretins. Monoiodotyrosine and diiodotyrosine were

noted in the serum of all 3 after ^{131}I. The deiodinating capacity of thyroid tissue removed from one of these cretins was tested by exposing radioactive D,L-diiodotyrosine as substrate to tissue slices from the gland (Querido et al. 1956). No deiodination of the diiodotyrosine occurred when the test tissue was from the cretin, whereas deiodination was readily demonstrable when thyroids removed surgically for nodular goitre or Graves disease, or when beef and sheep thyroids were used. These authors concluded that the thyroid glands of their patients were unable to conserve monoiodotyrosine and diiodotyrosine because they lacked the enzyme dehalogenase.

The abnormal loss of iodotyrosines in cases 9 and 10 appeared likely to be due to deficiency of dehalogenase (McGirr et al. 1956). Direct proof of the deficiency from study of the thyroid gland itself could not be obtained in 1956 because the only one of the patients in whom thyroidectomy was indicated on medical grounds, after requesting that her thyroid swelling be removed, refused operation. However, it seemed a reasonable tentative assumption that when the iodotyrosines became freed from

thyroglobulin they escaped into the circulation because there was no mechanism to remove their iodine in the thyroid gland. They were lost in the urine since there was no means of removing iodine in the periphery. A state of iodine want was thus established and hormone production was inadequate. Compensatory thyroid enlargement occurred but it failed to ensure sufficient hormone for the body's needs, and so the patients became hypothyroid. This hypothesis did not exclude the formation of some normal hormone. Indeed small amounts of thyroxine were identified in the serum of case 10. The clinical signs of hypothyroidism in this patient were reflected in the low serum $PB^{127}I$, namely 1.8 µg. per 100 ml, which confirmed that the production of hormone was inadequate.

The identification of thyroxine in the serum of case 10 disposes of an argument considered in the discussion of the Phase I tests, namely that no thyroxine was present in the serum of any of the cases. At the conclusion of the Phase II tests it was possible to state with some certainty that the misleadingly high values for serum $PB^{131}I$, that had been a common finding, had a two-fold explanation.

They were due in part to the precipitation by trichloracetic acid of monoiodotyrosine and diiodotyrosine with the proteins, and in part to the very rapid production and secretion of inadequate amounts of hormone with a large number of its molecules labelled with ^{131}I. It is presumably because of the high specific activity of the circulating hormone that its identification was possible despite the low serum $PB^{127}I$.

The identity of the unknown zones of radioactivity in the urine was not established. Stanbury et al. (1956 a) found similar zones in patients with impaired dehalogenase activity. It seemed likely that the chemical basis of this radioactivity was one or more of the conjugates of the iodotyrosines, probably with acetic acid, lactic acid and pyruvic acid. Unfortunately neither at this time nor later when similar zones of radioactivity were found in other patients of this family-group were such conjugates available for markers. Radioactivity in a similar site was later found in case 10 and in other affected tinker patients after the oral administration of radioactive monoiodotyrosine. It was also found in case VI (Chapter 17), who also had a

dehalogenase defect. The belief that its basis is a conjugate of an iodotyrosine appears to be a reasonable one (Chapter 7).

Stanbury et al. (1956 a) were of the opinion that the spot of radioactivity which they found in their patients near the origin of the chromatograms was a conjugate of monoiodotyrosine. I can add no information to confirm or refute their claim.

Conclusions

With proper techniques it was possible to identify monoiodotyrosine and diiodotyrosine in the serum and in the urine.

It appeared likely that the thyroid enlargement and hypothyroidism were due to the loss of these hormone precursors from the thyroid gland.

Their loss was probably due to deficiency of the enzyme dehalogenase, but direct proof of this hypothesis was lacking.

CHAPTER 7

PHASE III TESTS

Early experience suggested that very large 'tracer' doses of ^{131}I, such as $\frac{1}{2}$ - 1 millicurie, might be necessary if the chemical basis of plasma or serum radioactivity was to be identified. Such doses were discussed with the Isotopes Advisory Panel of the Medical Research Council. They were considered permissible only if thyroidectomy was to be undertaken.

Perusal of the literature about dehalogenase activity revealed that this enzyme was present in many other tissues besides the thyroid gland (Roche et al. 1952, 1953). If sporadic goitrous cretinism, particularly in the main tinker-group, was hereditary, as by this time was clear (Hutchison and McGirr 1956 and Chapter 9) and was caused by an abnormal gene then the basic genetic abnormality must be present from the moment of conception, and must later be passed on to every cell in the finally grown organism (Dent 1957). Hence the enzyme would probably be deficient or absent not only from the thyroid but from other tissues as well.

Stanbury et al. (1956 a & b) had made use of this knowledge when they had given radioactive diiodotyrosine intravenously to their patients and had observed the amount and chemical basis of the ^{131}I recoverable in the urine. In their 3 goitrous cretins diiodotyrosine was excreted unchanged in large amounts, for example over 50 per cent was excreted unchanged in the urine in the first 4 hours after the injection. Control studies in 15 normal subjects or patients with various diseases had shown that, with the exception of a patient with diabetes mellitus who excreted 7.3 per cent and 6.1 per cent in 2 tests, less than 4 per cent of the dose was excreted unchanged in the urine in 4 hours. The proportion of the ^{131}I excreted in the periods 0-1 hours, 1-2 hours and 2-4 hours present as diiodotyrosine did not exceed 18.1 per cent except in the patient with diabetes mellitus in whom it was 23.6 and 26.5 per cent in 2 tests.

Neither radioactive diiodotyrosine ($D^{131}IT$) nor radioactive monoiodotyrosine($M^{131}IT$) was readily available. It was clear that if either of these substances was going to be used, it would require to be prepared. Consideration was given to the relative ease of preparation of $M^{131}IT$ and $D^{131}IT$, and

it was decided to use $M^{131}IT$. Accordingly, whilst awaiting a patient of this tinker family-group in whom thyroidectomy was indicated so that fresh thyroid tissue would be available to study thyroid dehalogenase activity directly, a test was devised with $M^{131}IT$, which could be used to study dehalogenase activity indirectly. These tests proved to be a practical proposition because reasonably small amounts of ^{131}I, namely 5-25 μc, were used, and the $M^{131}IT$ could be given by mouth.

Methods

L-monoiodotyrosine labelled with ^{131}I was prepared by modification of the method of Pitt-Rivers (1956). L-tyrosine (181 mg., 1 m. mol.) was dissolved in 20 ml.of ammonia (sp. gr. 0.880), and was iodinated at room temperature by the slow addition of 2 ml. of N-iodine, containing 1 mc of $^{131}I_2$. The solution was evaporated to dryness in vacuo and then the residue was washed 3 times with iced water. The residue was dissolved in a weak ammonia solution from which an appropriate volume to give a dose of 5-25 μc was taken and diluted with water to mask the ammonia taste. Before use each batch of $M^{131}IT$ was checked by chromatography for purity. Contamination with ^{131}I as iodide was negligible.

An oral dose prepared in this way was then administered to the patients, and their urine was collected in the next 6 hours in 2 periods, 0-2 and 2-6 hours. The radioactivity in a 5 ml. sample from each period was measured and the total radioactivity in the period calculated. A sample from each period was examined by ascending chromatography in butanol acetic acid solvent after acidification to pH 2 with N-HCl and extraction with butanol. The chromatograms were run for 25 to 30 cm. The markers used were iodide (I^-) and monoiodotyrosine (MIT). The iodide was developed by spraying with 1 per cent palladium chloride; the monoiodotyrosine by spraying with diazotized sulphanilic acid, followed after drying by a spray of 10 per cent potassium carbonate. Each chromatogram was scanned for radioactivity by passing it between two end-window Geiger-Müller counters (G.E.C. GM4) connected through a rate-meter (EKCO type 1037A) with a recording milliammeter (Evershed and Vignoles). The chromatogram was then aligned with the milliammeter record, and the peaks of radioactivity on the record compared with the position of the markers on the chromatogram. The relative proportion of radioactivity in each peak was estimated by planimetry.

Clinical Material

Control studies were performed in 5 patients without thyroid disease, 3 patients with primary myxoedema, and 5 patients with non-toxic goitre.

Four goitrous cretins were studied. Two of these patients (cases 10 and 12) were members of the main tinker family-group. Two of them (cases 15 and 16) were also tinkers but there was no direct information that they were members of the main tinker-group. They came from a home for deprived children where they had been abandoned by their parents. Case 16 was euthyroid from thyroid medication at the time of the test (Chapter 3).

In addition 4 other members of the main tinker family cooperated voluntarily or with the agreement of their guardians in the test (Table VII, cases 17-20). Two of them (cases 17 and 18) were apparently healthy and euthyroid and neither had a goitre. Case 18, who was the husband of case 10, was also her cousin. Case 17 was the nephew of both cases 10 and 18. Unfortunately the information about the pedigree of cases 17 and 18 is inaccurate and they cannot be fitted into the family tree (fig.7). The third (case 19) was a ten-week-old infant; she was a niece

of cases 1-4. She had been admitted to hospital at 7 weeks with pyloric stenosis and had made a good recovery. She was euthyroid and non-goitrous. The fourth (case 20) was a seventeen-week-old infant; she was the second child of case 6, who had married outwith the tinker family-group. She had been admitted to hospital 2 weeks earlier with malnutrition. She was euthyroid and non-goitrous.

Results

The results are summarized in Table VII.

For the purpose of this test radioactivity in and beyond the thyroxine zone of the butanol acetic acid chromatograms (fig. 31) has been attributed to monoiodotyrosine conjugates. In chromatograms run in butanol ammonia there was a correspondingly high peak of radioactivity between the iodide spot and the thyroxine zone (fig. 32). Unfortunately markers for the acetic acid, lactic acid and pyruvic acid derivatives of monoiodotyrosine were not available so the precise identity of the excretion products other than iodide (I^-) and monoiodotyrosine (MIT) could not be established. Their presence in the urine after $M^{131}IT$ supports the belief that the unknown peaks of activity in the urine of cases 9 and 10 after

^{131}I were derivatives of the iodotyrosines (Chapter 6).

In the control patients under 6 per cent of the $M^{131}IT$ appeared in the urine as free or conjugated $M^{131}IT$ in the period 0-6 hours. The greatest proportion of the ^{131}I passed in either of the 2 periods present as $M^{131}IT$ was 17.6 per cent.

The findings in the 4 goitrous cretins studied are notably different. Twenty-eight per cent or more of the dose of $M^{131}IT$ appeared in the urine as free or conjugated $M^{131}IT$ in the period 0-6 hours. The relative proportion of the ^{131}I excreted as $M^{131}IT$ was equally strikingly elevated, particularly in the period 2-6 hours, when it was 64.6 per cent or more.

The results in the 4 apparently normal tinkers fall between those found in the 2 above groups. In the 2 adult subjects who were continent and gave full urine collections, the amounts of $M^{131}IT$ excreted as free or conjugated $M^{131}IT$, namely 6.7 and 9.7 per cent, were only slightly above the values of the control group. The two infants were incontinent and full and accurate collections were more or less impossible. As much urine as possible was collected and pooled in each period. Samples from each collection

were examined by chromatography as with the full collections of the adult patients. The proportion of ^{131}I excreted as free or conjugated $M^{131}IT$ was at least 31.7 per cent in one of the two collection periods for each of the 4 relatives.

Discussion

The results of the $M^{131}IT$ tests are similar to those obtained by Stanbury et al. (1956 b) with $D^{131}IT$ in a comparable series of patients. As far as these two series are concerned the $M^{131}IT$ and $D^{131}IT$ tests have been equally successful in revealing impaired ability to deiodinate an iodotyrosine, from which it is inferred that there is impaired dehalogenase activity. The two tests may not always be equally informative. Litvak and Stanbury (personal communication) have observed a 24-year-old man with cretinism and no detectable goitre (he had received full doses of thyroid for most of his life) who had excreted almost all of a dose of $D^{131}IT$ unchanged in the urine, yet on 2 occasions almost completely deiodinated $M^{131}IT$.

In the present investigation the $M^{131}IT$ test demonstrated impaired dehalogenase activity in 2 goitrous cretins of the main tinker family-group and in 2 goitrous cretins who were also

tinkers but were of unknown ancestry. It showed that the enzyme defect in the 2 groups is identical. Hence it may reasonably be concluded that cases 15 and 16 have a common ancestry with the main tinker family group.

The results of the $M^{131}IT$ tests in the 4 euthyroid relatives of the goitrous cretins suggested that their ability to deiodinate monoiodotyrosine was also impaired. These subjects were normal to outward appearances and there was no thyroid enlargement. Their biochemical defect was much less obvious than that of the goitrous members of the group. Admittedly the amount of $M^{131}IT$ excreted as free or conjugated $M^{131}IT$ by the 2 adults was only slightly above the values of the control group of patients but the proportion of ^{131}I excreted as free or conjugated $M^{131}IT$ in all 4 of the subjects was substantially higher than that found in any of the controls in at least one of the 2 collection periods. The evidence for a biochemical abnormality in the 4 relatives is perhaps rather slender. The absence of infant controls for cases 19 and 20 is particularly unfortunate in these circumstances. The explanation for their absenee is quite simple, namely a reluctance to expose a normal infant from healthy stock to any

radiation risk, however small.

The opinion that a minor defect in dehalogenase activity was present in the relatives was supported by the results of the $M^{131}IT$ test in an 11-year-old girl, who was euthyroid but goitrous, and in her mother (case VI, Chapter 17, and Table XIV). This is the only other patient I have encountered with a dehalogenase defect, apart from the tinker patients. Monoiodotyrosine and diiodotyrosine were identified in this girl's serum and urine after ^{131}I, and her thyroid gland failed to deiodinate $M^{131}IT$ in vitro. She excreted 54.3 per cent of a dose of $M^{131}IT$ as free or conjugated monoiodotyrosine in the first 6 hours after its ingestion; the proportion of the ^{131}I in her urine present as monoiodotyrosine or its conjugates was 97.3 in the period 0-2 hours, and 96.1 per cent in the period 2-6 hours. Her mother was a 29-year-old woman who was euthyroid and non-goitrous. She excreted 14.3 per cent of a dose of $M^{131}IT$ as free or conjugated monoiodotyrosine in the first 6 hours after its ingestion; the proportion of ^{131}I in her urine present as monoiodotyrosine or its conjugates was 43.4 per cent in the period 0-2 hours, and 1.0 per cent in the

period 2-6 hours. The values 14.3 per cent and 43.4 per cent contrast with comparable values of less than 6 per cent and 18 per cent in the control studies. The belief that the $M^{131}IT$ excretion test unmasked a minor biochemical abnormality in the 4 euthyroid relatives of the tinker patients also receives support from the investigations reported by Stanbury et al.(1956 b). Using $D^{131}IT$, they also found evidence of a minor biochemical abnormality in 4 of 5 relatives of one of their goitrous cretins who had a dehalogenase defect; these relatives were euthyroid, but were also goitrous. They excreted 4.5 to 10.9 per cent of an intravenous dose of $D^{131}IT$ unchanged in the urine in the period 0-4 hours compared with less than 4 per cent in the control patients. The proportion of the ^{131}I in the divided urine collections present as diiodotyrosine, which did not exceed 18.1 per cent in the controls, varied from 19.8 to 32.6 per cent.

It has been inferred from $D^{131}IT$ excretion studies that inability to deiodinate iodotyrosines peripherally in goitrous cretinism may be merely a secondary effect of the hypothyroidism (Mosier et al.1958, Trotter 1959). This belief is not supported by my experience with $M^{131}IT$. Normal amounts were excreted by 3 hypothyroid adult controls and excessive amounts by case 16 (Chapter 3) who was euthyroid from thyroid medication at the time of the test, and by other

euthyroid individuals, such as case VI (Chapter 17), her mother and the euthyroid relatives of the tinkers.

Reference will be made in more detail to the genetic aspects of dehalogenase deficiency in Chapter 9. Meanwhile it is opportune to state that genetic studies (Hutchison and McGirr 1956) had shown that the enzyme defect in the main tinker family group was dependent on the activity of a single autosomal gene. The defect was recessive. In the light of the results of the $M^{131}IT$ excretion tests it appeared to be a reasonable assumption that the patients with frank goitre and hypothyroidism were homozygous for the gene, while those relatives who showed only minor biochemical abnormalities were heterozygous carriers of the gene, which was incompletely recessive. The results with $M^{131}IT$ in case 20 confirmed this belief. She was a heterozygous carrier as she was the child of a goitrous cretin (case 6) and a normal male who was not a tinker. An abnormally high proportion of the ^{131}I in her urine was present as $M^{131}IT$.

The results with $M^{131}IT$ indicate that it should be possible to unmask the heterozygous carrier, as well as establish the enzyme defect in the frankly abnormal case. The self-contained existence of the tinker folk suggests that they would be an ideal group for genetic studies with a simple test such as we have

employed. Unfortunately to date it has proved difficult to win sufficient cooperation in a city remote from their usual haunts. Perhaps a field trial would be more successful.

Conclusions

Impaired ability to deiodinate $M^{131}IT$ given orally was demonstrated in two pairs of apparently unconnected goitrous cretins who were tinkers. This common disability supports the belief that the two pairs of tinkers have a common ancestry.

A similar but less marked disability was detected in 4 apparently healthy members of the main tinker-group. It is suggested that they are heterozygous carriers of the gene which is incompletely recessive.

The $M^{131}IT$ test offers a useful means of detecting impaired ability to deiodinate $M^{131}IT$ in other cases of goitre of unknown cause. It offers a means of detecting the heterozygous carrier of the enzyme defect, namely impaired dehalogenase activity, as well as of establishing the enzyme defect in the frankly abnormal case.

CHAPTER 8

PHASE IV TESTS

In the Summer of 1958 Mr. J.H.C. Philips, Consultant Surgeon to Kettering General Hospital, Northampton, reported that one of the 4 original patients, case 2, had recently come under his care, her family having migrated to England. She had previously been investigated in 1951 and again in 1955 and the results of these investigations have been recorded and discussed in Chapter 5 (Tables II-V and fig.18).

This patient was 11 years 7 months when goitrous cretinism was diagnosed in 1951 (fig.33) and treatment with dry thyroid, $1\frac{1}{2}$ grains daily, was started. The thyroid was taken irregularly but in 1955 her bone age was normal, and mentally she had vastly improved. She still had a large asymmetrical goitre (fig. 34). In 1958, because of the increasing size of her goitre, Mr. Philips decided that thyroidectomy was desirable. With his cooperation it was possible to study the iodine compounds in the serum, urine and thyroid gland after a dose of 1 millicurie of ^{131}I had been given orally

48 hours before operation on October 2nd., 1958. My assistant Miss W.Elspeth Clement went to Kettering General Hospital and studied the dehalogenase activity in the thyroid gland immediately following its removal. The rest of the thyroid gland, serum and urine samples were brought to Glasgow for examination by chromatography. A small portion of thyroid tissue was also prepared for electrophoresis of the thyroid proteins (Watson et al. 1959, and Chapter 13).

1. Study of dehalogenase activity of thyroid tissue.

Method

Immediately after the thyroidectomy two thin slices of fresh thyroid tissue, approximately 1 mm. thick, were removed from each lobe and from the isthmus. They were incubated overnight (18 hours) at 37°C. in Krebs-Ringer phosphate buffer (pH 7.4) which contained a trace of nicotinamide (final concentration = 2.5×10^{-3} M solution) and radioactive monoiodotyrosine ($M^{131}IT$). Enough radioactivity was used to give satisfactory chromatograms. After acidification to pH 2 with N-HCl a butanol extract of the buffer fluid was made. The extract was analysed

by ascending chromatography in butanol acetic acid solvent. Iodide (I^-) and monoiodotyrosine (MIT) markers were used. The experiment was performed in triplicate.

Experience had shown that this technique was satisfactory. The $M^{131}IT$ had been prepared and checked by chromatography to show that there was negligible contamination with ^{131}I as iodide or with $D^{131}IT$. Butanol extraction eliminated from the analysis any thyroglobulin that had leaked from the slices into the buffer fluid.

Results

Virtually all of the radioactivity persisted in the monoiodotyrosine zone of the chromatograms, and no radioactivity appeared in the iodide zone (fig. 35). In similar studies with thyroid tissue from 2 adult cases of non-toxic goitre and 2 children with sporadic goitres due to other enzyme defects the findings were entirely different. Over 90 per cent of the ^{131}I was removed from the $M^{131}IT$ and appeared in the iodide (I^-) spot of the chromatograms. A typical result is shown in fig. 36.

Discussion

The complete failure of fresh thyroid tissue under standard conditions in vitro to deiodinate $M^{131}IT$ indicates the absence of dehalogenase activity in the thyroid gland. It is unfortunate that it was not possible to control the test at the time with fresh normal thyroid tissue or with tissue from another case of goitre where preliminary studies had made a dehalogenase defect very unlikely. However, the technique had not failed to demonstrate dehalogenase activity in thyroid tissue from patients with goitres of various other kinds.

On only one other occasion in my experience have fresh thyroid slices failed to deiodinate $M^{131}IT$. This occurred with thyroid tissue from an 11-year-old girl who was euthyroid but goitrous. Her goitre was also due to a genetically determined dehalogenase defect. As has already been mentioned in Chapter 7 (see also case VI, Chapter 17) monoiodotyrosine and diiodotyrosine were identified in her serum and urine, and she deiodinated an oral dose of $M^{131}IT$ less well than the control subjects. Her mother, who was apparently normal and non-goitrous, also deiodinated oral $M^{131}IT$ less well than the controls.

These findings confirm that the fundamental biochemical defect in case 2, and the 12 other goitrous cretins who have been traced in her family group, is impaired or absent dehalogenase activity. Taken in conjunction with the indirect evidence of impaired dehalogenase activity found in cases 15 and 16 (Chapter 7) they support the belief that the various cretinous tinkers that have been studied have the same enzyme defect and owe their condition to the same recessive gene occurring in the homozygous state.

2. Chromatographic analysis of serum, urine and thyroid gland.

Methods

The patient was given by mouth 1 millicurie of ^{131}I approximately 48 hours before thyroidectomy. Blood samples were withdrawn at 3 hours and 48 hours after the dose of ^{131}I. Samples of serum of 10 ml. volume were used. They were brought to a pH of 2 with N-HCl, and were then extracted with 4 washes of N - butanol to remove all the extractable ^{131}I. The extracts were pooled in a crucible and evaporated to dryness in vacuo. A drop of methanol-ammonia (4 parts of methanol to 1 part of ammonia) was added. A drop was taken up in a capillary tube

and applied to Whatman No.1 filter paper. Ascending chromatograms were run in butanol acetic acid solvent[1]. After drying the paper and staining the markers the chromatograms were scanned for radioactivity by passing them between two Geiger-Müller counters (G.E.C. G.M.4) connected through a rate-meter (EKCO type 1037 A) to a recording milliammeter (Evershed and Vignoles).

Chromatograms of butanol extracts of urine passed in the periods 0-6, 6-24 and 24-48 hours were similarly prepared. They were run in butanol acetic acid[1], butanol ammonia[2], and collidine ammonia[3] solvents.

Portions of thyroid gland of approximately 0.5 g. weight were homogenized, and then digested overnight (18 hours) by trypsin in approximately the same volume of barbitone buffer at pH 8.6. Ascending chromatograms were prepared from the digests. They were run in butanol acetic acid[1], butanol ammonia[2], and amyl alcohol ammonia[4] solvents.

The chromatograms were run for 25-30 cm. Markers used included iodide (I^-) monoiodotyrosine (MIT), diiodotyrosine (DIT), 3-5, diiodothyronine (T2)*, triiodothyronine (T3) and thyroxine (T4).

* Supplied by Mrs. Pitt-Rivers.

Judicious selection of the markers to be run with the unknown spot was necessary. Experience had taught that care had to be exercised not to overload the spot, otherwise there was poor separation and smearing in the chromatogram. A marker applied to the paper alone did not usually run as well as the marker plus the unknown spot which appeared to carry it on. If there was sufficient material several runs were made with different markers. If there was only enough radioactivity in the specimen for a single run what was considered most likely to be the important marker was run with it and the other markers were run alongside. When the runs were completed the paper was dried by an electric heater, and the markers were stained by the application of the appropriate sprays [5,6].

Solvents

1. Butanol acetic acid.

   Butanol saturated with a mixture consisting of 1 part of glacial acetic acid and 5 parts of water. Beaker containing butanol and beaker containing glacial acetic acid placed on bottom of tank.

2. Butanol ammonia solvent.

   Butanol saturated with 2N-NH_4OH. This solvent is easier to use than butanol dioxane ammonia as it has not

the same tendency to become unsaturated.

Beaker containing butanol and beaker containing ammonia placed on bottom of tank.

3. Collidine ammonia solvent.

Collidine saturated with 2N-NH_4OH. Beaker containing collidine and beaker containing ammonia placed on bottom of tank.

4. Amyl alcohol ammonia solvent.

Tertiary amyl alcohol saturated with 2N-NH_4OH, boiled and the amyl alcohol layer separated.

Beaker containing amyl alcohol and beaker containing ammonia placed on bottom of the tank.

Sprays:

5. For iodide. 1% palladium chloride.

6. For iodotyrosines and iodothyronines. Spray of diazotized sulphanilic acid prepared from 0.05 M solution of sulphanilic acid in 9 per cent hydrochloric acid and 4.5 per cent sodium nitrate, placed together in equal volumes for 10 minutes at $0^{\circ}C$, followed, after drying, with a spray of 10% potassium carbonate.

Results

Serum studies.

At 48 hours 89 per cent of the total serum ^{131}I was protein-bound, and 86 per cent was extractable by 4 washes of butanol at pH 2; only 57 per cent was thyroxine-like (Table VI).

In the chromatogram of the 3 hour serum, iodide (I^-) and monoiodotyrosine (MIT) were identified, and there were traces of diiodotyrosine (DIT) and thyroxine (T4) (fig.37). The chromatogram of the 48 hour serum was technically poor; such radioactivity as was recorded was present in the diiodotyrosine (DIT) and thyroxine (T4) zones, but these zones were not well separated (fig.38).

Urine studies.

In each of the periods of collection 0-6, 6-24, and 24-48 hours, and in all the chromatograms iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT) were present (figs. 39-43). Other zones of radioactivity were presumably due to the presence of several unidentified conjugates of monoiodotyrosine and diiodotyrosine (Chapter 7).

In the butanol acetic acid chromatograms (figs.39-41)

the amount of iodide was most pronounced in the first period 0-6 hours, whereas the amount of conjugate towards the end of the strip, overlying the thyroxine-triodothyronine zone, was most pronounced in the final period 24-48 hours. Indeed these conjugate zones came to contain more radioactivity than any of the other zones.

In the butanol ammonia chromatograms the conjugate zone was sited just a little behind the thyroxine zone (fig.42). The colloidine ammonia chromatogram (fig.43) showed numerous peaks of radioactivity, some of which were no doubt due to derivatives of the iodotyrosines.

Thyroid gland studies

When the chromatograms were scanned for radioactivity monoiodotyrosine (MIT) and diiodotyrosine (DIT) were identified, but no thyroxine was detected (figs. 44-46). The MIT/DIT ratio varied from 2.9 in paranodular tissue (^{131}I concentration of 0.25 per cent of dose/g.) to 4.0 in nodular tissue (^{131}I concentration of 0.27 per cent of dose/g.) A zone of radioactivity, the chemical basis of which is not known, was present in chromatograms of the paranodular tissue digests. This zone, comprising 16.9 per cent of the radioactivity, was

located between diiodotyrosine (DIT) and thyroxine (T4) in the butanol acetic acid solvent (fig.44); it lay beyond the triiodothyronine (T3) zone in the butanol ammonia solvent (fig.45), and beyond the 3-5 diiodothyronine (T2) zone in the amyl alcohol solvent (fig.46). Elution experiments on chromatograms prepared from the thyroid gland of a case of familial goitre, in which similar zones of radioactivity were found, had established their common identity (Case VIII, Chapter 19).

Discussion

The serum and urine studies in case 2 confirmed the findings in cases 9 and 10 (Chapter 6), namely that monoiodotyrosine and diiodotyrosine were escaping from the thyroid gland into the circulation, and then were being excreted in the urine. Their urinary excretion products were apparently the free iodotyrosines and also several unidentified derivatives, probably various conjugates.

In the chromatograms prepared from the trypsin digests of the thyroid gland there were 3 findings of note.

Firstly, the amount of radioactivity in monoiodotyrosine greatly exceeded the radioactivity in diiodotyrosine. The MIT/DIT ratio varied from 2.9 to 4.0 in different portions of the thyroid gland. These ratios differ markedly from the ratio 0.5 - 0.75 found by Pitt-Rivers et al. (1957) in normal human thyroids. They also differ from the MIT/DIT ratios which I have found in rat thyroids at various times after an intraperitoneal injection of ^{131}I (Table VIII). In the rats the MIT/DIT ratios at 24 and 48 hours were 0.36 - 0.58, which

are similar to those found in humans.

The significance of the relative increase of monoiodotyrosine at the expense of diiodotyrosine, which is a deviation from the normal state of affairs, is obscure. It is clearly not a specific effect of a dehalogenase defect as I have found it in other cases of sporadic goitre due to other enzyme defects (Table XV). It has been reported by Pitt-Rivers et al. (1957) in adult cases of non-toxic nodular goitre, where the etiology was not defined but which were thought to be the result of a falling off of enzyme activity. I too, have found an elevated MIT/DIT ratio in 8 of 10 adult patients with nodular goitres (Table XVIII). It may well be merely an expression of a state of iodine lack, occasioned in case 2 by the loss of the iodotyrosines with their iodine from the thyroid. Such an explanation receives support from the studies of Bois and Larsson (1958) in the iodine deficient rat thyroid. However, the fact that a raised MIT/DIT ratio occurs in association with iodine deficiency does not establish that it is an effect peculiar to iodine deficiency. There might well be other explanations for it, just as there are other causes of goitre besides iodine deficiency. Knowledge of the $PB^{127}I$

content of the thyroid would be helpful in elucidating the rôle of iodine deficiency.

Secondly, no thyroxine was detected in the thyroid tissue though some had been detected in the serum. While this finding is consistent with difficulty in the oxidative coupling of two molecules of diiodotyrosine to form thyroxine, it is more probable that there was very rapid secretion of such thyroxine as was formed so that none was stored in the thyroid. Defective coupling of the iodotyrosines would require the deficiency of an oxidative enzyme. Absence of dehalogenase has already been established. It is contrary to the concepts of modern genetics that one gene could be responsible for two enzymes.

Finally, an unidentified zone of radioactivity was present in chromatograms run in each of the 3 solvents used. Elution experiments in another patient, whose thyroid showed similar zones of radioactivity, demonstrated that the three zones are due to a single substance. This zone has now in my experience been found in a goitrous cretin, a euthyroid patient with familial goitre (case VIII, Chapter 19), in a patient with Hashimoto's disease, and in 5 patients with

nodular goitre (Table XVIII). Traces of it have been found in rat thyroid (Table VIII). Unfortunately its chemical identity and significance are unknown.

3. Electrophoresis of thyroid proteins.

The method used for this investigation is detailed in Chapter 15 where it is more appropriately considered in relation to the cases of sporadic goitre, for it was to help in the investigation of one of them that the technique was devised.

The protein pattern in case 2 (fig.47 and Table XVI) was unremarkable, showing similar bands of protein (Q, T, Hb, Thy-g and X) to those usually found in a variety of thyroid conditions. Radioactivity was normally associated with the thyroglobulin (Thy-g) band. In this patient the radioactivity must have been due to monoiodotyrosine and diiodotyrosine constituents of the thyroglobulin as no thyroxine was detected in the gland chromatograms.

Conclusions

The direct demonstration that fresh thyroid slices failed to deiodinate M^{131}IT confirmed the hypothesis that the fundamental defect in the main tinker family group was impaired or absent dehalogenase activity.

Because of this enzyme defect the iodotyrosines escaped from the thyroid gland into the circulation and were then excreted free or in conjugated form by the kidney in the urine.

In consequence of the loss of the iodotyrosines and their iodine the thyroid glands of the tinker patients were unable to produce an adequate supply of thyroid hormone. One believes that the goitre is a compensatory enlargement of the thyroid, mediated through the anterior pituitary and increased production of thyrotrophin (TSH) in response to the inadequate output of hormone. The high TSH level found in the serum of case 11 (Chapter 3) supports this hypothesis.

Even considerable hyperplasia of the thyroid gland failed to achieve the production of sufficient hormone to meet the metabolic needs. As a result these patients were not only goitrous, they were also hypothyroid.

Presumably it was possible to identify the small amounts of hormone present in the blood because of its high specific activity. Failure to detect thyroxine in the gland was most probably the result of the rapid secretion of such thyroxine as was formed so that little or none was stored.

CHAPTER 9

HEREDITARY ASPECTS

Although there appears to be a hereditary tendency in the common type of sporadic 'athyroidic' cretinism, a familial prevalence is, in fact, distinctly uncommon (Childs and Gardner 1954). Consanguineous marriages amongst the parents of sporadic cretins are barely more common than among the parents of normal children. In striking contrast, familial prevalence and consanguinity of the parents are common in sporadic goitrous cretinism.

I have already referred to the endemic of cretinism which occurred in the village of Chiselborough in Somerset in the first half of last century (Norris 1848). Intermarriage existed to a greater extent in this parish than neighbouring parishes, and was considered to be one of a number of factors responsible for the endemic. The condition died out when sanitation was improved, the secluded valleys were opened up and frequent intermarriage among goitrous families ceased.

Osler (1897) was probably the first physician fully to appreciate the significance of the familial tendency in sporadic

goitrous cretinism and to comment upon the occurrence of consanguinity of the parents.

Analysis of the case records of the 134 sporadic goitrous cretins reported in the world literature between 1943 and 1957 reveals that 25 families, where siblings were affected, were responsible for 65 of the cases. Twenty-one cases occurred in 7 families in which the parents were definitely consanguineous. In addition there were 5 cases in 2 families in which the parents were almost certainly consanguineous. The influence of heredity is seen at its most marked in the family group of itinerant tinkers, the investigation of which I have just described and the hereditary aspects of which were described by Hutchison and McGirr in 1956.

The high familial incidence of sporadic goitrous cretinism and the frequent consanguineous marriages among the parents of the cretins led us (Hutchison and McGirr 1954) to the conclusion that the biochemical anomaly responsible for the hypothyroidism in these patients was an inborn error of metabolism of the type originally described by Garrod (1908),

and that it was probably transmitted by a recessive autosomal gene. A similar opinion was expressed by Wilkins et al. (1954) when they stated that the high familial incidence of this type of hypothyroidism suggested that there was an inborn defect in thyroid metabolism that was genetically transmitted. The occurrence of sporadic goitrous cretinism in identical twins (Frierson et al.1957) also suggests a genetic basis.

I now propose to examine the hereditary aspects of sporadic goitrous cretinism as it affects the main tinker family group in which my ^{131}I studies have shown absence of the enzyme dehalogenase.

The T - McP family group

Such details of the family tree as have been discovered are shown in fig.7. The members of this family-group are itinerant tinkers living chiefly in the West of Scotland in Kintyre, Argyll and the Isle of Islay. From time to time they travel in other parts of Scotland and occasionally they migrate temporarily to England. Very probably other members of the family group are scattered throughout the country, but it has not been possible to trace any of them with certainty.

These tinker folk are always on the move. They are usually on bad terms with the local police. These facts combined with the distance from Glasgow of their usual haunts have made collection of accurate data extremely difficult. Much of the information from which the family tree has been compiled was supplied by Dr.Catherine A.Brown, Executive School Medical Officer, Argyll County Council. We are well aware that the family tree is incomplete. It is as accurate as our pooled information allows. We have reason to believe that the family tree given here by no means exhausts the ramifications of the T - McP genes throughout Scotland.

The original male T came from Ireland to Scotland about 170 years ago. He settled in Southend, Kintyre, where he married his full cousin, a McP. A female T, (III 5) also married another McP, who was her full cousin. Our information is too scanty to give a satisfactory pedigree of the main McP family, which is probably scattered widely throughout the West and North of Scotland. As far as is known none of the marriages in generation II of the family was consanguineous, but in generations III and IV the amount of intermarriage is astounding. It is thought

to have been encouraged by the increasing isolation of these tinker folk from the other inhabitants of the country by their unique and self-demarcated mode of life.

The family tree (fig.7) shows that 13 known goitrous cretins (IV. 10; IV. 12-15; V. 6; V.7-10; and V.12-14) have appeared among 42 persons in 5 sets of siblings. Six were males and 7 females, an approximately equal sex distribution. All of these patients with the exception of the 13th. cretin (IV.14) have been investigated in hospital; 11 of them have had ^{131}I studies.

In addition in this family group there have been 4 cases of Werdnig-Hoffmann paralysis (V. 1-4) in another set of siblings.

None of the parents of the cretins or of the patients with Werdnig-Hoffmann paralysis showed any evidence of disease. Unfortunately not all of the supposedly healthy siblings of the cretins were available for examination.

Discussion

Goitrous cretinism in its various forms collectively shows a high familial incidence both in Europe and in the United States of America. Consanguineous marriages are

strikingly frequent among the parents of these cretins. The high familial incidence and frequent consanguineous marriages are particularly obvious in the main tinker family group. Their recognition enabled us (Hutchison and McGirr 1954) to forecast that this form of hypothyroidism must be genetically determined. It appeared likely that the hypothyroidism in this family group was due to a biochemical anomaly of the type originally studied by Garrod (1908) and that it was transmitted by a recessive autosomal gene. Garrod suggested that the immediate mechanism for such apparent abnormalities might be the absence of an enzyme essential for normal metabolism. It seemed to us that some forms of cretinism and myxoedema in childhood must be added to the growing list of inborn errors of metabolism.

In none of the tinker cases was there anything to suggest iodine deficiency or the ingestion of goitrogenic substances. Endemic cretinism usually appears in the family of a mother who is herself goitrous from iodine deficiency. All the parents of the tinker cretins were healthy. It is therefore extremely unlikely that the appearance of goitrous cretinism in the T - McP

family had anything to do with their common environment.

The pedigree of the family satisfies 4 of the criteria of simple recessive inheritance (Roberts 1940).

1. The parents of affected persons were normal to outward appearance.

2. There was a striking familial prevalence; in 3 of the 5 sets of siblings there was more than one cretin. In recessive inheritance the ratio of affected to normal is 1:3. The ratio was exceeded in this family, being 13:29 in the affected sibships. Such an excess is usual when there is adequate information only about sets of siblings in whom at least one person is affected, and the number of normals in unaffected sibship is unknown. It is not really surprising in the T - McP family because one of the goitrous cretins, case 11 (V.6), was the only child of unmarried parents. Further, inclusion of the children of the sibship with Werdnig-Hoffmann paralysis alters the ratio of affected to normal to 13:37 which is approximately 1:3.

3. All affected children were the offspring of consanguineous marriages.

4. The offspring of marriages of affected persons with normal persons are usually normal. Case 6 (IV.13) married a Cockney ex-boxer. They have 2 children who are apparently normal and non-goitrous. The younger of these children though normal to outward appearances has been shown to be a carrier of the enzyme defect (Table VII case 20).

   One additional criterion of autosomal recessive inheritance is not yet satisfied.

5. The offspring of marriages of two affected persons are all affected. In view of the predilection for consanguineous mating shown by this group of tinkers it may be that with adequate substitution therapy this position may alter with time.

Though it may properly be claimed that simple recessive inheritance cannot be proved on the strength of a single pedigree, the occurrence of familial prevalence and consanguinity in the families of other similar cases makes it extremely probable that the present cases were so determined. The appearance within the same pedigree of Werdnig-Hoffmann paralysis,

a disease known to be inherited as a Mendelian recessive (Gates 1946), is interesting. Here the first 3 criteria of recessive inheritance mentioned above are fulfilled.

In addition to the members of the main tinker group, 4 other tinker children with goitrous cretinism were studied (cases 13-16). Cases 13 and 14, who were referred from Dundee, were half-sisters with a common mother but a different father. The 2 other children of the mother's first marriage were normal. One of the 2 remaining children of her second marriage was also a goitrous cretin, but this child was not available for study. Case 15 and 16 were the only 2 affected siblings in a family of 6. Careful enquiry failed to produce any evidence that cases 13 and 14 were related to the T - McP family. Cases 15 and 16 were sent for investigation from a home for deprived children in Renfrewshire. Their parents could not be traced so no information about their family tree was available. In view of the unique and self-contained existence of the tinker folk with their predilection for intermarriage it seemed a probability that all the cretinous tinkers owed their condition to the same recessive gene. The $M^{131}IT$ test (Chapter 7) produced indirect

evidence of impaired dehalogenase activity in cases 15 and 16, as well as in cases 10 and 12 of the main tinker group. It confirmed the belief that they had the same enzyme defect and supported the opinion that they had a common ancestry.

The $M^{131}IT$ test demonstrated a similar but minor biochemical abnormality in 4 apparently normal relatives of the main tinker group. Using a similar test with $D^{131}IT$ Stanbury et al. (1956 b) in Leiden also demonstrated a minor biochemical abnormality in 4 of 5 euthyroid but goitrous relatives of one of their goitrous cretins who also had a dehalogenase defect.

The information provided by the family tree strongly supports the opinion that the enzyme defect in these tinker patients is genetically determined and that it is transmitted by a single autosomal recessive gene. The combined results of the biochemical and the genetic studies suggest that a single gene controls the presence or absence of a single deiodinating enzyme (Lancet 1956). The absence of the enzyme explains all the clinical features observed in these particular goitrous cretins.

It is a reasonable assumption that the patients with frank goitre and hypothyroidism were homozygous for the gene. The relatives who had a detectable but less obtrusive biochemical abnormality and either nothing in the way of clinical features or a goitre only were heterozygous carriers of the gene. As has already been pointed out (Chapter 7), the $M^{131}IT$ test in case 20 confirmed this assumption. She was obviously a heterozygous carrier, being the child of a goitrous cretin (case 6) and a normal male, who was not a tinker. Yet an abnormally high proportion of the ^{131}I in her urine was present as $M^{131}IT$.

If the disease is the result of a single enzyme defect, and if the enzyme is dependent upon a single recessive gene, the gene must be incompletely recessive for defects to occur in the heterozygous state (Neel and Schull 1954).

CHAPTER 10

GENERAL SURVEY OF THE VARIOUS BIOCHEMICAL DEFECTS DESCRIBED IN SPORADIC GOITROUS CRETINISM

Evidence has been presented to show that a fundamental biochemical defect, impaired dehalogenase activity, was responsible for the hypothyroidism and goitres in the affected members of a large tinker family-group. This defect is one of several that have been defined in sporadic goitrous cretinism. The evidence for these defects has recently been reviewed by Stanbury and McGirr (1957). They found that 3 mechanisms had been fairly well established.

1. The thyroid gland fails to utilize trapped iodide

The thyroid accumulates iodide with remarkable rapidity but it cannot produce iodotyrosines from tyrosine residues and iodide, presumably because of the lack of an oxidative enzyme. This defect is unmasked by an oral dose of potassium thiocyanate which discharges any iodide which has been accumulated by the gland but not incorporated into the organic form. Cases with this type of disability are apparently rare. It was first described by Stanbury and Hedge (1950) who studied 3 of 4 goitrous cretins in a family

of 7. Other cases with this defect have been reported by Stanbury (1951), Jackson (1954), Lelong et al.(1956), Schultz et al. (1957), Buchanan and Crooks (1959).

Stanbury et al. (1955 a) were able to study one of their patients again, and investigated the nature of the iodine compounds in the thyroid gland by methods such as chromatography. They found that iodide was present but there were no iodotyrosines or iodothyronines.

2. The thyroid gland fails to conserve the iodine of the iodotyrosines because of lack of the enzyme dehalogenase.

This defect was first suspected in the tinker patients whose investigation I have reported in this thesis (McGirr and Hutchison 1953, Hutchison and McGirr 1954, McGirr and Hutchison 1956, McGirr et al. 1956, McGirr et al. 1959 c). For defining the defect credit must be given to Stanbury and his colleagues working in Leiden (Stanbury et al. 1955 b, Stanbury et al. 1956 a & b, Querido et al. 1956). Other cases which may have had a similar defect have been reported by Burrell and Gairdner (1955) and Kunstadter et al.(1957).

In cases of this type monoiodotyrosine and diiodotyrosine escape from the thyroid gland into the blood because of lack

of the enzyme dehalogenase. The thyroid gland has to devote itself to the production and secretion of hormone precursors rather than to the formation of thyroxine and triiodothyronine. To compensate for their loss thyroid hyperplasia and enlargement occur in an attempt to ensure an adequate output of hormone.

3. The thyroid gland fails to couple iodotyrosines into iodothyronines with sufficient speed to secure an adequate supply of hormone. Such a defect was first postulated by Stanbury et al. (1955 a) in two goitrous sisters who showed the residual stigmata of cretinism. Thyroxine and triiodothyronine were present in the serum in normal amounts but the iodine in the thyroid gland was almost entirely present as mono- and di-iodotyrosine. Exhaustive chemical analysis of the entire thyroid gland of one of the sisters revealed only a trace of thyroxine. These authors suggested that the iodotyrosines were present in such large amounts that a small amount of iodinated thyronines was produced non-enzymatically. Presumably the primary failure was in the enzymatic coupling. As convincing proof of the existence of such an enzyme is as yet lacking it is not

surprising that there is no direct proof of its failure.

Lelong et al. (1956) gave a similar interpretation to their findings in one of their cases. Werner et al.(1957) also produced evidence that the defect in a 5-year-old goitrous girl with mild hypothyroidism was a failure of adequate coupling of iodotyrosines. The findings in the girl were somewhat unusual. Monoiodotyrosine and diiodotyrosine were identified in her serum and no good explanation of these findings was offered. The results of tests of dehalogenase activity were conflicting. On the one hand slices of fresh thyroid tissue were able to deiodinate $D^{131}IT$ normally in vitro. On the other hand there was some evidence to suggest slightly impaired dehalogenase activity in that an intravenous dose of $D^{131}IT$ was deiodinated less well than normal.

Mosier et al. (1958) also found evidence of impaired peripheral deiodination of $D^{131}IT$ in 2 goitrous cretins. Their thyroid glands contained only a trace of thyroxine and it was thought that their defect was an almost complete failure of iodothyronine formation. Homogenate of the only thyroid gland examined completely deiodinated $D^{131}IT$ in vitro. Peripheral deiodination of $D^{131}IT$ returned to normal in both

patients after thyroid replacement therapy. These findings, together with evidence of impaired peripheral deiodination of $D^{131}IT$ in adult patients with myxoedema (Stanbury and Litvak 1957) have suggested that the inability to deiodinate iodotyrosines, in particular diiodotyrosine, peripherally in goitrous cretinism is merely a secondary effect of the hypothyroidism (Trotter 1959). While the possibility exists that this explanation may on occasions account for the failure of the peripheral deiodination of $M^{131}IT$, it is certainly contrary to my experience (Chapter 7).

To date the presence of iodotyrosines in the serum and urine of patients with a so-called coupling defect has not been satisfactorily explained. It may be that the excessive accumulation in their thyroids of monoiodotyrosine and diiodotyrosine in conjunction with their hypothyroid state overwhelms a fundamentally normal dehalogenase system, which is temporarily at a disadvantage, or it may be that there is some link in the reactions of the enzymatic coupling of the iodotyrosines and their enzymatic deiodination.

4. Other, as yet ill-defined, defects.

It is clear that other cases of goitrous cretinism exist which cannot be explained by these defects. Some cases have

not been fully enough investigated to define the defect, often because of the limitations of technique at the time they were seen, yet the results obtained are of considerable interest. For example, chemical fractionation of the iodine components of thyroid tissue removed from the two patients described by Hamilton et al. (1943) showed that thyroxine-like iodine composed 11.4 to 16.4 per cent of the iodine present, and diiodotyrosine-like iodine 52 to 60.5 per cent. The chemical analysis suggested that thyroxine and diiodotyrosine were present in considerable, indeed adequate, amounts. Admittedly the identity of the thyroid iodine fractions was not indisputably established by chromatography, nor were the circulating iodine compounds measured. Their findings were, however, sufficient to lead the authors to the tentative conclusion that if the circulating thyroxine is extremely low or even absent in such patients the metabolic fault must lie in the inability to release, rather than in the inability to produce, the hormone.

Hubble (1953) described a family of 4 hypothyroid siblings each of whom had a goitre. Thyroxine was proved by chromatography to be present in the plasma and the thyroid gland of the eldest child. It was assumed that the amounts

of thyroxine formed by the thyroid glands of these children must be inadequate for their needs but no specific defect was defined. Di George and Paschkis (1957) described a 7½-year-old girl whose thyroid was discharging into her blood protein-bound iodine which was not butanol-extractable. Stanbury and McGirr (1957) also referred to 2 goitrous sisters seen in Glasgow who had an unidentified iodine compound in the serum, the behaviour of which was apparently similar to that described by Di George and Paschkis (1957). These 2 sisters are considered in detail in the second part of this thesis along with a younger sister who also had a goitre (cases VIII-X, Chapter 19). An unidentified iodine compound with similar characteristics was found in the serum of patients with thyroid carcinoma (Robbins et al.1955, Tata et al. 1956) and Hashimoto's thyroiditis (Owen and McConahey 1956). De Groot et al. (1958) reported the presence of an abnormal iodinated compound in the serum of a patient with congenital goitrous hypothyroidism. This material was precipitable with proteins, was non-dialyzable, was sedimented with proteins in the ultra centrifuge, and was insoluble in butanol. It moved with the serum albumin fraction on electrophoresis, but was apparently not part of, nor necessarily associated with serum

albumin, since it did not precipitate in the presence of albumin antiserum. Enzymatic hydrolysis liberated monoiodotyrosine, diiodotyrosine and thyroxine. They considered that their findings were consistent with the postulate that the defect in the patient was the formation of an abnormal thyroprotein in the gland, or abnormal fragmentation and release of normal thyroglobulin.

CHAPTER 11

GENERAL CONCLUSIONS

The investigations which have been described and discussed were undertaken in the first instance to establish the cause of goitrous hypothyroidism in 4 tinker siblings. Seven years elapsed before the enquiry could be rounded off by the direct demonstration of the enzyme defect responsible for their condition, namely impaired dehalogenase activity. It was shown that fresh thyroid tissue completely failed, under standard conditions in vitro, to deiodinate monoiodotyrosine labelled with ^{131}I. Meantime genetic studies had indicated that the biochemical anomaly, which was present in the family group of tinkers to which the original patients belonged and in which by this time there were 13 known cases of sporadic goitrous cretinism, was genetically determined and was transmitted by a single incompletely recessive autosomal gene.

Sporadic goitrous cretinism is a rare condition which can be explained on the basis of arrest at any stage of the synthesis of the thyroid hormone. The various defects that

have been established have been discussed already (Chapter 10). Among them the dehalogenase defect is unique in that the genetic aspects have also been worked out.

While the main purpose of the investigation has been achieved, namely to determine the cause of goitrous cretinism in a special group of patients, two incidental problems remain outstanding. They are:

1. The determination of the chemical basis and significance of a zone of radioactivity present in chromatograms of thyroid gland from one of the cases.
2. The precise identification of the various conjugates of monoiodotyrosine and diiodotyrosine present in the urine of these cases.

PART II

SPORADIC GOITRE

CHAPTER 12

SPORADIC GOITRE

Before embarking on studies in patients with non-toxic goitres but without other clinical evidence of thyroid dysfunction a preliminary survey was made of current knowledge about the aetiology of non-toxic goitre (Scottish Medical Journal 1959).

It emerged that iodine deficiency was widely accepted as the cause of endemic goitre in those regions of the world where its incidence was high, but it was also apparent that other factors might and on occasions must operate. These included excess lime in the water and soil, and an inherited predisposition resulting from consanguineous unions and inbreeding. Intermarriage in geographically isolated communities probably played a particularly important rôle in the causation of endemic cretinism. A biochemical anomaly consistent with a defect in hormone synthesis had been described in 3 cretins from an endemic area of Northern Italy (Costa et al. 1953). Suggestions for an infective origin were unconfirmed.

With regard to sporadic goitre, its aetiology in most cases was an enigma. While there was no evidence

incompatible with the iodine deficiency hypothesis there was no proof that iodine deficiency was a major or even an essential factor in its causation. Indeed one was forced to the conclusion that the precise rôle of iodine deficiency in the production of sporadic goitre remained unestablished. It certainly appeared a fair conclusion that if a low dietary intake of iodine played a part it was unlikely to be the sole cause. Clinical goitre might on occasions be due to the therapeutic use or accidental ingestion of substances with a goitrogenic action but in practice it appeared to have been rarely produced by the consumption of goitrogenic foods. As with endemic goitre so with sporadic goitre there was evidence for a hereditary factor.

Attempts had been made to reconcile the hypotheses of iodine lack and inherited predisposition (Brain 1927). It was suggested that in some cases of sporadic goitre iodine utilization was so defective that the amount derived from a normal environment was inadequate to prevent goitre. In other cases with a less pronounced defect goitre only developed when the available supplies of iodine were less

than normal, though the shortage was insufficient to cause goitre in persons not predisposed. In short, an environment suboptimal in iodine brought out an inherited predisposition. It was also suggested that the inherited element in goitre might be based on lack of an enzyme which controlled the transformation of tyrosine to thyroxine (Gates 1946).

The belief that goitre might be due to defects in the utilization of iodide, that the defects might be hereditary and that the inherited element might be based on the enzymes which control the stages in the synthesis of the thyroid hormone had been confirmed in sporadic goitrous cretinism. Because there was also evidence that heredity predisposed some individuals to goitre and not others living in the same environment it appeared a worthwhile proposition to examine the hypothesis that sporadic goitre might be due to disturbances of hormone synthesis similar to those found in sporadic goitrous cretinism, but of lesser degree.

As a first step in the examination of the hypothesis I decided to investigate adolescent or young adult patients who had large goitres present from an early age, and

especially those who had goitrous siblings.

CHAPTER 13

CLINICAL MATERIAL

Ten patients, each of whom had a goitre, were studied. Though their goitres were indistinguishable on clinical examination various biochemical abnormalities were found and they have been classified into 4 groups according to the defects. These were:-

I. Impaired ability to utilize trapped iodide (Chapter 16).

II. Impaired dehalogenase activity (Chapter 17).

III. Impaired ability to couple iodotyrosines to produce iodothyronines (Chapter 18).

IV. Production of an abnormal iodinated thyroid protein (Chapter 19).

A summary of the relevant clinical data in each group is presented in Table IX.

There were 5 patients in Group I, namely 2 sisters, 2 siblings and an isolated male, whose ages ranged from 10 to 19 years. Cases I, III and IV were each sent to hospital because of an enlarged thyroid gland, and this led to the discovery of cases II and V. The thyroids of

cases I, II and IV were so large that thyroidectomy was indicated. The thyroid of case III showed moderate enlargement. He had had a partial thyroidectomy at the age of 3 months to relieve pressure symptoms. The goitre of case V was small and it had not been previously noticed. In cases III and IV the goitre had been observed at or shortly after birth. In all cases the goitres were multinodular. None of the patients showed any signs of hypo- or hyperthyroidism. All were congenital deaf-mutes. Two deceased siblings of cases I and II had also been deaf-mutes.

In Group II there was only one patient, a 9-year old girl with a large goitre present since she was 7 years. She was admitted to hospital for thyroidectomy when she was 11 years old because the thyroid swelling had recently increased in size. She had then a large multinodular goitre. Clinically she appeared to be euthyroid. The histological picture was that of nodular goitre in childhood with adenocarcinomatous change in places.

There was only one patient in Group III, a 10-year old boy with a congenital goitre. He was admitted to hospital for thyroidectomy because the goitre had enlarged considerably in

the past year. His thyroid gland was markedly enlarged and was nodular. He showed no signs of hypo- or hyperthyroidism.

Group IV consisted of 3 sisters, aged 13 to 21 years when they came under my supervision at hospital. They all had very large multinodular goitres. They had had a goitrous brother who had died aged 9 years when he had diphtheria. The youngest girl, case VIII, had had a goitre from birth but she never showed any signs of hypo- or hyperthyroidism. The second girl's goitre (case IX) had been noticed when she was one year old; her initial development had been satisfactory but she had undoubtedly been hypothyroid by the time she went to school. She was almost certainly euthyroid when I saw her at the age of 18 years. The oldest girl's development (case X) had been retarded from infancy. A goitre had first been noticed when she was 7 years old. She was still hypothyroid when I saw her at the age of 21 years. Earlier attempts at thyroidectomy in cases VIII and X had been abandoned because of technical difficulties, but the operation was successfully performed when they were 19 and 21 years old. Case IX also had a successful thyroidectomy when she was 18 years old.

None of the parents of any of these patients had goitres and they were unrelated in all cases. A paternal great-aunt of cases IV and V, and a maternal aunt of case VI had had a goitre.

None of the patients came from an area where goitre is endemic, and there was no history to suggest the consumption of any goitrogenic food or drug.

Response to Treatment

Thyroidectomy was performed on all the patients with the exception of cases III and V. In each case as much thyroid tissue as possible was removed, varying from 100 g. in case VI to 560 g.in case VIII, and substitution treatment with dry thyroid or sodium l-thyroxine was given to prevent the recurrence of thyroid enlargement and to keep the patient euthyroid. Doses of dry thyroid from $\frac{1}{2}$ - 4 grains daily or their equivalent of sodium thyroxine were used. In only one patient (case I) did thyroid swelling recur. Following her discharge from hospital she had neglected to take any treatment, yet she remained euthyroid. She was re-started on treatment 16 months after operation. Eight months later she was still euthyroid but the recurrent thyroid enlargement

was little altered in size. Likewise thyroid medication had little effect on the size of the goitre of case V. On the other hand the enlargement of the thyroid of case III diminished, though the goitre did not disappear after 7 months treatment with the biggest dose used, namely 4 grains dry thyroid daily. All the patients remained euthyroid while under observation for periods ranging from 6 months to 3 years.

A more detailed description of each patient is presented when each of the 4 groups is dealt with separately (Chapters 16-19).

CHAPTER 14

ROUTINE ^{131}I INVESTIGATIONS

Evaluation of thyroid uptake of ^{131}I, urinary excretion of ^{131}I and serum ^{131}I.

The simple routine ^{131}I investigations gave results which followed a pattern common to all the cases, and are therefore best dealt with together. They are of interest because they suggest thyroid overactivity in the absence of the clinical features of thyrotoxicosis.

Methods

The methods used were similar to those already described in Chapter 5. In addition in most of the cases the thyroid plasma ^{131}I clearance was estimated after an intravenous dose of 25 μc ^{131}I (Berson et al.1952).

Results

The uptake of ^{131}I by the thyroid glands, the urinary excretion of ^{131}I and the values for serum ^{131}I are summarized in Tables X, XI and XII.

Discussion

Uptake of ^{131}I by the thyroid gland (Table X).

The ^{131}I tests demonstrated the unusual avidity for iodide which has been consistently found in various groups of sporadic goitrous cretins. The initial thyroid clearances which ranged from 360 to 1170 ml. per min. per 1.73 sq.metres in 7 of the 8 patients in whom clearances were carried out were greatly increased compared with an upper value of 80 ml. per min. per 1.73 sq. metres in normal subjects. The 8th. patient (case IV) had recently received Lugol's iodine and there was no detectable accumulation of ^{131}I in her thyroid gland. The values of the uptake of ^{131}I by the thyroid at 24 hours were also above the upper limit of normal, namely 50 per cent of the dose, in 6 of the 8 patients in whom such estimates were made. In the remaining 2 patients the clearance values were abnormally high. In one of them (case III) there was little change between the half-hour uptake of 38 per cent and the 24 hour uptake of 43 per cent found on different occasions. In the other(case VI), who had a dehalogenase defect (Chapter 17) there was a substantial fall in the ^{131}I content of the thyroid from the half-hour value of

74 per cent to the 24 hour value of 35 per cent. This result follows the pattern already described in the tinker patients with a dehalogenase defect (Chapter 5).

Urinary excretion of ^{131}I (Table XI).

Urine studies were made on only 4 of the patients. The amount of ^{131}I excreted in 0-24 hours fell below 20 per cent of the dose in 3 of them and so, like the clearance and uptake values, lay in the thyrotoxic range. Likewise in these same patients the amounts excreted in 6-24 hours were in or almost in the unequivocal thyrotoxic range of 0-5 per cent. The T factor, despite the inaccuracies that must have resulted from the small amounts of ^{131}I excreted and the difficulties in urine collections in a general medical ward, gave a more accurate reflection of the clinical status. It was normal in 3, and in one (case X) lay in the equivocal zone between the hypothyroid and normal ranges.

Serum ^{131}I studies (Table XII).

The 48 hour serum protein-bound ^{131}I values were normal in 2 (that is, less than 0.40 per cent of the dose per litre) and elevated in 6 of the 8 patients in whom they were estimated. It may be a chance finding that among Group I patients only 1 in 3

had an anomalous $PB^{131}I$ pre-operatively, compared with 5 out of 5 of the patients in Groups II-IV. On the other hand it may reflect in part their difficulty in the organic binding of iodine (Chapter 16). This explanation though certainly a possibility does not appeal to me as being very likely, for in case I the $PB^{131}I$ increased from 0.22 to 0.66 per cent of the dose after operation, presumably due to an even further lowering of the organic iodine pool from loss of most of the thyroid gland at operation.

The explanation of the anomalously elevated $PB^{131}I$ values may be twofold:

1. A rapid turnover of ^{131}I in the thyroid gland associated with a low organic iodine pool.
2. The inclusion with thyroxine of some other protein-precipitable organic ^{131}I compound.

The first explanation alone almost certainly accounts for the elevated $PB^{131}I$ in case III, and in case II post-operatively. It almost certainly does not completely account for the elevated $PB^{131}I$ in case VI in whom a dehalogenase defect was found and iodotyrosines identified in

her serum (Chapter 17). No doubt iodotyrosines were included with the thyroxine and increased the $PB^{131}I$ value. As will become clear later (Chapter 19) it does not account for the elevated $PB^{131}I$ in cases VIII-X for in their serum some organic ^{131}I compound which was protein-precipitable but not butanol-extractable was present.

The conversion factor (the percentage of the serum ^{131}I which is protein-precipitable) was abnormal in the 8 patients with $PB^{131}I$ values. Values above 50% are often accepted as evidence of thyrotoxicosis, but as I have shown already, they also proved to be a common finding in the tinker patients with sporadic goitrous cretinism (Chapter 5).

Conclusions

The gland uptake, urinary excretion and serum ^{131}I studies in this group of patients stress the dangers of interpreting the results of such tests without knowledge of the clinical problem. They could too readily be accepted as evidence for thyrotoxicosis. Of these simple tests the urinary T factor, as in sporadic goitrous cretinism, most closely reflected the clinical picture.

CHAPTER 15

SPECIAL INVESTIGATIONS

In addition to the routine ^{131}I studies the special techniques, which had been used to establish the cause of the goitrous cretinism in the tinker patients, were also employed to discover the cause of the sporadic goitres. These techniques were:

1. Effect of anion block on the ^{131}I content of the thyroid gland.

Potassium thiocyanate (2 g.) or potassium perchlorate (600/800 mg.) was given by mouth approximately 30 minutes after an intravenous dose of ^{131}I. Its effect on the ^{131}I content of the thyroid gland was noted.

These anions not only block any further uptake of ^{131}I by the thyroid, they discharge any ^{131}I which persists as iodide in the gland and which in consequence does not become protein-bound. This test showed whether or not the thyroid had any difficulty in utilizing trapped iodide, thereby indicating (Group I, cases I-III and V) or eliminating (Group II, case VI; Group III, case VII and Group IV, case VIII) impaired peroxidase activity.

The results are summarized in Table XIII.

2. Study of dehalogenase activity .

(a) of thyroid tissue

The technique has already been described in Chapter 8. Thyroid tissue from case II (Group I) and case VIII (Group IV) deiodinated more than 90 per cent of the $M^{131}IT$ *in vitro*, whereas thyroid tissue from case VI (Group II) failed to deiodinate $M^{131}IT$ *in vitro*.

(b) of other tissues

Details of the $M^{131}IT$ excretion test are given in Chapter 7. It revealed impaired deiodination of an oral dose of $M^{131}IT$ in case VI (Group II), and normal deiodination in case VII (Group III) and cases VIII and X (Group IV).

The results are summarized in Table XIV.

3. Chromatography of serum, urine and thyroid gland .

The techniques used have been described in Chapter 8. Twenty-four or 48 hours before thyroidectomy an oral dose of ^{131}I was given; 100 μc ^{131}I was given to cases I, II, VI, and VII, and 1 mc. ^{131}I to case VIII. This large dose of ^{131}I was given after careful consideration of the risks in relation to the size of her thyroid gland (560 g) and after consultation with the Isotopes Advisory Panel of the Medical Research Council.

(a) Serum and urine

With the smaller dose of ^{131}I the serum radioactivity was barely enough to allow identification of its chemical basis. However, in case VI iodotyrosines, which are not normally detectable in the serum and urine, were found in the serum as well as in the urine. Thyroxine was also identified in the serum. In case I, though the basis of the radioactivity in the serum was not identified, the urine was examined and found to contain only iodide. In case VIII after 1 mc ^{131}I thyroxine and a trace of triiodothyronine were identified in the serum and iodide in the urine.

(b) Thyroid gland

The techniques used in the individual cases varied until a satisfactory method, using the direct examination of enzyme digests of gland homogenate, was established. In case I thyroid tissue was hydrolyzed by boiling for 16 hours with 2N-NaOH. In cases II, VI and VII it was digested by trypsin in barbitone buffer at pH 8.6 for 18 hours. In case VIII trypsin and chymotrypsin were used. In cases II, VI and VIII the digests were examined directly; in cases I and VII butanol extracts of hydrolysate

and digest respectively were used after acidification to pH 2.

The results are summarized in Table XV. Various abnormalities were found.

(i) Absence of thyroxine.

Thyroxine was identified in one of the Group I cases (case I), but was absent from the other examined by chromatography (case II). It was also found in case VI (Group II). It was found in case VIII (Group IV) but only after alkaline hydrolysis of an unusual zone (zone C) of radioactivity at the origin of the chromatograms. Thyroxine was not found in case VII (Group III).

(ii) The MIT/DIT ratio deviated from the normal in all cases. It was increased in glands from GroupsI-III, but was diminished in case VIII (Group IV).

(iii) Abnormal ^{131}I compounds.

Two abnormal compounds were present in the gland of case VIII. One of these (zone C) at the origin of the butanol acetic acid chromatograms was not butanol-extractable, proved resistant to enzyme digestion, but broke down with alkaline hydrolysis thus revealing the only thyroxine detected in this gland. This compound

will be considered in detail when Group IV cases are discussed. The second unknown zone of radioactivity (Y) between diiodotyrosine (DIT) and thyroxine (T4) was characterized in different solvent systems but its identity was not established.

The discovery of the unusual compound (zone C) in case VIII, which suggested that an abnormal thyroid protein might be present, stimulated an enquiry into the findings on electrophoresis of the thyroid proteins.

4. Electrophoresis of thyroid proteins.

After trial and error a standard technique was evolved (Watson et al. 1959). A small portion of thyroid tissue was homogenized in approximately half its volume of barbitone buffer (pH 8.6). The best results were achieved if rough handling of the tissue was avoided. It was snipped into tiny pieces with scissors, and then agitated in a pipette shaker for 30 minutes. The homogenate was centrifuged and small amounts of the supernatant liquid (0.05 and 0.02 ml.) were applied to strips of Whatman No.1 filter paper. Electrophoresis was performed at 120 volts,

current strength 1 ma. per 5 cm. breadth of paper for 16 hours. The paper was dried and the proteins stained with aqueous bromphenol blue. It was scanned for radioactivity in the scanner used for the chromatographic strips.

Experience in 23 thyroid glands of different types established the presence of a number of protein bands (fig. 48). These bands are:

1. Q band at or just beyond the starting point present in 22 out of the 23 glands.

2. T band, probably gamma globulin, present in 11 out of the 23 glands.

3. Hb band, due to haemoglobin present in all of the glands.

4. Thy-g band, due to thyroglobulin, present in 20 glands, probably present in 2 glands and not detected in 1 gland.

5. X band, almost certainly albumin, present in all of the 23 glands.

In glands from patients with non-toxic nodular goitres (8 cases examined) the radioactivity was found chiefly in the thyroglobulin zone (fig. 48) and sometimes to a lesser extent in the albumin zone (fig. 49).

The results in cases II and IV (Group I), case VI (Group II) and case VIII (Group IV) are summarized in Table XVI. Cases II and VI followed the pattern found in non-toxic nodular glands.

In case IV who had been taking Lugol's iodine no Q protein was found and no radioactivity was present in her gland. The remarkable finding however was the association of radioactivity with the Q band in case VIII.

Comment on Special Investigations

The results of the special investigations established differences in the different groups of patients. Thus, cases in Group I were readily acceptable as a distinct group whose thyroids had difficulty in oxidizing iodide to iodine and combining it to protein. Case VI was unique in that she was unable to conserve iodotyrosines within her thyroid, and her thyroid and other tissues were unable to deiodinate $M^{131}IT$. Case VII, who was one of the earliest patients with sporadic goitre investigated, differed in that both the initial utilization of iodide and the conservation of the iodotyrosines within the thyroid were normal, as was his ability to deiodinate an oral dose of $M^{131}IT$. No thyroxine was detectable in his thyroid gland. The findings in this case are consistent with a defect in coupling iodotyrosines to produce iodothyronines. Group IV

cases differ clearly from Groups I and II. They had no apparent difficulty in the early utilization of iodide or in the conservation of iodotyrosines, but there is evidence which suggests that an abnormal thyroid protein was present in the thyroid gland.

[illegible] (Fig. 50)

CHAPTER 16

GROUP I CASES

IMPAIRED ABILITY TO UTILIZE TRAPPED IODIDE (fig.50)

Case reports

Case I. M.McG. (fig. 51). A girl, aged 15 years, was admitted to hospital on March 6th., 1957. She was the fourth child of a family of 8, 6 of whom were by her mother's first husband. The parents had been unrelated and there was no family history of goitre or deaf-mutism. Of the first 6 children 2 suffer from goitre and deaf-mutism (cases I and II), and 2 who died in early childhood were deaf-mutes.

At the age of 2 years congenital deafness was diagnosed. She went to a special school from the age of 3 years and had learned to lip-read. Goitre was first noted at the age of 10 years. For the past 2 years it had been causing increasing dysphagia. The menses started at 13 years and were normal.

She was a normally proportioned adolescent. Her height was 161.3 cm. and her weight was 56.1 Kg. Pulse rate 80 per minute. Blood pressure 120/80 mm.Hg. She appeared to be of average intelligence in spite of deaf-mutism. There was a large goitre with numerous firm or cystic nodules. No bruit was audible over the gland. There were no signs

of hypo- or hyper-thyroidism. The haemoglobin level was 13.2 g. per 100 ml.; red cells 4.04 million per c.mm. Radiographs of the skeleton confirmed that ossification was normal for her age. The E.C.G. was normal. Serum cholesterol was 170 mg. per 100 ml. The B.M.R. was 12 per cent below standard.

Pre-operative ^{131}I tests. After an intravenous dose of ^{131}I on March 11th., 1957, the accumulation of ^{131}I by the thyroid gland was unusually rapid. Thyroid plasma clearance in the first 5 minutes was equivalent to a clearance of 637 ml. per minute per 1.73 sq. metres. After 5 minutes there was a dramatic fall in the rate of accumulation of ^{131}I. The corrected* uptake of ^{131}I by the thyroid gland at 5 minutes was 40 per cent, at 30 minutes 48 per cent and at 24 hours it was 56 per cent. Blood values 48 hours after the dose of ^{131}I showed that the total serum ^{131}I was 0.31 per cent of the dose per litre and the protein-bound ^{131}I was 0.22 per cent of the dose per litre. Urine excretion figures were 18.5 per cent

* The uptake observed over the thyroid less the contribution due to general body radioactivity.

of the dose in the period 0-6 hours, 12.3 per cent in the period 6-24 hours, and 4.2 per cent in the period 24-48 hours.

Chromatography of a butanol extract of urine showed that the radioactivity extracted was confined to iodide.

Another intravenous test was performed on March 25th., 1957. The accumulation of ^{131}I by the thyroid gland followed a similar pattern. On this occasion she was given 2g. potassium thiocyanate orally after 30 minutes. An almost immediate fall in the radioactivity of the gland followed, from a corrected* value of 48 per cent of the dose to 14 per cent (fig.52; Table XIII). After 24 hours 13 per cent of the dose of ^{131}I remained in the gland.

Treatment and progress. An extensive subtotal thyroidectomy was performed by Mr. Ian McLennan on April 2nd., 1957. The removed thyroid tissue weighed 215 g. Dry thyroid, $\frac{1}{2}$ grain daily, was started after operation to diminish the likelihood of recurrence and to obviate the development of hypothyroidism. She was discharged from hospital on April 16th., 1957. She did not report for follow-up until August 18th., 1958. Although she had not

been taking dry thyroid as had been advised she was still euthyroid and had a small goitre.

Chromatography of thyroid gland. Twenty-four hours before thyroidectomy 100 μc of ^{131}I was given by mouth. Portions of the gland were hydrolyzed for 16 hours with 2N-NaOH. After acidification to reduce the pH to 2, butanol extracts of the hydrolyzate were made. Ascending chromatograms were run in butanol acetic acid and butanol ammonia solvents. After drying and staining of the markers the chromatograms were scanned for radioactivity. Iodide (I^-), monoiodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T4) were identified (figs. 53 and 54; Table XV).

Post-operative ^{131}I tests. On August 26th., 1958, another intravenous dose of ^{131}I was given which revealed persisting avidity of the thyroid remnant for ^{131}I (Tables X and XII). Rapid accumulation of ^{131}I in the gland occurred in the first 16 minutes giving a thyroid plasma clearance value of 188 ml. per minute per 1.73 sq. metres. The corrected* uptake of ^{131}I at 16 minutes was 27 per cent of the dose, at 30 minutes 36 per cent and at 24 hours 44 per cent. After 48 hours the total serum ^{131}I was

0.97 per cent of the dose per litre and the protein-bound ^{131}I was 0.66 per cent of the dose per litre.

Further treatment. She was restarted on thyroid and has remained euthyroid on sodium thyroxine 0.05 mg.t.i.d. The thyroid swelling has not appreciably altered in size.

Case II. B. McG. A girl, aged 19 years, the sister of case I, was seen as an out-patient on August 19th., 1958. She was recognised to be deaf about the age of 2 years and she attended a special school until the age of 16 years. She had always been smaller than her contemporaries at school. Menstruation started when she was 14 years of age. Shortly after taking up employment a goitre was first noticed.

Inspection revealed a small stockily-built young woman. Her height was 148 cm. and her weight was 55.9 Kg. Pulse-rate 84 per minute. Blood pressure 115/80 mm. Hg. She was a deaf-mute, rather emotional but of average intelligence. There was a concomitant convergant strabismus. Her thyroid gland was markedly enlarged, lobular and firm. No bruit was audible. She was euthyroid. Radiographs of the skeleton confirmed that bone age was consistent with

chronological age. The E.C.G. was normal.

^{131}I tests. After an intravenous dose of ^{131}I on August 25th., 1958, there was a rapid accumulation of ^{131}I by the thyroid gland. The results are summarized in Tables X and XII.

In the first 14 minutes the thyroid plasma clearance was equivalent to 360 ml. per minute per 1.73 sq. metres. The corrected* uptake of ^{131}I by the gland was 41 per cent of the dose at 14 minutes, 47 per cent at 30 minutes and 91 per cent after 24 hours. Blood values at 48 hours showed that the total serum ^{131}I was 0.20 per cent of the dose per litre and the protein-bound ^{131}I was also 0.20 per cent of the dose per litre.

The intravenous dose of ^{131}I was repeated on September 1st.,1958, when the pattern of accumulation by the thyroid gland was confirmed. After 30 minutes 800 mg. of potassium perchlorate was given by mouth. This was followed by a rapid but incomplete discharge of ^{131}I from the thyroid gland, the corrected* uptake falling from 47 per cent to 21 per cent of the dose (fig. 55; Table XIII).

Treatment and progress. An extensive subtotal thyroidectomy was performed by Mr. Ian McLennan on October 22nd., 1958. 225 g. of thyroid tissue was removed. Dry thyroid was started one week after operation. She was discharged from hospital on October 30th., 1958. She has remained euthyroid and thyroid enlargement has not recurred on sodium thyroxine 0.05 mg. t.i.d.

Chromatography of thyroid gland. Forty-eight hours before thyroidectomy 100 μc ^{131}I was given by mouth. Portions of the gland were digested by trypsin in a barbitone buffer at pH 8.6. Ascending chromatograms of the digest were run in butanol acetic acid and butanol ammonia solvents. When the chromatograms were scanned for radioactivity iodide (I^-) monoiodotyrosine (MIT) and diiodotyrosine (DIT) were identified. No thyroxine was detected (figs. 56 and 57; Table XV).

Electrophoresis of thyroid proteins.

Electrophoresis of thyroid proteins revealed Q, T, Hb, Thy-g and X bands of protein. Radioactivity was associated chiefly with thyroglobulin, and to a minor degree with albumin (fig. 58; Table XVI).

Dehalogenase activity of thyroid gland.

Several thin slices of thyroid tissue were incubated for 24 hours at 37°C in Krebs-Ringer phosphate buffer (pH 7.4), which contained a trace of nicotinamide and $M^{131}IT$. Chromatography of a butanol extract of the buffer fluid showed that the radioactivity was present almost entirely in the iodide zone. (fig. 59). Dehalogenase activity in the thyroid gland was normal.

Case III. P.S. A boy, aged 10 years, was admitted to hospital on September 3rd., 1958. He was the first child of 4. Two younger sisters are in good health. The fourth child was stillborn. His parents are in normal health. They are unrelated. There is no history in their families of goitre or deafness. At the age of 3 months he was referred to Mr. Matthew White at the Royal Hospital for Sick Children, Glasgow, with stridor. A diffuse firm goitre was discovered. There was no evidence of cretinism. The serum cholesterol level and radiographs of wrists and lower extremities were within normal limits. A considerable portion of the right thyroid lobe was removed. This was followed by treatment with dry

thyroid until the age of 6 months, when it was recorded that "neck swelling is now negligible".

His subsequent development was retarded. His retardation was attributed, at least in part, to deafness which was recognised when he went to school. He has been seen on several occasions by otologists and psychiatrists. His intelligence quotient lies between 59 and 61. Attempts to assess his hearing have never been very successful. A child psychiatrist is convinced that he has a sensory aphasic disturbance and also an emotional disorder. He attends a special school.

On August 10th., 1958, his grandmother noticed a "sudden" swelling of his neck.

In hospital he appeared as an underdeveloped boy. His height was 127.5 cm. and his weight was 25.7 Kg. Pulse-rate 96 per minute. Blood pressure 105/75 Hg. There appeared to be no hearing in the right ear. He could hear quite well with his left ear with the help of a hearing aid. Speech was not abnormal in quality although limited in vocabulary. The thyroid gland showed moderate visible enlargement, particularly of the left lobe. No bruit was audible.

There were no signs of hypothyroidism. The haemoglobin level was 15 g.. per 100 ml. Radiographs showed a normal bone age. Serum cholesterol was 137 mg. per 100 ml.

^{131}I tests. After an intravenous dose of ^{131}I on September 12th., 1958, the thyroid gland trapped the ^{131}I with excessive rapidity. In the first 4 minutes the thyroid plasma clearance was equivalent to 471 ml. per minute per 1.73 sq. metres. The corrected* uptake of ^{131}I by the gland was 34 per cent of the dose after 10 minutes and 38 per cent at 30 minutes (Table X). Potassium perchlorate 600 mg. was given by mouth at 38 minutes. There was a rapid but incomplete discharge of ^{131}I from the thyroid gland. The content of ^{131}I was 21 per cent of the dose at 90 minutes (fig. 60; Table XIII).

On September 22nd., 1958, he was given an oral dose of ^{131}I (Tables X and XII). The uptake by the thyroid gland corrected for residual radioactivity after 24 hours was 43 per cent of the ingested dose. The total serum ^{131}I at 48 hours was 0.49 per cent of the dose per litre; the protein-bound ^{131}I was 0.42 per cent of the dose per litre.

Treatment and progress. He was started on dry thyroid by mouth. Seven months later he was taking 1 grain q.i.d. He was euthyroid and his goitre was smaller.

Case IV. A.A. A girl, aged 18 years, was admitted to hospital on October 30th., 1958. She was the second child of a family of 5. The parents were unrelated. A paternal great-aunt had had a goitre. There was no family history of deaf-mutism. The patient and her elder brother (cases IV and V) both suffered from goitre and deaf-mutism.

Her early development was satisfactory, but at 15 months it became apparent that she was deaf. She attended a special school from 5 to 16 years. Her school reports showed normal progress for a deaf-mute child, and she learned to lip read. Menstruation started when she was 15 years of age. Fullness of the neck, "like a roll of fat", had been apparent from birth. In the past 3 years thyroid enlargement had progressively increased, leading ultimately to inspiratory stridor which was most noticeable in recumbency. She was treated with iodine in milk for a short spell when she was 15 years; shortly before admission iodine had been

re-started.

She was a well-built normally developed young woman. Her height was 162.6 cm. and her weight was 56.6 Kg. Pulse-rate was 84 per minute. Blood pressure was 120/80 mm. Hg. She was bright and alert, and was apparently of average intelligence despite her deaf-mutism. There was a large goitre with numerous nodules or cysts. She was euthyroid. Hb. was 13.6 g. per 100 ml.; red cells 5.04 million per c.mm. Radiographs showed normal bone age. The E.C.G. was normal. Serum cholesterol was 147 mg. per 100 ml. B.M.R. was standard.

^{131}I tests. After an intravenous dose of ^{131}I on November 4th., 1958, the thyroid gland failed to accumulate any ^{131}I, presumably due to recent iodine medication.

Treatment and progress. Subtotal thyroidectomy was performed on November 12th., 1958, by Mr. Ian McLennan. The post-operative course was uneventful. She was started on sodium l-thyroxine one week after operation. She has remained euthyroid on sodium thyroxine 0.05 mg.t.i.d. and there has been no recurrence of thyroid enlargement 6 months after operation.

Electrophoresis of thyroid proteins.

T, Hb, Thy-g and X bands of protein were present (Table XVI). No radioactivity was detected in any zone (fig. 61).

Case V. J.A. A youth aged 19 years, a brother of Case IV, was seen as a hospital out-patient on November 7th., 1958. He had a difficult forceps birth and had a transient facial palsy. His development was normal, but like his sister, when he was 15 months old it was realised that he was deaf. He attended a special school from 5 to 16 years. He was described as "a bit lazy" at school, but his ultimate performance was satisfactory, and he learned to lip-read. No goitre had been noted.

He was a well-built youth, of good physique. His height was 172.7 cm. and his weight was 69.8 Kg. Pulse-rate was 72 per minute. Blood pressure 150/80 mm.Hg. He was deaf-mute but of average intelligence. He had a small goitre with a cystic nodule in the isthmus. He was euthyroid.

^{131}I tests. After an intravenous dose of ^{131}I on November 12th., 1958, there was rapid accumulation of ^{131}I by the thyroid gland (Table X). In the first 10 minutes the thyroid plasma clearance was equivalent to 645 ml. per minute per 1.73 sq. metres. The corrected* uptake of ^{131}I by the gland was 53 per cent at 10 minutes, and 67 per cent at 30 minutes. After 30 minutes 600 mg. of potassium perchlorate was given by mouth. This was followed by a rapid but incomplete discharge of ^{131}I from the thyroid gland. The corrected* uptake fell to 20 per cent (fig. 62; Table XIII).

Treatment and Progress.

He was started on sodium thyroxine with a view to preventing further enlargement of his thyroid gland. Six months later on sodium thyroxine 0.05 mg. t.i.d. its size was unchanged.

Discussion

Clinical features.

The patients have lived their lives in two large cities in the West of Scotland, Glasgow and Paisley, where goitre

is non-endemic. It is hardly likely that their goitres could be due to a primary deficiency of iodine. The familial incidence of the goitre in 4 of the cases, and its appearance within a few weeks of birth in the fifth suggests an inborn defect in the synthesis of the thyroid hormone. The euthyroid state of the 5 patients is in keeping with the belief that their hyperplastic thyroids were able to compensate for their intrinsic inadequacy by the increase in size and were able to produce enough hormone to keep the patients euthyroid.

Radioactive iodine studies.

The rapid discharge, induced by potassium thiocyanate or perchlorate of a substantial amount of the half-hour ^{131}I content of the thyroid glands of cases I-III and V indicated a failure to utilize most, but not all, of the ^{131}I which had been so eagerly trapped. The process of incorporating the iodide into a protein complex was defective presumably due to an intrinsic deficiency in the peroxidase enzyme system. A biochemical abnormality of this type has also been demonstrated in one type of goitrous cretinism (Stanbury and Hedge 1950; Stanbury 1951; Jackson 1954; Lelong et al. 1956; Schultz et al. 1957; Buchanan and Crooks 1959). More recently, Clayton et al.(1958)

have described four siblings, in a negro family of six children, who were euthyroid although goitrous, and whose thyroid glands also showed a defective ability to combine iodide to protein. Unlike the present cases the negro children were not deaf. Morgans and Trotter (1958) have also recently reported evidence of a partial defect in the organic binding of iodine in two siblings who had goitres. These children were euthyroid and they were also deaf-mutes.

Chromatographic studies of thyroid gland.

The chromatograms prepared from the alkaline hydrolysate and the trypsin digest of the thyroid glands in cases I and II respectively are interesting. Remarkably little iodide was found in case I, but a substantial amount was present in case II. In both glands an appreciable amount of the ^{131}I which had been trapped by the gland was, in fact, incorporated into the thyroglobulin. Monoiodotyrosine, diiodotyrosine and thyroxine were identified in case I, and monoiodotyrosine and diiodotyrosine in case II. The amount of radioactivity in thyroxine in case I, namely 14.7 per cent, was normal. However, the amount of radioactivity in monoiodotyrosine exceeded the

amount in diiodotyrosine. In case I the ratio could not be accurately determined because of poor separation of monoiodotyrosine and diiodotyrosine in the chromatograms. In case II the ratio varied from 1.7 to 2.1 according to the portion of gland examined (Table XV), which contrasts with the ratio value of 0.5 - 0.75 found by Pitt-Rivers et al. (1957) in normal human thyroid glands and the ratio value of 0.36 - 0.58 which I have found in normal rat thyroid glands (Table VIII).

In a goitrous cretin whose thyroid gland showed a similar enzyme defect, Stanbury et al. (1955 a) failed to reveal any iodinated tyrosines or thyronines by chromatographic analysis of the thyroid tissue. The presence of iodinated amino-acids in the present cases accords well with the clinical finding that the patients were euthyroid, and confirms the clinical deduction that the biochemical defect was not absolute.

Electrophoresis of thyroid proteins. The protein pattern in the electrophoretic strips of thyroid tissue from case II was unremarkable, being similar to that found in adult cases of nodular goitre. Q protein was absent in the gland of case IV;

it is not known if this has any significance or is related to the medication with iodine. This was the only gland in 23 of various types examined that Q was absent. It was also the only one from a patient who recently had iodine.

Genetics and deafness. The familial incidence of this particular defect is in keeping with a genetic basis for it. The idea that sporadic goitre may be genetic in origin has been difficult in the past to sustain because it has not been possible to exclude the possibility that familial incidence is due not to a common heredity but to a common environment. It is now possible to argue that it is most unlikely that impaired activity of one specific enzyme in several members of several families (cases I and II, IV and V; Clayton et al. 1958; Morgans and Trotter 1958) could have an environmental origin. Further the environment is unlikely to have had much to do with the goitre of an infant only a few weeks old (case III).

The association of goitre and cretinism with deaf-mutism, which is well known often to be hereditary, has been appreciated for a very long time (Bircher 1883), but the relationship

between them has remained unexplained. It is, however, impossible to accept that deaf-mutism is a direct sequel to goitre or cretinism.

Of particular interest in relation to the present series of cases is the description by Brain (1927) of a series of 12 deaf-mute patients belonging to 5 families who had sporadic goitres. In every case the deafness long antedated the goitre. None of the families came from an area where goitre was endemic and the parents of the affected individuals were all normal to outward appearances. Brain (1927) considered that the goitres were explained by a simple recessive inheritance. He suggested that goitre might "depend on a relative inability on the part of the individual to absorb or utilize iodine". Johnsen (1958) has recently described 12 cases of deafness and goitre in 2 families. In 8 at least of his patients the deafness long antedated the goitre.

The association of goitre with deaf-mutism may have 2 explanations. It may be due to a single gene with multiple effects. As the 2 conditions are met with singly

more frequently than in combination, it might also be that 2 closely linked genes are involved (McGirr et al.1959 a).

Conclusions

^{131}I studies in 5 euthyroid deaf-mute patients with sporadic goitres, 2 of whom were sisters and 2 siblings, have demonstrated a partial defect in the utilization of the ^{131}I trapped by the thyroid glands.

There is now evidence that an identical defect in the utilization of iodide by the thyroid may cause sporadic goitres, as well as sporadic goitrous cretinism.

This defect is presumably due to impaired activity of the enzyme peroxidase.

Its familial incidence suggests that it has a genetic basis.

This enzyme deficiency and the deaf-mutism were probably determined by the activity of 2 closely linked genes.

CHAPTER 17

GROUP II CASE

IMPAIRED DEHALOGENASE ACTIVITY (fig.63)

Case report

Case VI. S. McC. A girl, aged 9 years, was seen as an outpatient at hospital on February 7th., 1956.

She was the eldest of 3 children. The parents were unrelated. A maternal aunt had had a thyroidectomy for goitre. She was a normal full-time baby who developed normally. Her progress at school was satisfactory. A goitre had been present for 2 years.

She was a normally proportioned child whose general nutrition and development were satisfactory. She weighed 34.9 Kg. and her height was 136.5 cm. She had a definite goitre on the surface of which firm nodules and cysts were palpated. No bruit was audible over it. Pulse rate was 84 per minute. Blood pressure was 120/75 mm. Hg. The texture of skin and hair was normal. Radiography of bones confirmed normal bone development.

Her progress was observed carefully. She continued to grow normally. When seen on June 20th.,1958, it was apparent that her thyroid had increased in size. She was admitted to hospital on August 7th., 1958, for thyroidectomy. Her weight was then 36.1 Kg. and her height was 142.9 cm.

Pulse rate was 84 per minute. Blood pressure was 100/55 mm.Hg. Skin and hair were normal. She appeared to be euthyroid. Radiography of the skeleton revealed a normal bone age. Serum cholesterol was 232 mg. per 100 ml. Electrocardiogram was within normal limits.

^{131}I studies. After an intravenous dose of ^{131}I on December 18th., 1957, the ^{131}I accumulation by the thyroid was unusually rapid (fig.64). The thyroid plasma ^{131}I clearance was equivalent to 433 ml. per min. per 1.73 sq.metres. The corrected* uptake at 30 minutes was 74 per cent of dose and at 24 hours was 35 per cent (Table X). At 48 hours the total serum ^{131}I was 0.93 per cent of the dose per litre and protein-bound ^{131}I 0.71 per cent (Table XII). The intravenous test was repeated on March 9th., 1958, but on this occasion she was given an oral dose of 600 mg. potassium perchlorate at 30 minutes. There was no loss of ^{131}I from the thyroid gland (Table XIII).

Treatment and progress. A subtotal thyroidectomy was carried out on August 16th., 1958, by Mr. Ian McLennan. Approximately 100 g. of thyroid tissue was removed. Her post-operative course was

satisfactory. She has since kept well and has remained euthyroid on sodium thyroxine 0.05 mg. three times a day. No thyroid tissue has been felt in the neck and no lymph nodes have been palpated.

Chromatographic studies. Forty-eight hours before thyroidectomy she was given by mouth 100 μc of ^{131}I.

Serum. At 48 hours 88.4 per cent of the ^{131}I in the serum was extracted by 4 washes of butanol after acidification to pH 2. A chromatogram was prepared from the butanol extract of the serum and run in butanol ammonia solvent. When it was scanned for radioactivity monoiodotyrosines (MIT) and thyroxine (T4) were identified (fig. 65). A trace of triiodothyronine (T3) was probably also present.

Urine. Chromatograms were prepared from a butanol extract of the urine passed in the period 0-24 hours following the dose of ^{131}I. They were run in butanol acetic acid, butanol ammonia, and collidine ammonia solvents. Most of the radioactivity was present as unidentified substances most probably iodotyrosine conjugates. They were located at the

thyroxine-triiodothyronine (T4 - T3) zone in the butanol acetic acid chromatogram (fig.66) and just preceding thyroxine (T4) in the butanol ammonia chromatogram (fig.67). Diiodotyrosine (DIT), monoiodotyrosine (MIT) and iodide (I^-) were also present. In the chromatograms run in collidine ammonia solvent (fig.68) there were several unidentified peaks of radioactivity.

Thyroid tissue. Numerous chromatograms were prepared from homogenized portions of thyroid tissue before and after trypsin digestion in barbitone buffer (pH 8.6). The chromatograms were run in butanol acetic acid and butanol ammonia solvents.

The radioactivity of the undigested gland remained at the origin of the chromatograms. After digestion with trypsin monoiodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T4) were found in the paranodular tissue (figs. 69 and 70; Table XV). The ratio of radioactivity present in monoiodotyrosine to that in diiodotyrosine was abnormally high, namely 2.0, compared with 0.5 - 0.75 found by Pitt-Rivers et al.(1957) in normal human thyroids and the ratio 0.36 - 0.58 which I have found in normal rat thyroid (Table VIII). Thyroxine was not found in the

nodules: monoiodotyrosine and diiodotyrosine were detected and there was an even more marked change in the MIT/DIT ratio, namely 14.4 (figs.71 and 72; Table XV).

Electrophoresis of thyroid proteins.

Q, Hb, Thy-g, and X bands of protein were present. Radioactivity was associated with thyroglobulin and to a lesser extent with albumin (fig.73; Table XVI).

Dehalogenase activity of thyroid gland.

Dehalogenase activity in the thyroid gland was investigated by placing thin slices of thyroid tissue from both paranodular and nodular areas in Krebs-Ringer phosphate buffer (pH 7.4) which contained a trace of nicotinamide and $M^{131}IT$. They were incubated at 37°C for 18 hours. The thyroid failed to deiodinate the monoiodotyrosine (fig.74).

Radioactive (^{131}I) monoiodotyrosine ($M^{131}IT$) excretion studies in patient and her mother.

Eleven days after operation on August 27th., 1958, the patient was given by mouth 20 μc of $M^{131}IT$. In the first 6 hours following its administration 54.3 per cent of the dose of monoiodotyrosine appeared in the urine as free or conjugated

monoiodotyrosine. Monoiodotyrosine or its conjugates constituted 97.3 per cent of the ^{131}I present in the urine excreted in the period 0-2 hours, and 96.1 per cent in the period 2-6 hours (Table XIV and figs. 75 a b and c).

The patient's mother, a 29 year old woman who was euthyroid and non-goitrous, cooperated in a similar test. She excreted 14.3 per cent of the dose of monoiodotyrosine as free or conjugated monoiodotyrosine in the period 0-6 hours. Monoiodotyrosine or its conjugates constituted 43.4 per cent of the ^{131}I present in the urine in the period 0-2 hours, and 1.0 per cent in the period 2-6 hours (Table XIV).

Pathology

The gland resected at operation (weight 100 g.) was very nodular. Some of the nodules, which varied from 0.5 cm. to 2.0 cm. in diameter, were fleshy, while others were cystic and contained blood stained fluid.

The histological appearances were of nodular goitre in childhood with carcinomatous change in places, the tumour presenting in the main the features of a papillary adenocarcinoma (McGirr et al. 1959 b).

Discussion

Clinical features.

When the patient was first seen early in 1956 her goitre was thought to be simple or non-toxic, and probably due to a defect in hormone synthesis. The diagnosis of neoplasm was considered because of the patient's youth and because her goitre was nodular. It was discarded because the whole gland was obviously involved and there was no cervical lymphadenopathy. On palpation the goitre was similar to goitres found in sporadic goitrous cretins with inborn defects in the synthesis of the thyroid hormone. Thyroid hyperplasia mediated through the anterior pituitary was considered to be its explanation. The euthyroid state of the patient was presumed to show that her enlarged hyperplastic thyroid had, by its increase in size, compensated for its intrinsic inadequacy. Even when thyroidectomy was decided upon in June 1958, neoplasm was not thought to be a serious hazard. Nor did the appearance of the gland at operation arouse anxiety. The evidence, which makes the

histological diagnosis of cancer unavoidable, re-emphasizes the importance of viewing the nodular goitre of childhood with concern. Hitherto thyroidectomy has not been considered to be indicated when there is biochemical evidence of a defect in synthesis unless the goitre is very large. If there are further reports of neoplastic change in such goitres this opinion will have to be revised.

Radioactive iodine studies.

The failure of potassium perchlorate to discharge any ^{131}I from the thyroid gland demonstrated that the initial steps in the utilization of iodide were normal, and that peroxidase activity was apparently normal.

The presence of iodotyrosines in the serum and urine, and the failure of thyroid tissue under standard conditions *in vitro* to deiodinate $M^{131}IT$ demonstrated the absence of dehalogenase activity within the thyroid gland. The recovery of substantial amounts of monoiodotyrosine and its conjugates in the urine after an oral dose of $M^{131}IT$ indicated that dehalogenase activity was absent or impaired in other tissues as well as in the thyroid.

Comparison of the urinary chromatograms after $M^{131}IT$ (figs. 75 a b and c) with the urinary chromatograms after ^{131}I (figs. 66, 67 and 68) reveals similar peaks of radioactivity. These findings strengthen the conviction that the unidentified peaks of radioactivity after ^{131}I in the urine of patients with a dehalogenase defect such as case 2 and case VI, are due to derivatives, probably conjugates, of the iodotyrosines.

The mother of the patient also showed evidence of some impairment of dehalogenase activity. In the first 6 hours after an oral dose of $M^{131}IT$ she excreted 14.3 per cent of the dose as free or conjugated monoiodotyrosine compared with less than 6 per cent in normal subjects (Chapter 7). Likewise the proportion of ^{131}I excreted as monoiodotyrosine was 43.4 per cent in the period 0-2 hours, compared with an amount not greater than 17.6 per cent in either of the periods 0-2 hours or 2-6 hours in control subjects.

Impaired ability to deiodinate $D^{131}IT$ peripherally has been imputed to hypothyroidism rather than a specific enzyme defect. As has been shown in Chapter 7 this has not been my experience with $M^{131}IT$. For example, hypothyroidism

could have nothing to do with the excretion of excessive amounts of $M^{131}IT$ by case VI and her mother who were both euthyroid.

Chromatographic studies of thyroid gland.

The concentration of ^{131}I in the paranodular tissue (0.55 per cent of the dose /g.) of the thyroid gland was greater than in the nodular tissue (0.12 per cent of dose /g.) though each was substantially below the values of 2.8 and 1.6 per cent/g. which Pitt-Rivers et al. (1957) found in 2 normal glands. It is perhaps significant that thyroxine was found in the paranodular tissue and not in the nodular tissue, and that the MIT/DIT ratio, though elevated in both tissues, was less markedly abnormal in the paranodular (2.0) than in the nodular (14.4).

Electrophoresis of thyroid proteins.

The protein pattern was unremarkable being similar to that found in adult cases of nodular goitre.

Genetics.

Evidence for the genetic basis of dehalogenase deficiency in sporadic goitrous cretinism has already been presented in chapter 9. The evidence of a similar defect in mother and child leaves little doubt that it was hereditary in the

present case. It has proved impossible to get any information about the thyroid which was removed from the patient's aunt. Despite this the biochemical and genetic evidence strongly favours the belief that dehalogenase deficiency was the fundamental defect, that it preceded and caused the patient's goitre and was not secondary to it.

Pathology. The histological diagnosis of neoplastic or hyperplastic thyroid lesions is often difficult since so many of the criteria acceptable in other organs as histological evidence of malignancy may be present in non-malignant as well as in malignant states of the thyroid gland. Thus, for example, the presence of epithelial cells in the capsule of individual nodules or of the gland itself, pleomorphism and the presence of epithelial cells in the lumina of large blood vessels may be seen in the thyroids of goitrous cretins and thyrotoxic patients. These were all present in this case, but, in addition, sections stained for elastic tissue showed mural invasion of thick-walled veins by thyroid epithelium which also infiltrated small vascular channels in the adventitial coat of a large

artery. It would appear that these vascular findings, which have not been seen in non-malignant states of the thyroid, are of more value in the recognition of neoplastic change than the intraluminar 'invasion' of large vessels.

Pathogenesis.

The presumption is that the child's goitre was due to impaired dehalogenase activity and that this defect was inherited. In consequence the thyroid gland had difficulty in producing enough thyroid hormone for the child's metabolic needs because of the constant leak of iodotyrosines from the thyroid into the blood and their subsequent loss in the urine. To compensate for this loss the thyroid enlarged in an attempt to ensure an adequate output of hormone. Presumably the thyroid hyperplasia and enlargement were brought about through the anterior pituitary by increased thyrotrophin production. Compensation was satisfactory in this patient to the extent that sufficient thyroid hormone was produced to keep her euthyroid.

Neoplasia developed in the hyperplastic thyroid. The evidence regarding the exact relationship of the hyperplasia

and the neoplasia is inconclusive. Either the neoplasia was an incidental development in a gland which was already hyperplastic, or it was the end result of a sequence of changes which had been initiated to secure an adequate output of hormone in the face of production difficulties due to impaired dehalogenase activity. If the second explanation is the correct one then the neoplasia was due ultimately to an enzyme defect. The opinion that the neoplasia was responsible for the enzyme defect appears untenable; there is the evidence of the enzyme deficiency in the mother as well as the child, and the genetic basis has been established independently in sporadic goitrous cretinism (Chapter 9). Further the defect was not confined to the thyroid gland. The $M^{131}IT$ excretion test demonstrated the deficiency in tissues other than the thyroid.

Conclusions

An 11-year old girl had a thyroidectomy for a goitre which had been present for $3\frac{1}{2}$ years. She was euthyroid. Radioiodine studies revealed an unusually active thyroid from which

iodotyrosines were escaping into the blood and were excreted in the urine.

The thyroid gland was multinodular and showed in areas papillary adenocarcinomatous change.

Impaired dehalogenase activity was demonstrated in thyroid tissue slices and in other tissues. Her mother had a similar enzyme deficiency.

In view of the evidence that enzyme defects of this nature are genetic in origin, it appears reasonable to conclude that the sequence of events in case VI was : impaired dehalogenase activity, compensatory thyroid hyperplasia and goitre, neoplasia.

CHAPTER 18

GROUP III CASE

IMPAIRED ABILITY TO COUPLE IODOTYROSINES TO PRODUCE IODOTHYRONINES (fig.76)

Case report

Case VII. S.S. (Fig. 77). A boy aged 10 years 3 months was admitted to hospital on June 21st., 1957. He was the oldest of 3 children. The parents were unrelated and there was no family history of goitre.

At birth he appeared healthy apart from a swelling in the anterior aspect of the neck. He developed normally, and he got on well at school. The neck swelling had enlarged considerably in the past year, until its size was an embarrassment.

On admission he was well nourished. His physical and mental development were normal. His height and weight, 135 cm. and 28.6 Kg., were a little under the average for his age. The skin and hair were of normal texture. Pulse rate varied from 70-90 per minute. Hb. was 13.2 g. per 100 ml. and red cells were 4,300,000 per c.mm. There were no signs of hypo- or hyperthyroidism. The thyroid gland, which was markedly enlarged, was nodular and firm. Radiography of the skeleton confirmed that his ossification was normal for his age. The electrocardiogram was normal. Serum cholesterol was 210 mg. per 100 ml.

^{131}I studies. (Tables X-XII). After an oral tracer dose the uptake of ^{131}I in the thyroid gland at 24 hours was 67 per cent of the dose, and at 48 hours 61 per cent of the dose. The total serum ^{131}I at 48 hours was 1.26 per cent of the dose per litre; the protein-bound ^{131}I was 1.01 per cent. The urinary excretion values were 7.9 per cent of the dose in the period 0-6 hours, 5.5 per cent in 6-24 hours, and 7.2 per cent in 24-48 hours.

After an intravenous tracer dose of ^{131}I the ^{131}I accumulation by the thyroid was unusually rapid. The thyroid plasma ^{131}I clearance in the first 5 minutes was equivalent to a clearance value of 676 ml. per minute per 1.73 sq. metres. The corrected* thyroid uptake at 5 minutes was 49 per cent of the dose; at 30 minutes it was 65 per cent. He was given 2 g. potassium thiocyanate orally at 40 minutes. It had no apparent effect on the ^{131}I content of the thyroid gland (Fig.78).

Treatment and progress.

Because the goitre was very large and nodular thyroidectomy was performed on July 4th., 1957. 160 g. of thyroid tissue was removed. Thyroid medication was started one week after operation.

he has remained euthyroid and there has been no recurrence of glandular enlargement on dry thyroid, 1 grain B.D.

Chromatography of thyroid gland. Twenty-four hours before thyroidectomy he was given by mouth 100 μc ^{131}I. Portions of thyroid tissue were homogenized and digested by trypsin in a barbitone buffer at pH 8.6. After acidification to reduce pH to 2 butanol extracts of the digest were made. Ascending chromatograms of the butanol extracts were run in butanol acetic acid (Fig. 79) and butanol ammonia (Fig.80) solvents. When the chromatograms were scanned for radioactivity monoiodotyrosine (MIT) and diiodotyrosine (DIT) were identified. The MIT/DIT ratio was 0.9. No thyroxine was detected (Table XV).

Post-operative ^{131}I studies. In February,1958, he was given by mouth 15 μc of M^{131}IT. Only 3.3 per cent was excreted unchanged in the urine in the first 6 hours following its ingestion. In the pooled samples for 0-2 and 2-6 hours the greatest proportion of radioactive monoiodotyrosine was 14.1 per cent (Table XIV).

Discussion

Clinical features. Like the patients with sporadic goitre in Groups I, II and IV this patient had lived in a city where goitre is non-endemic. Unlike them, however, his was the only goitre in the family. It had been noted at birth yet he had developed normally and had remained euthyroid. If the assumption is a correct one that the goitre is a compensatory enlargement of the thyroid gland to overcome production difficulties then in this case it had successfully achieved its purpose and had secured an adequate output of hormone. Ultimately, however, the enlargement had become unsightly and an embarrassment to the patient.

Radioactive iodine studies. The failure of potassium thiocyanate to discharge any ^{131}I from the thyroid gland showed that the initial steps in the utilization of iodide were normal. Iodide had been converted to iodine and incorporated within the tyrosine of thyroglobulin. Peroxidase activity was apparently normal.

The deiodination of an oral dose of $M^{131}IT$ was normal, indicating normal dehalogenase activity in the tissues of the body, and presumably also in thyroid tissue.

Chromatographic studies of thyroid gland.

The chromatograms showed radioactivity in zones corresponding to monoiodotyrosine and diiodotyrosine, but no thyroxine was detected in the thyroid tissue examined. Now the euthyroid state of the patient is presumptive evidence that enough hormone was produced to meet his growth and metabolic needs.

In the absence of any other explanation for this patient's goitre the failure to detect thyroxine suggested that the disability was a partial failure to couple iodotyrosines into iodothyronines. The thyroid gland was able to produce and secrete an adequate amount of hormone but it did not produce enough to store any appreciable amount of it.

A coupling defect was first postulated by Stanbury et al. (1955 a) in one of 2 goitrous sisters who showed the residual stigmata of cretinism. Thyroxine and triiodothyronine were identified in the serum but the iodine in the thyroid gland was almost entirely present as monoiodotyrosine and diiodotyrosine.

Exhaustive chemical analyses of the thyroid gland revealed only a trace of thyroxine. The authors suggested that the iodotyrosines were present in such large amounts that a small amount of iodinated thyronines was produced non-enzymatically. Lelong et al. (1956) gave a similar interpretation to their findings in one of their cases. Werner et al. (1956) also produced evidence that the defect in a 5-year old goitrous girl with mild hypothyroidism was a failure of adequate coupling of iodotyrosines, but there were some anomalous features in this patient. These have already been discussed in Chapter 10.

While the findings in case VII are compatible with a partial coupling defect the data are inadequate to substantiate this diagnosis. It is my experience that thyroxine may not be stored in the thyroid when the goitre is due to other fairly well defined defects. For example, no thyroxine was found in the thyroid of case 2 of the tinker patients and her condition was due to impaired dehalogenase activity (Chapter 8). Though she was a cretin some thyroxine was identified in her serum.

Likewise in case II (Chapter 16) no thyroxine was found in her thyroid gland, and there is good evidence for another defect, namely impaired peroxidase activity. These particular defects have been eliminated in case VII, but no doubt others as yet unrecognized might occur and no thyroxine be found in the thyroid gland.

The gland of case VII was one of the earliest glands to be chromatographed; hence a butanol extract was used. This means that any non butanol-extractable ^{131}I compound, such as zone C later found in the thyroid of case VIII, would have been eliminated from the analysis. Whether this possibility is excluded by the differences in the MIT/DIT ratios in case VII and case VIII is not known. In case VII the ratio was increased and in case VIII it was diminished (Table XV), in comparison with the values of 0.5 - 0.75 found by Pitt-Rivers et al.(1957) in normal glands.

Conclusions

^{131}I studies in a 10-year old euthyroid boy with a congenital goitre have shown that his thyroid gland eagerly accumulated iodide which it quickly

incorporated into iodotyrosines. Iodotyrosines were demonstrated in the thyroid gland but no thyroxine was detected.

His euthyroid state is presumptive evidence that he produced enough hormone for his metabolic needs.

He may have had a partial coupling defect which made production of iodothyronines from iodotyrosines difficult. He produced only enough hormone for his needs, but not enough to store in amounts that could be detected.

CHAPTER 19

GROUP IV CASES

PRODUCTION OF AN ABNORMAL IODINATED THYROID PROTEIN (fig.81)

Case reports

Case VIII. C.K. A girl, aged 13 years, was admitted to hospital on November 24th., 1952. She was the seventh child of a family of 8, of whom 4 including the patient had a goitre. Two of her affected sisters are also considered in this chapter (cases IX and X). No details are available about the fourth affected sibling; he was the fourth child and died aged 9 years when he had diphtheria. The parents are unrelated; neither has a goitre. There was no other family history of goitre.

The patient was a full time baby. Her mental and physical development had been normal. She cut her first tooth at 5 months, walked at 11 months, and talked at 16 months. A goitre had been present at birth. It had remained symptomless. She had been seen elsewhere at 6 years because of the goitre. Her bone age was then normal. She was treated with dry thyroid from 1945 to 1948. During this period her goitre became smaller; normal development was maintained. She received Lugol's iodine for the first two months of 1949. Thereafter she received no further

treatment. The enlargement of the thyroid became more marked, especially in the two years preceding her first admission to hospital. Her progress at school had been average.

On admission her nutrition, physical and mental development, were normal. Her height was 150 cm. and her weight was 40.4 Kg. The striking finding was the presence of a very large nodular goitre, over which a thrill was palpable and a bruit was audible. She was normally bright and alert. Her skin and hair were normal. Pulse rate was 90 per minute; blood pressure was 110/70 mm.Hg. Hb. was 11.5 g. per 100 ml. and red cells numbered 4,200,000 per c. mm. Radiography of the skeleton showed a bone age in keeping with her chronological age. The basal metabolic rate was 9 per cent above standard (Aub and Du Bois).

Treatment and progress. An attempt was made to perform thyroidectomy but the operation had to be abandoned because of technical difficulties. She continued to develop normally, and had no complaints apart from her goitre. Menarche occurred when she was 15 years old. She was readmitted to hospital

on June 28th., 1955, on account of fever, productive cough, and breathlessness of 12 days duration. Examination revealed an extensive left-sided pleural effusion of tuberculous origin. Her goitre was still markedly enlarged. She received a twelve weeks course of 1 g. streptomycin daily and 18 g. para-amino salicylic acid daily with excellent effect. Satisfactory clearing of the effusion occurred, the only residual sign observed in the X-ray being minimal obliteration of the left costo-phrenic angle.

She was readmitted to hospital on March 20th.,1958, (Fig. 82). Her general condition was satisfactory. The chest was clear. B.S.R. was 11 mm. in 1 hour. The goitre remained enormously enlarged, particularly the left lobe, which displaced the carotid artery backwards. A purring thrill and a loud bruit were present over the gland. An almost complete thyroidectomy was performed on March 31st. 1958, by Mr. Ian McLennan. He experienced considerable difficulty because of the gland size, its adherence to surrounding structures, and the grossly hypertrophied blood vessels. 560 g. of thyroid tissue was removed. Blood

loss necessitated the transfusion of 2 pints of blood during the operation. There was considerable effusion of blood into the wound area following the operation but it absorbed satisfactorily and she was allowed home on April 17th., 1958. She was put on thyroid by mouth post-operatively and has remained well and shown no recurrence of thyroid enlargement on a maintenance dose of sodium thyroxine 0.05 mg. three times daily.

^{131}I studies (Tables X - XII).

In June 1957 she was given an intravenous tracer dose of 25 µc ^{131}I (fig.83). Unusually rapid thyroid accumulation of ^{131}I occurred. Because of the continuous falling off of the accumulation gradient only an approximate value for thyroid plasma ^{131}I clearance could be calculated. Over the first 5 minutes the clearance rate was 1170 ml. per minute per 1.73 square metres. The corrected* thyroid uptake at 5 minutes was 72 per cent of the injected dose; at 30 minutes it was 80 per cent. The uptake at 4 hours was 86 per cent, at 24 hours 77 per cent, and at 72 hours 66 per cent. The total serum ^{131}I at 48 hours was 0.54 per cent of the dose per litre; the protein-bound fraction was 0.55 per cent. Only

0.23 per cent per litre was extractable by 4 washes of butanol after acidification to pH 2, virtually all of which, namely 0.20 per cent was thyroxine-like. The intravenous clearance was repeated in December 1957. An identical uptake curve was obtained. At the end of 20 minutes the patient was given an oral dose of 600 mg. potassium perchlorate. It led to a cessation of uptake, but no fall in the ^{131}I content of the thyroid was noted in the succeeding 30 minutes (fig.84).

Chromatography of serum, urine and thyroid gland.

On March 29th., 1958, 48 hours before thyroidectomy, she was given 1 mc. of ^{131}I by mouth, for the purpose of studying the various iodine compounds present in her serum, urine and thyroid gland.

Serum. In serum withdrawn before the operation 83 per cent of the total ^{131}I was precipitable with the proteins; 72 per cent was butanol-extractable. The latter value was little changed by overnight digestion of the serum with trypsin and chymotrypsin (Table XVII). Ascending chromatograms were prepared from butanol extracts of the serum which had been acidified to pH 2. They were run in butanol acetic acid (fig.85)

and butanol ammonia (fig.86) solvents. After staining of the markers they were scanned for radioactivity. Most of the radioactivity was identified with thyroxine (T4) but there appeared to be also a trace of triiodothyronine (T3)and iodide (I^-).

Urine. In chromatograms of urine extracted by butanol after acidification only iodide was found.

Thyroid tissue. Numerous chromatograms were prepared from portions of thyroid tissue homogenized in barbitone buffer. Both undigested gland, and gland digested in barbitone buffer at pH 8.6 by trypsin and chymotrypsin were used. The chromatograms were run in various solvents including butanol acetic acid, butanol ammonia, and amyl alcohol and ammonia. To help establish the chemical identity of the various zones of radioactivity found when the chromatograms were scanned, they were eluted and then re-run in alternative solvents.

The radioactivity of the undigested gland remained almost entirely at the origin of the chomatograms, a trace only of iodide being detected (fig.87). After digestion with trypsin or chymotrypsin the presence of iodide (I^-, zone B) monoiodotyrosine (MIT, zone Z) and diiodotyrosine (DIT, zone X) was demonstrated (fig. 88; Table XV). The MIT/DIT ratio

varied from 0.1 - 0.5 in different portions of thyroid tissue. No thyroxine (T4) nor triiodothyronines (T3) was found after trypsin or chymotrypsin digestion. The identity of the iodide, monoiodotyrosine and diiodotyrosine zones was confirmed by a series of elution experiments in which the radioactivity was eluted from the particular zone of a chromatogram and re-run in the same or a second solvent system (figs. 89-91).

There were certain unusual zones of radioactivity. Zone Y was shown by elution to correspond to a zone at the end of and beyond triiodothyronine in the butanol ammonia chromatograms (figs. 92 and 93). It retained its characteristics after boiling with 2 N-NaOH for 1 hour (fig. 94). At first it was thought that it might be due to 3,5-diiodothyronine* but this was disproved by its position in amyl alcohol ammonia chromatograms (figs. 95 and 96) where it was quite apart from the diiodothyronine. Its identity remained unestablished.

* 3,5-diiodothyronine was kindly supplied by Mrs.R.Pitt-Rivers.

Elution studies suggested that zone A in part came from zone B, which was iodide, but no opinion was reached about the origin of the rest of its activity (fig.97). At first zone C, which comprised 24.5 - 53.9 per cent of the radioactivity in the chromatograms, was considered most probably to be due to thyroglobulin, which had remained undigested in unusually large amounts. In my experience not more than 16 per cent of radioactivity remains in this zone (Tables VIII, XV and XVIII) and I have tacitly assumed that it is due to undigested thyroglobulin. Like thyroglobulin zone C was not extractable by butanol, but unlike thyroglobulin it proved resistant to redigestion with trypsin when the origin of the chromatogram was placed in barbitone buffer at pH 8.6. Repeated attempts at redigestion proved unsuccessful (figs. 98 and 99). The activity of the trypsin and the pH of the buffer were carefully checked. When zone C was boiled with 2N-NaOH for 1 hour, 80 per cent of its radioactivity became butanol extractable after acidification. Chromatograms run in butanol acetic acid (fig.100) and butanol ammonia (fig. 101) showed that zone C had broken down chiefly to thyroxine, but monoiodotyrosine and diiodotyrosine were also present. It appears significant that the thyroxine released

by alkaline hydrolysis of zone C was the only thyroxine detected in the gland (McGirr et al.1959d).

Electrophoresis of thyroid tissue. The unusual findings in the serum and thyroid gland of this patient suggested that useful information might be obtained by electrophoresing the thyroid tissue to see how such proteins as were present behaved. The electrophoresis was done by Dr. W.C.Watson, of the University Department of Medicine, Royal Infirmary, Glasgow.

The gland from case VIII was the first gland to be examined by electrophoresis, and as a result the technical result is not completely satisfactory (fig.102). Nevertheless several bands of protein are recognizable, namely Q, T, haemoglobin and X or albumin. Q and T bands are present but are faint and are difficult to show clearly in the photographic reproduction. Virtually no stainable protein is present in the thyroglobulin zone. Radioactivity is present in two zones. There is a broad band present in the areas normally occupied by thyroglobulin and albumin. A lesser more compact zone corresponds to the Q band of protein.

Dehalogenase activity of thyroid gland.

Dehalogenase activity in the thyroid gland was investigated by placing two thin slices of fresh thyroid tissue approximately

1 mm. thick, in Krebs-Ringer phosphate buffer (pH 7.4), which contained a trace of nicotinamide and $M^{131}IT$. They were incubated at 37°C for 18 hours. At the end of this period virtually no monoiodotyrosine was detectable, as it had been almost completely deiodinated and its radioactivity was now present in the iodide spot of the chromatogram prepared after the incubation (fig.103). The experiment was repeated in triplicate with slices of thyroid tissue from different areas of the gland.

$M^{131}IT$ excretion studies.

Ten months after thyroidectomy, on January 2nd., 1959, she was given by mouth 25 µc of $M^{131}IT$. Only 1.8 per cent of the radioactive monoiodotyrosine was excreted unchanged in the urine in the first 6 hours. In the pooled samples of 0-2 and 2-6 hours the greatest proportion of radioactive monoiodotyrosine was 16.5 per cent, the rest having been deiodinated to iodide. (Table XIV).

Case IX. M.K. A girl aged 18 years was admitted to hospital on May 20th., 1955.

She was the sixth member of the family. She sat up at 5 months, cut her first tooth at 6 months and walked and

talked at 1 year. At school she was slow and was less lively than other children. When she left at the age of 15 years she obtained unskilled employment, but she had lost two jobs because she was unduly slow. A goitre had first been noted when she was about 1 year old. At first it had been unobtrusive but since the age of 7 years it had gradually increased in size. She had been seen elsewhere at 9 years because of the goitre. Her bone age was then just within normal limits. She was treated with dry thyroid from 1945 to 1948 but it had little effect on goitre size. She then received Lugol's iodine for the first two months of 1949. Her appetite had always been good and her bowels were regular. Her periods, which started at the age of 16 years, were regular.

On admission she appeared rather pale, but her general nutrition and development were normal. Her height was 160 cm. and she weighed 53.5 Kg. Cerebration was somewhat slow. The striking finding was the presence of an enormous nodular goitre, more marked on the right side of the neck, over which a thrill could be readily felt and a bruit was audible. The texture of her skin and hair was normal. Pulse rate

was 90 per minute; blood pressure was 110/65 mm. Hg. Hb. was 9.3 g. per 100 ml.; red cells were 4,450,000 per c.mm. Radiography of the skeleton showed slightly delayed ossification; her bone age was approximately 17 years. X-ray of the heart confirmed its normal size. Electrocardiography showed no significant abnormality. Blood cholesterol concentration was 138 mg. per 100 ml. The basal metabolic rate was 5 per cent above standard (Aub and Du Bois). Protein-bound iodine was 4.5 µg. per 100 ml.

^{131}I studies. (Tables X - XII, and fig.104). After an oral dose of ^{131}I the maximum uptake of ^{131}I by the thyroid, namely 90 per cent, was reached in 4 hours and was maintained for 24 hours. By 48 hours a fall was appreciable; it was then 80%. On the tenth day 55 per cent still remained in the thyroid. The total serum ^{131}I at 48 hours was 0.62 per cent of the dose per litre; the protein-bound ^{131}I fraction was 0.49 per cent; the total butanol-extractable ^{131}I was 0.35 per cent and the thyroxine-like ^{131}I was 0.20 per cent. The urine excretion figures were 0.7 per cent in the 0-6 hour period, 4.4 per cent in 6-24 hours, and 3.7 per cent in the 24-48 hour period.

Treatment and progress. She was put on iron and ammonium citrate gr. 30 three times daily and was given a transfusion of 2 pints of blood in preparation for operation. Thyroidectomy was performed on June 18th., 1955, by Mr. Ian McLennan. An almost complete thyroidectomy was performed; 530 g. of thyroid tissue was removed and only a small stump of thyroid tissue was left on the right side. Her general state necessitated a transfusion of 2 pints of blood during the operation. During her post-operative course there was transient swelling in the wound area and she developed symptoms of tetany, complaining of paraesthesia in the fingers and leg cramps. Her serum calcium was low on June 24th., 1955, being 3.3 m.equiv./litre. The tetany responded to treatment with calcium.

She was observed periodically as an out-patient. She requires a maintenance dose of sodium thyroxine of 0.05 mg. t.i.d. She is subject to epistaxis and has had on occasion to have iron for a definite iron deficiency anaemia. There has been no recurrence of thyroid enlargement.

Case X. E.K. A female, aged 21 years, was admitted to hospital on August 26th., 1955.

She was the fifth member of the family.

Her development had always been backward. Dentition and speech were delayed. She did not walk until she was 3 years old. She had attended a special school for mentally handicapped children which she left when she was 16 years. She was unfit to take a job but helped her mother, under supervision, in the home. A goitre had first been noted when she was 7 years old; it had progressively increased in size. From 1945 to 1948 she had been treated elsewhere with dry thyroid with little benefit. At the beginning of 1949 she had had a brief course of Lugol's iodine. Thyroidectomy had been attempted in 1952 but it had been abandoned because of technical difficulties. Following the operation the thyroid had continued to enlarge and recently she had complained of constriction of the throat, dyspnoea and dysphagia. Her appetite was good and her bowels moved regularly. Her periods started at the age of 16 years, and were regular. She had had otitis media, following whooping cough as a child.

On examination her general nutrition and physical development were satisfactory. Her height was 162.5 cm. and she weighed 48.7 Kg. Her expression was dull and she was slow-witted. Her speech was slurred and rather indistinct. She was deaf in both ears because of chronic otitis media. The striking finding was the presence of a very large nodular goitre, more marked on the left side, with an overlying operation scar. The texture of the skin and hair was normal. Pulse rate was 80 per minute; blood pressure was 100/70 mm.Hg. Hb.was 12.1 g. per 100 ml.; red cells were 4,010,000 per c.mm. Radiography of the skeleton showed a bone age of 17-18 years. X-ray of the heart confirmed its normal size. Electrocardiography showed elevation of the ST interval in leads I, II, VI and V2; it was depressed in V3. T wave was inverted in V3. Blood cholesterol concentration was 190 mg. per 100 ml. The basal metabolic rate was 2 per cent below standard (Aub and Du Bois). Protein-bound ^{127}I was 3.6 µg. per 100 ml.

^{131}I studies. (Tables X-XII, fig.105). After an oral dose of ^{131}I the maximum uptake of ^{131}I by the thyroid, namely

88 per cent, was reached in 6 hours and was maintained until 48 hours. At 96 hours it was 73 per cent. The total serum ^{131}I at 48 hours was 0.62 per cent of the dose per litre; the protein-bound ^{131}I fraction was 0.48 per cent. The total butanol-extractable ^{131}I was 0.27 per cent and the thyroxine-like ^{131}I was 0.08 per cent. The urine excretion figures were 0.7 per cent in the 0-6 hour period, 6.0 per cent in the 6-24 hour period, and 4.2 per cent in the 24-48 hour period.

Treatment and progress. Thyroidectomy was performed on September 20th., 1955, by Mr. Ian McLennan. 170 g. of thyroid tissue was removed. She was started on desiccated thyroid, later changed to sodium thyroxine, and remained under supervision as an out-patient. She requires a maintenance dose of 0.05 mg. three times daily. In March 1956 she had an iron deficiency anaemia (Hb 11.0 g. per 100 ml.) which responded to oral iron therapy. In February 1959 she had to be readmitted to hospital with anaemia due to blood loss following dental extraction; her Hb. which was 9.0 g. again responded to oral iron therapy. There has been no recurrence of the goitre.

Further investigations. On 23rd. January, 1959, she was given by mouth 25 μc of $M^{131}IT$. Only 3.8 per cent of the radioactive monoiodotyrosine was excreted unchanged in the urine in the first 6 hours following its ingestion. In the pooled samples for 0-2 and 2-6 hours the greatest proportion of radioactive monoiodotyrosine was 18.2 per cent (Table XIV).

Discussion

Clinical features.

The chief clinical interest in the K family is the evidence that within the same family the goitrous members may be either euthyroid or hypothyroid. E.K.(case X) had undoubtedly been hypothyroid in childhood and in consequence her early development had been slow. Persistent stigmata were mental retardation and simple-mindedness, and delayed ossification which was approximately 4 years behind her chronological age when she was first seen. When she was admitted to hospital in 1955 there were no gross myxoedematous features. Her serum protein-bound iodine was 3.6 μg. per 100 ml., which was a borderline value between the euthyroid and hypothyroid ranges. C.K. (case VIII) had developed normally

and had remained euthyroid. M.K. (case IX) had almost certainly been hypothyroid as a child. She was clinically euthyroid when she was admitted to hospital in 1955. The basal metabolic rate and the serum protein-bound iodine were also normal. It appears that the evidence of hypothyroidism diminished or disappeared in cases X and IX respectively when the increased demands of the period of active growth and development at and around puberty were over.

C.K.(case VIII) had a goitre from birth, yet she remained euthyroid. A goitre had been noticed in M.K. (case IX) at approximately 1 year, before there was any evidence to suggest hypothyroidism. By contrast E.K. (case X) was obviously hypothyroid before her goitre was observed. It is apparent that thyroid hyperplasia and enlargement at times compensate adequately for inefficient function, and maintain the patient in a euthyroid state. In these circumstances it is a goitre that will be first noted. At other times when the thyroid cannot enlarge sufficiently to compensate for its intrinsic inadequacy evidence of impaired function in the form of the clinical features of hypothyroidism is likely to attract attention before a goitre is detected. On these

occasions either the hypothyroidism precedes the goitre or, more probably, the goitre passes unobserved until the features of hypothyroidism are sufficiently marked to arouse interest. It is easy to overlook a goitre in the short thick neck of a cretinous child.

Radioiodine studies.

The failure of potassium perchlorate to discharge any ^{131}I from the thyroid gland of case VIII showed that the initial steps in the utilization of iodide and hence peroxidase activity were normal. Dehalogenase activity also was normal, as was shown directly by the efficient deiodination of $M^{131}IT$ in vitro by thyroid slices from case VIII, and indirectly by the efficient deiodination of an oral dose of $M^{131}IT$ by cases VIII and X.

The behaviour of the serum radioactivity in these patients aroused my interest because it is unusual to find that only 43 to 57 per cent of the serum ^{131}I is butanol-extractable at a time when 77 to 100 per cent is precipitable with the proteins. It is obvious that some abnormal iodine compound is included with the protein-bound ^{131}I. The finding of normal dehalogenase activity virtually eliminated any possibility that some of the

radioactivity precipitated with the proteins was due to free iodotyrosines, which would in any case have been butanol-extractable (Table VI). An unidentified compound with somewhat similar characteristics has been found in the serum of patients with thyroid carcinoma (Robbins et al. 1955, Tata et al. 1956) and Hashimoto's thyroiditis (Owen and McConahey 1956). Di George and Paschkis(1957) described a similar substance in the blood of a $7\frac{1}{2}$-year old girl with goitrous hypothyroidism. De Groot et al. 1958 investigated in some detail the properties of a similar iodinated compound which was present in the serum of a 27-year old woman with goitrous hypothyroidism. Enzymatic hydrolysis of the serum of this patient liberated monoiodotyrosine, diiodotyrosine and thyroxine. De Groot and his colleagues considered that the defect in their patient was either the formation of an abnormal thyroprotein in the gland, or abnormal fragmentation and release of normal thyroglobulin.

Detailed study of the abnormal serum compound was hampered by the fact that at the time C.K. (case VIII) had her thyroidectomy in 1958 practically all the protein-bound ^{131}I (83 per cent of the total serum ^{131}I) was butanol-extractable

^{131}I (72 per cent of the total serum ^{131}I), whereas earlier in 1957 the protein-bound ^{131}I was 100 per cent of the serum ^{131}I and the butanol-extractable ^{131}I was only 43 per cent of the serum ^{131}I. Enzyme digestion of the serum in 1958 produced no definite increase in the amount of ^{131}I which was butanol-extractable, hence chromatography of the digested serum could not be expected to reveal any additional compounds.

Some information relevant to the problem of the identity of the protein-bound ^{131}I in cases VIII - X may conveniently be referred to here. At the request of Dr. James H. Hutchison I investigated in 1959 3 goitrous cretins aged 1 year 3 months (H. McL.), 5 years (T.McL.) and 9 years (A. McD.) H. McL. and T. McL. were brothers. Most of the radioactivity present in their serum 48 hours after 10 μc ^{131}I was protein-bound, namely 84 to 100 per cent, but only a small fraction of it, namely 16 to 37 per cent was butanol-extractable (Table XVII). While the butanol-extractable fraction was not materially increased when the serum of one of them was digested with trypsin, in this patient (A. McD.) and in the other one so studied (H.McL.) the percentage of the butanol-extractable ^{131}I rose markedly

when the serum was digested with chymotrypsin. In one of them (A. McD.) the butanol-extractable fraction increased from 24 per cent to 58 per cent, of which 28 per cent was thyroxine-like and 30 per cent iodotyrosine-like. In the other (H.McL.) the butanol-extractable fraction increased from 37 per cent to 76 per cent, of which 31 per cent was thyroxine-like and 45 per cent was iodotyrosine-like. It is of interest that De Groot et al. (1958), who found a similar iodinated compound in the serum of a 27-year old woman with goitrous hypothyroidism, also found that enzymatic digestion of her serum, particularly with chymotrypsin liberated monoiodotyrosine, diiodotyrosine and thyroxine. Like Owen and McConahey (1956) I have also found a similar iodinated compound in the serum of 4 cases of Hashimoto's thyroiditis in which I have made these observations (Table XVII).

I established by the oral $M^{131}IT$ test that the dehalogenase activity of the goitrous cretins(H.McL., T.McL and A.McD) was normal. It would therefore appear that any iodotyrosines incorporated within the abnormal iodinated protein are not affected by dehalogenase as are free iodotyrosines. If

this surmise is correct it supports the belief that any thyroxine present in the abnormal protein found in the serum of cases VIII - X might also be unaffected by the action of the appropriate extra-thyroidal deiodinating enzyme.

Chromatographic studies of thyroid gland.

The iodine compounds present in only one of the thyroids (case VIII) were studied by chromatographic analysis after ^{131}I. The chromatograms prepared from enzyme digests of thyroid tissue homogenate showed that monoiodotyrosine and diiodotyrosine were formed. The MIT/DIT ratio varied from 0.1 to 0.5 which is low compared with the normal ratio of 0.5 - 0.75 found by Pitt-Rivers et al. (1957) in normal thyroids and 0.36 - 0.58 by myself in rat thyroids (Table VIII). Such a low ratio is unique in my experience. I do not know its explanation. My immediate reaction was to explain it away by suggesting that the iodine stores of the thyroid tissue were well stocked, for depletion of the iodine stores was shown to increase the MIT/DIT ratio in rats fed on iodine-deficient diets (Bois and Larsson 1958). Without knowledge of the PB^{127}I content of the thyroid tissue it is probably unwise to speculate further about its significance.

No thyroxine was found in the chromatograms prepared from the enzyme digests of thyroid tissue. Such thyroxine as was present was a constituent of an iodine containing compound which remained at the origin of the chromatograms. Like thyroglobulin this compound was not butanol-extractable; unlike thyroglobulin it resisted digestion by trypsin and chymotrypsin. Alkaline hydrolysis, however, rendered 80 per cent of its radioactivity butanol-soluble and it was then shown to be made up chiefly of thyroxine, and smaller amounts of monoiodotyrosine and diiodotyrosine.

It is tempting to conclude that these results indicate the presence of an unusual thyroid protein which is unusually resistant to enzymatic hydrolysis. It may be that the compound which was intermittently present in the serum of C.K. (case VIII) and which was also detected in the serum of both her sisters was in some way related to it. If this surmise is correct then the unknown substance, at least when present in the thyroid gland, differed from the compound described by De Groot et al. (1958) in the serum of their 27-year old patient with goitrous hypothyroidism in that it resisted enzymatic digestion. Likewise it differed from the

compound in the goitrous cretins (H. McL., T.McL. and A.McD.) to whom I have referred earlier, and to that in at least one of the cases of Hashimoto's thyroiditis (Table XVII, case 20, Watson et al. 1959). It might possibly be that the iodinated protein yields to the action of chymotrypsin when it reaches the serum because it has already been subjected to the proteolytic activity of thyroid protease before liberation from the thyroid gland.

The chemical basis of the radioactivity in zone Y of the chromatograms prepared from the thyroid gland of case VIII was not established. A similar zone of radioactivity has now been found in a goitrous cretin with a dehalogenase defect (Chapter 8), in 5 patients with non-toxic nodular goitres (Table XVIII), in one patient with Hashimoto's thyroiditis, and occasionally in normal rat thyroid (Table VIII).

Electrophoresis of thyroid proteins.

The results of the electrophoresis of the thyroid proteins of cases 2 (Chapter 8), II and IV (Chapter 16), VI (Chapter 17) and VIII have been recorded because they demonstrate the type of record that is to be expected when electrophoresis is applied to the study of goitre. Several proteins are present in most goitres (fig.48); in order, from the origin of the

electrophoretic strips, they are recognized as:

1. Q, an unexpected band, of unknown significance
2. Gamma globulin
3. Haemoglobin
4. Thyroglobulin
5. Albumin.

The extent to which blood proteins contribute to the protein pattern of thyroid tissue is uncertain but presumably they account for the haemoglobin and probably for some of the gamma globulin. Thyroglobulin is a distinctive thyroid protein. Albumin is probably mostly derived from thyroid tissue, for it is present in amounts, relative to gamma globulin, greatly in excess of those in the plasma. Q protein is not present in plasma.

Q protein is almost certainly a normal thyroid protein. It is hoped in future that it may be possible to identify it with a particular cellular fraction by differential centrifugation. At present little more may be written about it than that it was recognized in 22 out of 23 glands studied, including those of cases 2 (fig.47), II (fig.58), VI (fig. 73), and VIII (fig.102), but not that of case IV (fig.61), who had had recent iodine medication, and that it was

unusual to find ^{131}I apparently associated with it. In case VIII (fig.102), however, there was a peak of radioactivity close to the origin of the electrophoretic strips, apparently related to Q protein. Radioactivity was also found in this zone of strips from certain other goitres. In Hashimoto's thyroiditis (5 cases) radioactivity was virtually exclusively close to the origin, but the background radioactivity of the strips was inexplicably high (fig.106). In thyrotoxicosis prepared for operation with methyl thiouracil only (3 cases) there was a peak of radioactivity at the origin but most of it was normally present in the thyroglobulin zone (fig.107). In all of the samples with radioactivity near the origin the amount of stainable thyroglobulin was negligible.

Radioactivity was not present near the origin of a strip from the gland of a thyrotoxic patient who died 5 days after 10 mc. ^{131}I (fig.108). Its absence in this case suggests that the methyl thiouracil and not the thyrotoxicosis was responsible for its unusual location in the methyl thiouracil treated cases. It is, therefore, of interest that Roche et al. (1951) found a protein, which they considered abnormal, but which may be Q protein, in thiouracil treated dogs.

Experience with electrophoresis of thyroid proteins is still toolimited to form any definite opinion about these findings. Present evidence supports the following interim judgments:

1. Q protein is not denatured protein, for, if it were, it might be expected that there would be some relationship between its ^{131}I content and that of thyroglobulin and/or albumin, and this does not appear to be so.
2. The ^{131}I occasionally responsible for an early radioactive peak is due to organically bound ^{131}I and not to ^{131}I as iodide absorbed to protein. No radioactivity was present near the start of the electrophoretic strips of case II, in whose gland up to 48.8 per cent of the ^{131}I present was iodide.
3. There is no proof that the radioactivity near the origin of the electrophoretic strips is definitely or necessarily only associated with Q protein, and not with some other protein of similar electrophoretic mobility. For example, in Hashimoto's thyroiditis in which there is an auto-immune reaction, it is possible that radioactivity

may be associated with a protein-complex formed by the reaction of thyroglobulin with antibody. Preliminary studies (Goudie, personal communication) show that serum from cases of Hashimoto's thyroiditis reacts with thyroglobulin from non-toxic nodular goitres and alters its electrophoretic mobility in this way. It is not known whether these observations have any relevance to the findings in the thiouracil treated cases of thyrotoxicosis or in case VIII, but it is of interest that, as in case VIII, an iodine fraction was present in the serum of the 4 cases of Hashimoto's thyroiditis so studied, which was precipitable with the proteins but was not butanol extractable (Table XVII). An alternative explanation which suggests itself is that various mechanisms which may be either inherited (case VIII) or acquired (methyl thiouracil or Hashimoto's thyroiditis), interfere with the biosynthesis or storage of thyroxine and its precursors and that Q protein may to a greater or lesser extent become concerned with the synthesis or binding of these substances.

4. As yet there is no evidence that Q protein plays any

part normally in intrathyroidal iodine metabolism. Hence there is no evidence that the apparent association of ^{131}I with it is an exaggeration of a normal process which has been revealed because the conditions enumerated above have interfered with the transport of organic ^{131}I compounds from it to thyroglobulin.

Case VIII had had a course of para-amino salicylic acid, 18 g. daily, for 12 weeks in the Summer of 1955. Like thiouracil PAS has a blocking effect on thyroid hormone synthesis. It is unlikely that its use would be responsible for unusual chromatographic and electrophoretic findings in the thyroid 3 years later. It certainly could not explain the abnormal protein-bound ^{131}I in the serum of her 2 sisters.

Genetic studies. Four out of the 8 children in the family were goitrous. The familial incidence of the defect is obvious, and is in keeping with a genetic basis for it. The family lived in Glasgow where goitre is non-endemic. It is unthinkable that the goitres were due to primary iodine deficiency, and the environment is unlikely to have had anything to do with a goitre which was present at birth (case VIII) when the mother

was perfectly healthy and non-goitrous. A similar anomaly was also found in the serum of 3 goitrous cretins, to whom reference has already been made. Two of them were brothers.

Conclusions

The chief metabolic error in cases VIII - X was the formation of an unusual iodinated thyroid compound, probably a protein, from which thyroxine was released with difficulty. At times this compound, or a derivative of it, was present in the blood.

The result of the defect was difficulty in supplying enough thyroid hormone to the tissues. If the compensatory thyroid enlargement secured an adequate output of hormone the patient remained euthyroid (case VIII); if it failed she became hypothyroid (case X). As the metabolic demands changed with age so the thyroid gland coped with them less or more successfully and the patient who had been for a time hypothyroid became euthyroid (case IX).

It is of interest that affected patients in the one family may be either euthyroid or hypothyroid.

The familial incidence of the goitre suggests that the defect has a genetic basis.

CHAPTER 20

GENERAL DISCUSSION

In the selected group of 10 young patients with sporadic goitres various biochemical abnormalities, similar to those already described in sporadic goitrous cretinism, have been defined. There is also evidence that these defects have a genetic basis. These findings support the hypothesis that inherited disturbances of hormone synthesis are responsible for some cases of sporadic goitre. It is obviously premature to attempt to assess how frequently a genetically determined defect in synthesis is responsible for sporadic goitre. The results of the present investigations have been sufficiently fruitful to encourage further research along similar lines.

Additional encouragement comes from the findings of Pitt-Rivers et al. (1957) in adults with non-toxic nodular goitres. They administered ^{131}I to patients who were

about to undergo surgery and made a chromatographic analysis of the various iodine fractions in thyroid tissue removed at operation. They found a diminished concentration of ^{131}I, and a lower content of thyroxine and a higher ratio of monoiodotyrosine (MIT) to diiodotyrosine (DIT) than they found in normal thyroid glands. They suggested that nodular goitre probably resulted from the inability of the thyroid gland to synthesize sufficient hormone for the body's needs and for storage. They concluded that the biochemical insufficiency was probably due to a falling off of the activity of the enzymes involved in the various stages of synthesis but they did not know what initiated this.

My own experience in 10 adult patients with goitres which were nodular is similar to that of Pitt-Rivers et al. (1957) (Table XVIII). The concentration of ^{131}I per g. of thyroid tissue in 8 of them was substantially lower than the values of 2.8 and 1.6 per cent that Mrs. Pitt-Rivers and her colleagues found in 2 normal thyroids examined 24 and 48 hours after an oral dose of ^{131}I. A measurable amount of thyroxine ^{131}I was present in only one of them, and the MIT/DIT ratios were above 0.75 in 8 of them.

The concentration of ^{131}I per g. of thyroid tissue, less than 0.8 per cent of the dose, was also substantially lower in the thyroid glands of the cases of sporadic goitre described in this thesis than the values that Pitt-Rivers et al. (1957) found in normal thyroid glands. Interpretation of the significance of such deviations from normal is probably worthless without a knowledge of the $PB^{127}I$ content of the thyroid tissue. They do suggest, however, that despite the apparent avidity of the enlarged glands for ^{131}I, the glands remain fundamentally inefficient per unit mass of tissue.

The failure to detect thyroxine in a gland with a well-defined defect, such as case II (Chapter 16) with impaired peroxidase activity, despite the clinical evidence that enough hormone was being produced to keep the patient euthyroid is a warning not to accept too readily absence of thyroxine in the thyroid as evidence of a coupling defect. The absence of detectable amounts of thyroxine in the thyroid may simply be due to the rapid secretion of such hormone as is produced in an attempt to secure an adequate output of hormone so that little or none is available to store. This belief is perhaps supported by the chromatographic findings

in cases 2 (Chapter 8) and VI (Chapter 17), each of whom had a dehalogenase defect. No thyroxine was detected in the thyroid of case 2, who was hypothyroid, although thyroxine was identified in her serum. Presumably a small amount of high specific activity thyroxine was secreted into the blood but the amount was inadequate and none was stored. On the other hand in case VI, in whom thyroid hyperplasia had apparently successfully compensated for the dehalogenase defect and had achieved sufficient hormone output to keep her euthyroid, thyroxine was not only identified in her serum, it was also found in the paranodular tissue of her thyroid.

This simple explanation of the absence of thyroxine in the presence of an established defect of peroxidase or dehalogenase commends itself to me in preference to the suggestion that there may also be a deficiency of coupling enzyme. It may be that impairment of coupling enzyme activity has been too readily accepted as the explanation of the goitre in case VII (Chapter 18). Serum studies and direct examination of the enzyme digest as in case VIII (Chapter 19) might have revealed some abnormality. The deficiencies in

the investigations of case VII underline the importance of using all techniques available in the investigation of these cases.

An increased MIT/DIT ratio in thyroid tissue was a finding common to 3 of the 4 groups of patients with sporadic goitre. Two groups have well-defined defects, namely impaired peroxidase and impaired dehalogenase activity. The increased MIT/DIT ratio is therefore more likely to be a non-specific effect, rather than a specific effect of dyshormonogenesis. It may be the consequence of depleted organic iodine stores (Pitt-Rivers et al. 1957, Bois and Larsson 1958) rather than an indication that the enzyme which activates diiodotyrosine synthesis is relatively inefficient (Pitt-Rivers et al. 1957).

While the biochemical studies have established certain abnormalities some of which are related to impaired activity of a specific enzyme, not all the defects found have been fully defined, and presumably there are other as yet undiscovered defects. More work is needed in cases such as VIII - X to identify any abnormal thyroid compound which is unusually resistant to enzyme digestion and to elucidate the significance

of such a finding. Likewise the significance of the protein, designated Q, in the electrophoretograms of the proteins of a variety of goitres, and the occasional apparent association of organically bound ^{131}I with it, as in case VIII (Chapter 19) remain to be explained. Rall (1956) observed that at that time no syndrome had been described which was characterized by an abnormal thyroglobulin. Cases VIII - X may qualify to be so classified.

It is apparent that the clinical effects of each defect and perhaps the completeness of each defect may vary not only from one group of patients to another, but also within the same family and perhaps even within the same thyroid gland.

For example, impaired ability to utilize trapped iodide, presumably due to impaired peroxidase activity, may lead to goitres with (Stanbury et al.1955 a) or without (cases I-V, Chapter 16) hypothyroidism. Stanbury and his colleagues failed to find any iodinated tyrosines or thyronines in the thyroid of their patient who was hypothyroid, suggesting that the block to synthesis was in effect complete.

Iodinated amino acids were present in the thyroids of both cases I and II, who were euthyroid, confirming the clinical deduction that the defect was not complete.

There is evidence too that other defects may vary in their clinical effect, though it is less clear why this should be so. For example, the dehalogenase defect led to goitrous hypothyroidism in the tinker family-group (cases 1-16, Chapter 3), while case VI (Chapter 17) had a large goitre only, but remained euthyroid. On the basis of the biochemical studies, either the direct demonstration of impaired dehalogenase activity in fresh thyroid slices (Chapters 8 and 17) or its indirect demonstration by the $M^{131}IT$ excretion test (Chapters 7 and 17), the enzyme defect was no more severe in cases 1 - 16 than in case VI. There is little doubt that loss of the iodotyrosines accounts for the clinical state of these patients, but precisely how it does this is unknown. It may be due to a secondary state of iodine deficiency consequent upon the loss of the iodine of the iodotyrosines. The iodine lack may vary; it may be so extreme that it causes goitrous cretinism or it may be less severe and cause goitre without hypothyroidism. This

hypothesis does not explain why the degree of iodine lack should vary, so that, even if it is correct, it is also possible that additional factors are involved. The fact that minor biochemical abnormalities were demonstrated in some of the apparently normal and non-goitrous relatives of both cases 1 - 16 and case VI suggests that the enzyme defect may vary in degree according to its inheritance for presumably these relatives were heterozygous while the affected patients were homozygous for the gene (Chapter 9).

The clinical effect of a particular defect may vary in the same family. All the tinker patients (Chapter 3) were hypothyroid as well as having goitres but the severity of the hypothyroidism varied and so did the ages of onset of recognizable clinical features. Amongst group IV patients (Chapter 19), who were apparently producing and secreting an abnormal thyroid protein, there was an even more obvious variation in the clinical effect of the defect. Thus, case VIII had always been euthyroid; case IX had been hypothyroid in childhood but was euthyroid when she was seen at the age of 18 years; case X had clearly been hypothyroid since infancy, and was still hypothyroid when she was seen aged 21 years.

There is some evidence too that the biosynthesis of the thyroid hormone may be disorganized to varying degrees in different areas of the same thyroid gland. This effect is perhaps most obvious in the results of the chromatographic studies of the ^{131}I compounds in the thyroid gland of case VI (Chapter 17). The absence of thyroxine in the nodules and the very high MIT/DIT ratio there suggests a more severe derangement than in the paranodular tissue where thyroxine was present and the MIT/DIT ratio was less markedly elevated. In neither the nodular nor paranodular tissue was dehalogenase activity demonstrated by the in vitro test with fresh thyroid slices.

It is unfortunate that difficulties in the assay of thyrotrophin (TSH) have daunted most investigators from attempting to measure the level of TSH in serum or urine of patients with simple goitre or sporadic goitrous cretinism. Such an assay is desirable in the investigation of these patients. Indeed it is essential to confirm or refute the belief that the goitre is produced by compensatory thyroid hyperplasia mediated through the

anterior pituitary and increased TSH production. The investigations of cases I - X are also incomplete in this respect, but as noted in Chapter 3, the level of TSH in the serum of one of the tinker children with goitrous cretinism (case 11) was unusually high.

Another problem that remains outstanding is the relationship of the neoplasia to the hyperplasia in the thyroid gland of case VI (Chapter 17). This is a problem of practical as well as of theoretical importance. Should further experience show that the risks of neoplastic change are more than a remote hazard then thyroidectomy rather than thyroxine therapy may be the treatment of choice in all nodular goitres in childhood, whether or not a defect in synthesis is demonstrated.

CHAPTER 21

SUMMARY AND CONCLUSIONS

Evidence for inherited disturbances of hormone synthesis in selected cases of sporadic goitre as well as in sporadic goitrous cretinism has been presented and discussed.

Such defects in synthesis are considered to be the cause of the goitres, and of the hypothyroidism in these cases.

The findings encourage further research to establish the truth or falsity of the hypothesis that inherited defects in hormone synthesis have a significant rôle in the pathogenesis of goitre.

Implicit in the hypothesis which seeks to explain the pathogenesis of goitre on the basis of a defect in hormone synthesis is the belief that the goitre is the result of a compensatory effort mediated through the anterior pituitary and increased production of thyrotrophin (TSH) (Table XIX). The thyroid becomes hyperplastic and

enlarges, and with less or more success makes good the difficulties in hormone production. If the goitre fails to produce enough hormone hypothyroidism as well as goitre results, as in sporadic goitrous cretinism. Alternatively, the hyperplastic gland, though perhaps still inefficient per unit mass of tissue, may, by its increase in size, compensate for its intrinsic inadequacy and produce enough hormone to keep the patient euthyroid.

Defects at various levels of synthesis occur in varying degrees of severity, from the more or less complete which produces goitrous cretinism recognizable in infancy to the minimal, which only comes to light when special tests, such as the $M^{131}IT$ excretion test, are used to detect it. It is not difficult to imagine that minor defects may occur and account for goitres which develop later in life. Depending on their severity they may operate alone or they may require additional factors to augment their effects.

The belief that inherited defects in hormone synthesis may lead to goitre does not conflict with other theories of

goitrogenesis. Indeed, it complements them and helps to explain the rather uncertain rôle of some of the traditional factors in the causation of sporadic goitre. No doubt iodine deficiency and goitrogens may act alone, but they probably do so rarely in this country. It appears reasonable to expect that increased metabolic demands, for example at puberty and during pregnancy, iodine deficiency, and even at times exogenous goitrogens, should contribute to the evolution of goitre more readily in the gland that already has to overcome an inherited defect in hormone synthesis.

Sporadic goitre, as it is usually met with in practice, shows a marked predominance in females who usually out-number males by approximately 8 to 1. By contrast, amongst the special cases of goitrous cretinism and sporadic goitre discussed in this thesis there were 17 females and 9 males giving a much lower relative incidence in females. The cause of the marked predominance of sporadic goitre, as it is usually met with, in the female sex is uncertain. Perhaps it is due, in part at least, to the altered metabolic demands and the other endocrine changes which occur at puberty, during reproductive life including during pregnancy, and at the

menopause. Such factors presumably exert a relatively more important influence in patients with minor enzyme defects than in those with more severe defects, and so lead to the different sex ratios in the two groups.

CHAPTER 22

EPILOGUE

Though sporadic goitrous cretinism is a rare condition its study has been rewarding. The immediate satisfaction has been in establishing the cause of sporadic goitrous cretinism in a unique family group of tinkers. This was the task set me in 1951 by Dr. Hutchison when he asked me to cooperate with him in the investigation of 4 children, members of one tinker family, who were hypothyroid and had goitres. The significance of the findings in our tinker patients and in similar patients extends beyond their immediate value in elucidating the cause of sporadic goitrous cretinism. They have been a stimulus to enquiry into the cause of other cases of sporadic goitre, and indeed of goitre in general. It well might have been of patients with sporadic goitrous cretinism that Garrod wrote "they are of great interest - for they are, so to speak, Nature's experiments, by the study of which valuable light is thrown upon the normal working of the metabolic processes in man". Or it might have been of them that

William Harvey wrote in a letter in 1657: "Nature is nowhere accustomed more openly to display her secret mysteries than in cases where she shows traces of her workings apart from the beaten path; nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual law of Nature by careful investigation of cases of rarer forms of disease. For it has been found, in almost all things, that what they contain of useful or applicable nature is hardly perceived unless we are deprived of them, or they become deranged in some way".

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I wish to thank my colleagues who helped me in various ways and who referred patients for investigation. In particular, I am indebted to Dr. James H. Hutchison, Royal Hospital for Sick Children, Glasgow, who provided me with the opportunity to study sporadic goitrous cretinism and with whom I have continued a happy collaboration in the investigation of many other cases of cretinism and goitre. My thanks are due to Dr. W.C. Watson, University Department of Medicine, Royal Infirmary, Glasgow, who carried out the electrophoretic studies; to Dr. A.R. Currie, University Department of Pathology, Royal Infirmary, Glasgow, for his opinion on the pathology of the thyroid gland of

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REFERENCES

BEACH, F. (1896). The etiology of sporadic cretinism. Brit. med. J., 2, 620.

BENUA, R.S., DOBYNS, B.M. (1955). Iodinated compounds in serum, disappearance of radioactive iodine from thyroid, and clinical response in patients treated with radioactive iodine. J. clin. Endocr., 15,118.

BERNHEIM, M., BERGER, M. (1954). Le diagnostic et le mécanisme des insuffisances thyroidienne de l'enfant étudies a la faveur des epreuves au radio-iode. Sem.Hôp. Paris, 30, 3575.

BERSON, S.A., YALOW, R.S., SORRENTINO, J., ROSWIT, B. (1952). The determination of thyroidal and renal plasma ^{131}I clearance rates as a routine diagnostic test of thyroid dysfunction. J. clin. Invest., 31, 141.

BIRCHER, H. (1883). Der endemische kropf und seine beziehungen zur taubstummheit und zum kretinismus. Basel. Benno Schwabe.

BLACKIE, G.S. (1855). Cretins and cretinism; a prize thesis of the University of Edinburgh. Edinburgh. Maclachlan and Stewart.

BLOM, P.S. TERPSTRA, J. (1953). High PBI^{131} concentration in blood of patients with myxedema; preliminary report. J. clin. Endocr., 13,989.

BOIS, I, LARSSON, L-G. (1958). Studies on the ratio between labelled monoiodotyrosine and labelled diiodotyrosine in the thyroid gland of rat after radioiodine administration. Acta endocr. (Kbh.), 29, 102.

BRAID, F. (1951). Hypothyroidism in childhood. Brit. med.J., 1, 1169.

BRAIN, W.R. (1927). Heredity in simple goitre. Quart J. Med., 20, 303.

BUCHANAN, J., CROOKS, J. (1959). Goitrous cretinism. Scot. med. J., 4, 115.

BURRELL, C.D., GAIRDNER, D. (1955). Cretinism in three siblings. Proc. roy. Soc. med., 48, 1026.

CHAIKOFF, I.L., TAUROG, A. (1948). Symposium on the use of isotopes in biology and medicine. Madison. University of Wisconsin Press.

CHILDS, B., GARDNER, L.I. (1954). Etiologic factors in sporadic cretinism. An analysis of ninety cases. Ann. hum. Genet., 19, 90.

CLAYTON, A.W., SMITH, J.D., LEISER, A. (1958). Familial goiter with defect in intrinsic metabolism of thyroxine without hypothyroidism. J. Pediat., 52, 129.

COSTA, A., COTTINO, F., FERRARIS, G.M., MARCHIS, E., MAROCCO, F., MORTARA, M., PIETRA, R. (1953). Richerche sulla patogenesi del cretinismo endemico. Medicino (Parma), 3, 455.

DAVENPORT, C.B. (1932). The genetical factor in endemic goiter. Carnegie Institute of Washington Publication No.428.

DEBRÉ, R., TUBIANA, M., MICHEL, R., ROYER, P. (1956). Goitre myxoedémateux par trouble congénital de la synthèse hormonale. Soc. Pédiat. Paris, sous presse Arch. franç. Pediat. cited by Lelong et al. 1956.

DE GROOT, L.J., POSTEL, S., LITVAK, J., STANBURY, J.B. (1958). Peptide-linked iodotyrosines and iodothyronines in blood of a patient with congenital goiter. J. clin. Endocr., 18, 158.

DENT, C.E. (1957). Symposium on inborn errors of metabolism. Amer. J. Med., 22, 671.

DE ROBERTIS, E., GRASSO, R. (1946). Peroxidase activity of the thyroid gland under normal and experimental conditions. Endocrinology, 38, 137.

DI GEORGE, A.M., PASCHKIS, K.E. (1957). Sporadic hypothyroidism associated with goiter. J. clin. Endocr., 17, 645.

DINGLEDINE, W.S., PITT-RIVERS, R., STANBURY, J.B. (1955). Nature and transport of iodinated substances of blood of normal subjects and of patients with thyroid disease. J. clin. Endocr., 15, 724.

DOBYNS, B.M., HIRSCH, E.Z. (1956). Iodinated compounds in the lymphatic pathways leading from the thyroid. J. clin. Endocr., 16, 153.

FAGGE, C.H. (1871). On sporadic cretinism occurring in England. Med.-chir. Trans., 54, 155.

FAWCETT, D.M., KIRKWOOD, S. (1953). The synthesis of organically bound iodine by cell-free preparation of thyroid tissue. J. biol. Chem., 205, 795.

FRASER, R., HOBSON, Q.J.G., ARNOTT, D.G., EMERY, E.W. (1953). The urinary excretion of radioiodine as a clinical test of thyroid function. Quart. J. Med., 22, 99.

FRIERSON, H.F., HAWK, J.C.JR., JENKINS, M.Q. (1957). Sporadic cretinism with goiter occurring in identical twins. J. Pediat., 51, 704.

GARROD, A.E. (1908). The Croonian lectures on inborn errors of metabolism. Lancet, 2, 142.

GATES, R.R. (1946). Goiter in Human Genetics. New York. The MacMillan Company.

GOODWIN, J.F., MACGREGOR, A.G., MILLER, H., WAYNE, E.J., (1951). The use of radioactive iodine in the assessment of thyroid function. Quart. J. Med., 20, 353.

GROSS, J., PITT-RIVERS, R. (1952). Identification of 3:5:3'-L-triiodothyronine in human plasma. Lancet, 1, 439.

GROSS, J., PITT-RIVERS, R. (1953). 3:5:3'-triiodothyronine. I. Isolation from thyroid gland and synthesis. Biochem. J., 53, 645.

GROSS, J. (1954). Thyroid hormones. Brit. med. Bull., 10, 218.

HAMILTON, J.G., SOLEY, M.H., REILLY, W.A., EICHORN, K.B.(1943). Radioactive iodine studies in childhood hypothyroidism. Amer. J. Dis. Child., 66, 495.

HARINGTON, C.R. (1926). Chemistry of Thyroxine. II. Constitution and synthesis of diiodothyronine. Biochem. J., 20, 300.

HARINGTON, C.R., BARGER, G. (1927). Chemistry of Thyroxine. III. Constitution and synthesis of thyroxine. Biochem. J., 21, 169.

HAYLES, A.B., KENNEDY, R.L.J., WOOLNER, L.B., BLACK, B.M.(1956). Nodular lesions of the thyroid gland in children. J. clin. Endocr., 16, 1580.

HORST, W., HARNACK, G.A. Von. (1953). Hypothyreosen im kindesalter. Dtsch. med. Wschr., 78, 1259.

HUBBLE, D. (1953). Familial cretinism. Lancet, 1, 1112.

HURXTHAL, L.M., MUSULIN, N.(1946). Cretinism. Amer. J.Med., 1, 56.

HUTCHINSON, J. (1855). The Aldeberg Hospital for Cretins. Med. Tms (Lond.), 32, 117.

HUTCHISON, J.H., McGIRR, E.M.(1954). Hypothyroidism as an inborn error of metabolism. J. clin. Endocr., 14, 869.

HUTCHISON, J.H., McGIRR, E.M. (1956). Sporadic non-endemic goitrous cretinism. Hereditary transmission. Lancet,1, 1035.

JACKSON, A.D.M. (1954). Non-endemic goitrous cretinism. Arch. Dis. Childh., 29, 571.

JOHNSEN, S. (1958). Familial deafness and goitre in persons with a low serum level of protein-bound iodine. Acta otolaryng. (Stockh.), Suppl., 140, 168.

KEATING, F.R., JR., POWER, M.H., BERKSON, J., HAINES, S.F.(1947). The urinary excretion of radioiodine in various thyroid states. J. clin. Invest., 26, 1138.

KENDALL, E.C. (1915). The isolation in crystalline form of the compound containing iodine which occurs in the thyroid; its chemical nature and physiological activity. Trans. Ass. Amer. Phycns, 30, 420.

KUNSTADTER, R.H., KOHLENBRENER, R.M., OLINER, L. (1957). Thyroid dysfunction in goitrous children. A.M.A. Amer. J. Dis. Child., 94, 682.

LANCET (1956) Annotation: One gene: one enzyme? Lancet, 2, 1035.

LELONG, M., JOSEPH, R., CANLORBE, P., JOB, J.-C., PLAINFOSSE, B. (1956). L'hypothyroidie par anomalie congénitale de l'hormonogénèse. Arch.franç. Pédiat., 13, 342.

LERMAN, J., JONES, H.W., CALKINS, E.(1946). Studies on 2 sporadic cretinous brothers with goiter, together with some remarks on relation of hyperplasia to neoplasia. Ann. intern. Med., 25, 677.

LEVY, R.P., KELLY, L.W., JEFFERIES, W.McK. (1956). The effects of thyrotropin and desiccated thyroid upon hypothyroidism with goiter. Amer. J. med. Sci., 231, 61.

LOGAN, J. (1957). Sporadic goitrous cretinism in xeroderma pigmentosum. J. clin. Endocr., 17, 151.

LONDON MEDICAL GAZETTE (1851). Annotation. London.med.Gaz. 12 (new series), 1054.

McCARRISON, R. (1917). The Thyroid Gland in Health and Disease. London. Ballière, Tindall & Cox.

McCONAHEY, W.M., KEATING, F.R., JR., POWER, M.H. (1949). The behavior of radioiodine in the blood. J. clin.Invest., 28, 191.

McGIRR, E.M., HUTCHISON, J.H. (1953). Radioactive iodine studies in non-endemic goitrous cretinism. Lancet,1,1117.

McGIRR, E.M., HUTCHISON, J.H. (1956). Sporadic cretinism with goitre. Lancet, 1, 106.

McGIRR, E.M., HUTCHISON, J.H., CLEMENT, W.E. (1956). Sporadic non-endemic goitrous cretinism. Identification and significance of monoiodotyrosine and diiodotyrosine in serum and urine. Lancet, 2, 906.

McGIRR, E.M., HUTCHISON, J.H., CLEMENT, W.E. (1959a). Sporadic goitre due to dyshormonogenesis: impaired utilization of trapped iodide. Scot. med. J., 4, 107.

McGIRR, E.M., CLEMENT, W.E., CURRIE, A.R., KENNEDY, J.S. (1959b). Impaired dehalogenase activity as a cause of goitre with malignant change. Scot. med. J., 4, 232.

McGIRR, E.M., HUTCHISON, J.H., CLEMENT, W.E., (1959c). Sporadic goitrous cretinism. Demonstration of dehalogenase deficiency in the thyroid gland of a goitrous cretin, and in heterozygous carriers among her relatives. Lancet, (In Press).

McGIRR, E.M., CLEMENT, W.E., WATSON, W.C., HUTCHISON, J.H., CURRIE, A.R. (1959d). Goitre due to dyshormonogenesis. J. Endocr., 18, xxiii.

MAN, E.B., KYDD, D.M., PETERS, J.P.(1951). Butanol-extractable iodine of serum. J. clin. Invest., 30, 531.

MASON, A.S., OLIVER, R. (1949). Urinary excretion of radioactive iodine as a diagnostic aid in thyroid disorders. Lancet, 2, 456.

MEDICAL TIMES AND GAZETTE. (1855). An English cretin. Med. Tms (Lond.), 32, 266.

MORGANS, M.E., TROTTER, W.R. (1958). Association of congenital deafness with goitre. Lancet, 1, 607.

MOSIER, H.D., BLIZZARD, R.M., WILKINS, L. (1958). Congenital defects in the synthesis of the thyroid hormone. Pediatrics, 21, 248.

MURRAY, I.P.C., McGIRR, E.M., (1959). Radioactive iodine studies in the diagnosis of Hashimoto's thyroiditis. Brit. med. J., (In Press).

MURRAY, M.M., RYLE, J.A., SIMPSON, B.W., WILSON, D.C.(1948). Thyroid enlargement and other changes related to the mineral content of drinking water. Memor.No.18 med. Res. Coun.(Lond.). London: H.M. Stationery Office.

NEEL, J.V., SCHULL, W.J. (1954). Human Heredity. Chicago. University of Chicago Press.

NORRIS, H. (1848). Notice of a remarkable disease, analogous to cretinism, existing in a small village in the West of England. Med.Tms (Lond.), 17, 257.

OSLER, W. (1897). Internal secretions considered in their physiological, pathological and clinical aspects. Trans. Congr. Amer. Phycns Surg., 4, 169.

OWEN, C.A., JR., McCONAHEY, W.M. (1956). An unusual iodinated protein of the serum in Hashimoto's thyroiditis. J. clin. Endocr., 16, 1570.

PARKER, W.R., (1896). An obsolescent variety of cretinism. Brit. med. J., 2, 506.

PFAUNDLER, M.V. (1924). Uber die entstehungsbedingungen von endemischen kropf und kretinismus. Jb.Kinderheilk, 105, 223.

PICKERING, D.E., SHELINE, G.E., CRANE, J.T. (1957). Sporadic familial goitrous hypothyroidism. Amer.J. Dis. Child., 93, 510.

PITT-RIVERS, R. (1948). The action of antithyroid substances on the formation *in vitro* of acetylthyroxine from acetyldiiodotyrosine. Biochem. biophys. Acta, 2, 311.

PITT-RIVERS, R. (1956). A simple preparation of monoiodotyrosine. Chem. and Ind. (Rev.) 21.

PITT-RIVERS, R., HUBBLE, D., HOATHER, W.H., (1957). Chromatographic study of thyroidal iodine metabolism in non-toxic nodular goiter. J. clin. Endocr., 17, 1313.

QUERIDO, A., STANBURY, J.B., KASSENAAR, A.A.H., MEIJER, J.W.A. (1956). The metabolism of iodotyrosine. III. Diiodotyrosine deshalogenating activity of human thyroid tissue. J. clin. Endocr., 16, 1096.

RAILTON, T.C., (1891). Sporadic cretinism. Brit.med.J., 1, 694.

RALL, J.E., (1950). Iodine compounds in blood and urine of man. J. clin. Endocr., 10, 996.

RALL, J.E. (1956). The role of radioactive iodine in the diagnosis of thyroid disease. Amer. J. Med., 20, 719.

REID, J. (1836). On the history and causes of bronchocoele or goitre. Edinb. med. surg. J., 46, 40.

RIGGS, D.S. (1952). Quantitative aspects of iodine metabolism in man. Pharmacol. Rev., 4, 284.

ROBBINS, J., RALL, J.E., RAWSON, R.W. (1953). A unique serum iodine component in certain patients with thyroid carcinoma. J. clin. Endocr., 13, 852.

ROBBINS, J., RALL, J.E., RAWSON, R.W. (1955). A new serum iodine component in patients with functional carcinoma of the thyroid. J. clin. Endocr., 15, 1315.

ROBERTS, J.A.F. (1940). An introduction to medical genetics. London. Oxford University Press.

ROCHE, J., MICHEL, O., DELTOUR, G.H., LISSITSKY, S. (1951). Thyroglobuline marquée ou artificiellement iodée sans denaturation. Formation dans des conditions expérimentales diverses et proprietés. Biochim. biophys. Acta, 6, 572.

ROCHE, J., MICHEL, R., MICHEL, O., LISSITZKY, S. (1952). Sur la déshalogénation enzymatique des iodotyrosines par le corps thyroide et sur son rôle physiologique. Biochim. biophys. Acta, 9, 161.

ROCHE, J., MICHEL, O., MICHEL, R., GORMAN, A., LISSITZKY, S.(1953). Sur la déshalogénation enzymatique des iodotyrosines par le corps thyroide et sur son rôle physiologique. II. Biochim. biophys. Acta, 12, 570.

ROCHE, J., MICHEL, R. (1955). Nature, biosynthesis and metabolism of thyroid hormones. Physiol. Rev., 35, 583.

ROSENBERG, I.N. (1951). Nature of circulating thyroid hormone in Graves' disease. J. clin. Invest., 30, 1.

SCHULTZ, A.L., FLINK, E.B., KENNEDY, B., ZIEVE, L. (1957). Exchangeable character of accumulated I^{131} in the thyroid of a goitrous cretin. J. clin. Endocr., 17, 441.

SCOTTISH MEDICAL JOURNAL (1959). Leading article. The aetiology of simple or non-toxic goitre. Scot. med. J. 4, 142.

SEXTON, D.L., MACK, R.(1954). Cretinism, with or without goiter, in 5 of 10 siblings. J. clin. Endocr., 14, 747.

SHELINE, G.E., CLARK, D.E. (1950). Index of thyroid function; estimation by rate of organic binding of I^{131}. J. Lab.clin. Med., 36, 450.

SILVERMAN, S.H., WILKINS, L. (1953). Radioiodine uptake in the study of different types of hypothyroidism in childhood. Pediatrics, 12, 288.

SLINGERLAND, D.W., (1955). The influence of various factors on the uptake of iodine by the thyroid. J. clin. Endocr., 15, 131.

STANBURY, J.B., HEDGE, A.N. (1950). A study of a family of goitrous cretins. J. clin. Endocr., 10, 1471.

STANBURY, J.B. (1951). Cretinism with goiter: a case report. J. clin. Endocr., 11, 740.

STANBURY, J.B., OHELA, K., PITT-RIVERS, R.(1955a). The metabolism of iodine in two goitrous cretins compared with that in two patients receiving methimazole. J. clin. Endocr., 15, 54.

STANBURY, J.B., KASSENAAR, A.A.H., MEIJER, J.W.A., TERPSTRA, J. (1955b). The occurrence of mono- and di-iodotyrosine in the blood of a patient with congenital goiter. J. clin. Endocr., 15, 1216.

STANBURY, J.B., KASSENAAR, A.A.H., MEIJER, J.W.A., TERPSTRA, J. (1956a). The metabolism of iodotyrosines. I. The fate of mono- and di-iodotyrosine in normal subjects and in patients with various diseases. J. clin. Endocr., 16, 735.

STANBURY, J.B., MEIJER, J.W.A., KASSENAAR, A.A.H. (1956b). The metabolism of iodotyrosines. II. The metabolism of mono- and di-iodotyrosine in certain patients with familial goiter. J. clin. Endocr., 16, 848.

STANBURY, J.B., LITVAK, J. (1957). The metabolism of iodotyrosines. IV. Metabolism of l-diiodotyrosine in patients with hypothyroidism. J. clin. Endocr., 17, 654.

STANBURY, J.B., McGIRR, E.M. (1957). Sporadic or non-endemic familial cretinism with goiter. Amer. J. Med., 22, 712.

STEVENSON, L.E. (1896). Goitre and cretinism. Brit. med.J., 2, 974.

TATA, J.R. RALL, J.E., RAWSON, R.W. (1956). Studies on an iodinated protein in the serum of subjects with cancer of the thyroid. J. clin. Endocr., 16, 1554.

TAUROG, A., CHAIKOFF, I.L. (1948). Nature of circulating thyroid hormone. J. biol. Chem., 176, 639.

TAUROG, A., CHAIKOFF, I.L., TONG, W. (1949). On the occurrence of monoiodotyrosine in the thyroid gland. J. biol. Chem., 178, 997.

TROTTER, W.R. (1959). Diseases of the Thyroid in The Thyroid Hormones. Edited by R. Pitt-Rivers and J.R. Tata, London. Pergamon Press.

UZAN, R. (1955). Le myxoedème congénital: recherches étiologiques, cliniques et biologiques. Thèse Lyon. Bosc. Cited by Lelong et al. 1956.

VEALL, N. (1948). Geiger-Müller counter for measuring beta-ray activity of liquids and its application to medical tracer experiments. Brit. J. Radiol., 21, 347.

WATSON, W.C., YEUNG, R., McGIRR, E.M. (1959). Electrophoresis of the thyroid proteins. Scot. med.J., (In Press).

WERNER, S.C., BLOCK, R.J., MANDL, R.H., KASSENAAR, A.A.H. (1957). Pathogenesis of a case of congenital goiter with abnormally high SPI and with mono- and diiodotyrosine in the serum. J. clin. Endocr., 17, 817.

WHITELAW, M. J., THOMAS, S., REILLY, W.A. (1956).
A non-goitrous cretin with a high level serum PBI and thyroidal I^{131} uptake. Abstract. J. clin. Endocr., 16, 983.

WILKINS, L., CLAYTON, G.W., BERTHONG, M. (1954).
Development of goiters in cretins without iodine deficiency: hypothyroidism due to apparent inability of the thyroid gland to synthesize hormone. Pediatrics, 13, 235.

ZIEVE, L., VOGEL, W.C., SCHULTZ, A.L. (1956). Determination of protein-bound radioiodine with an anion exchange resin. J. Lab. clin. Med., 47, 663.

ZONDEK, H., LESZYNSKY, H.E., ZONDEK, G.W. (1955).
Triiodothyronine in myxedema and familial sporadic cretinism with goiter. Acta endocr. (Kbh.), 18, 117.

ZONDEK, H., LESZYNSKY, H.E. (1956). The genesis of thyroid adenomas. Lancet, 1, 77.

GOITRE DUE TO DYSHORMONOGENESIS

WITH PARTICULAR REFERENCE TO SPORADIC GOITROUS CRETINISM

AND

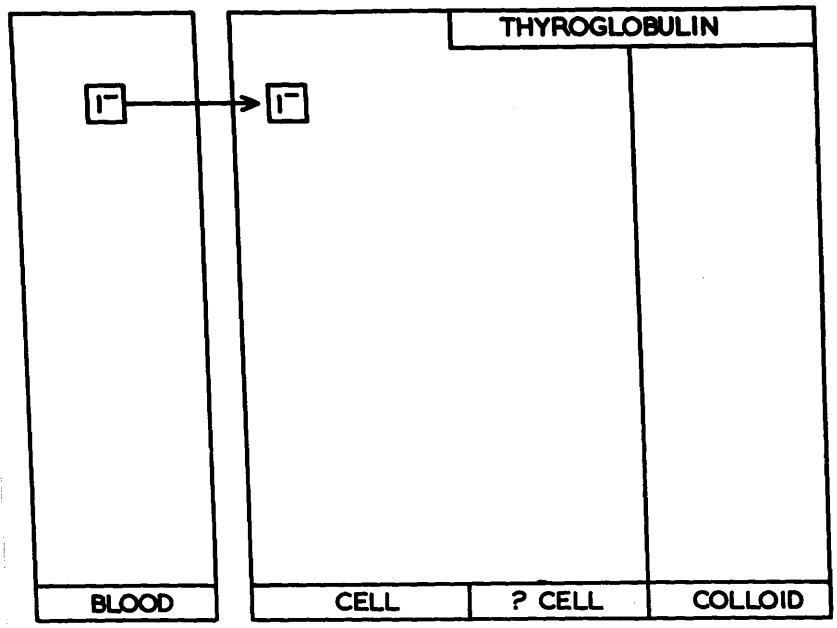
SPORADIC FAMILIAL GOITRE IN YOUNG PATIENTS

VOLUME II

CONTENTS

VOLUME II

FIGURES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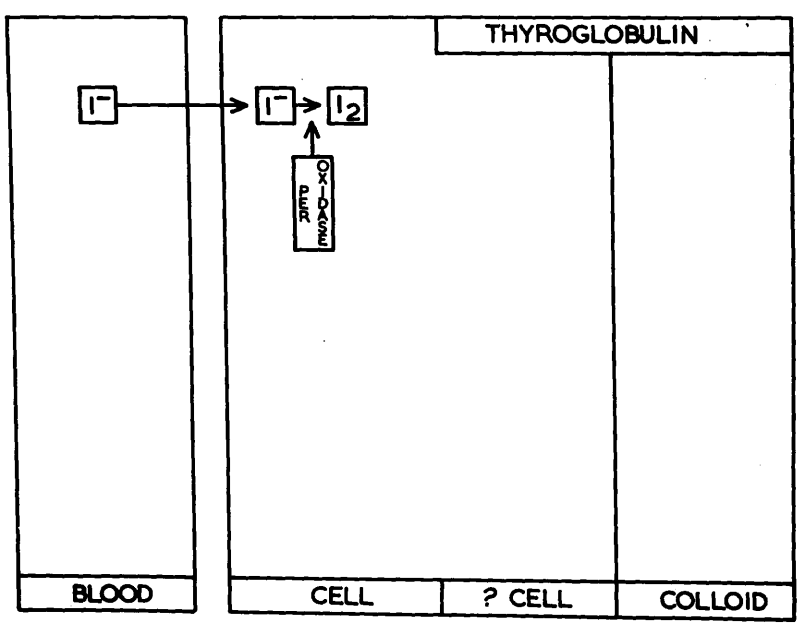
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Fig.1

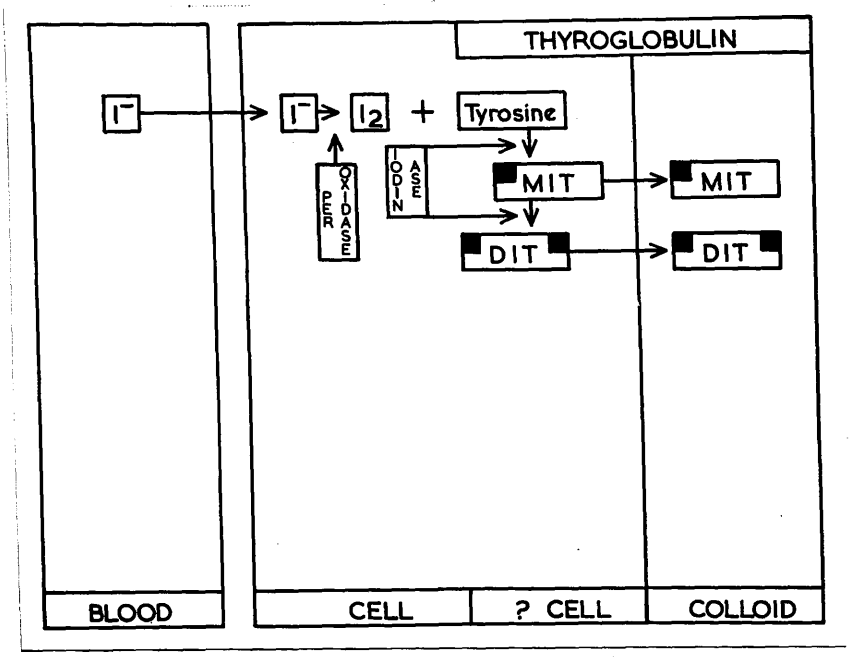
Biosynthesis of the thyroid hormone.

Stage I. Trapping of iodide by thyroid.

Fig.2

Biosynthesis of the thyroid hormone.

Stage II. Oxidation of iodide to iodine.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? CELL
COLLOID

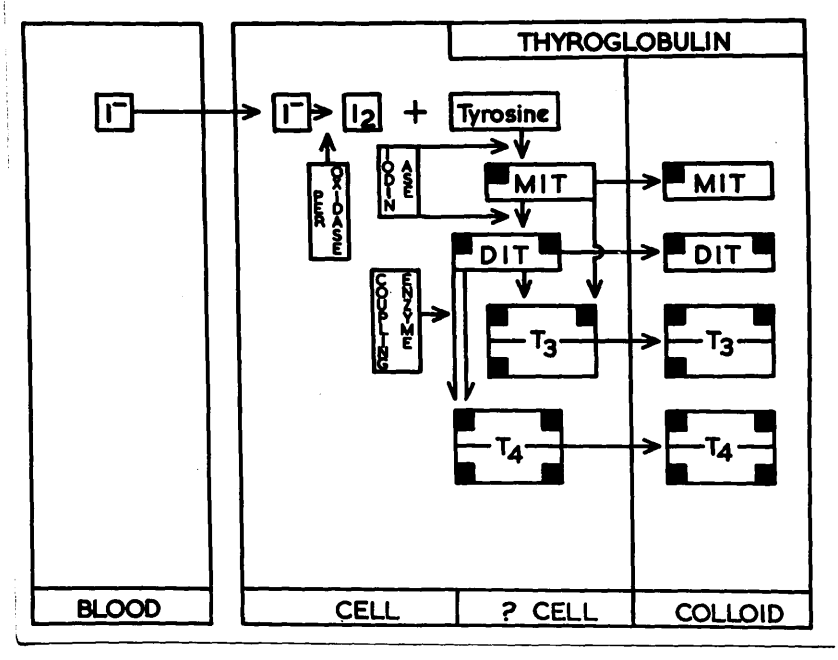

THYROGLOBULIN
I⁻
I⁻
I₂
+
Tyrosine
PEROXIDASE
IODINASE
MIT
MIT
DIT
DIT
COUPLING ENZYME
T3
T3
T4
T4
BLOOD
CELL
? CELL
COLLOID

Fig.3

Biosynthesis of the thyroid hormone.

Stage III. Iodination of tyrosine.

Fig.4

Biosynthesis of the thyroid hormone.

Stage IV. Coupling of iodotyrosines.

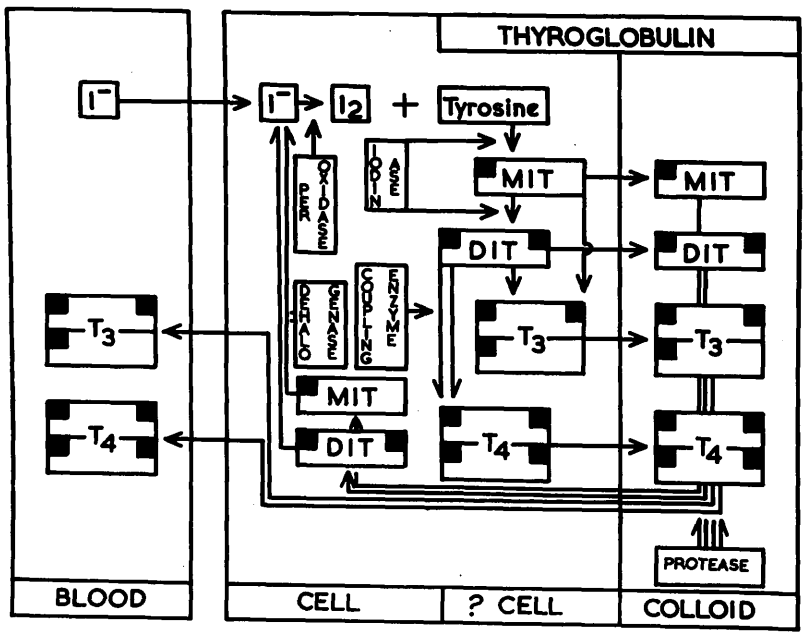
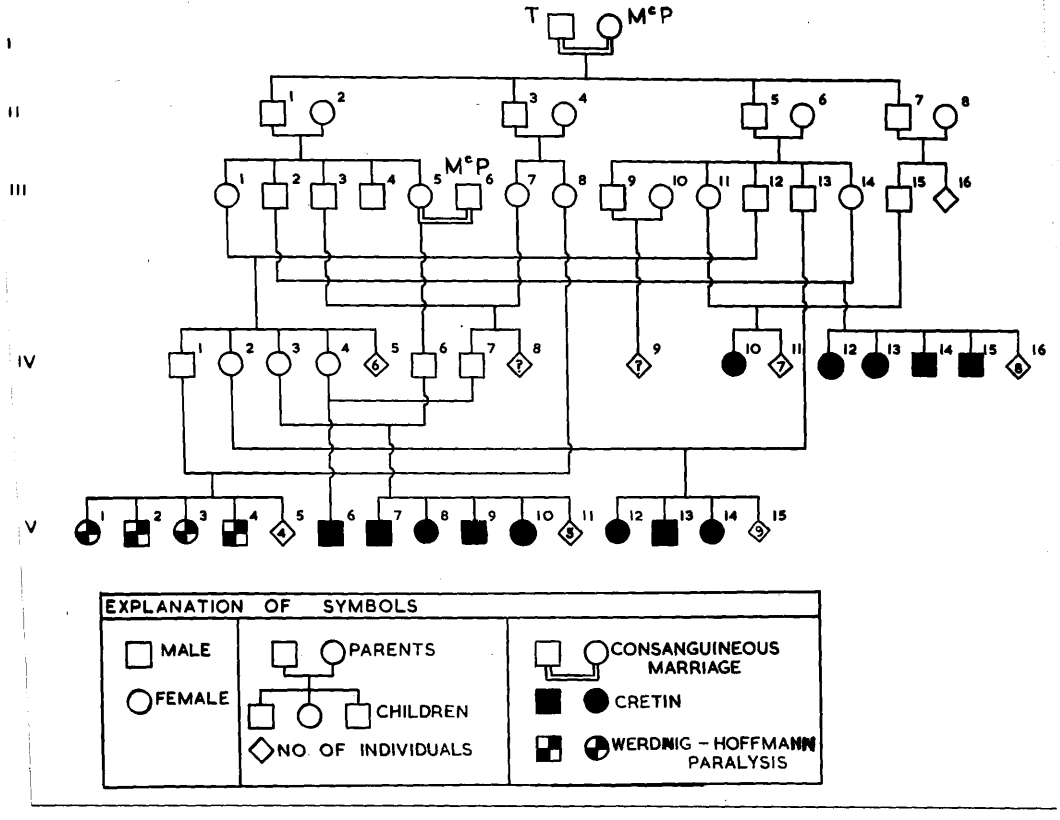

THYROGLOBULIN
I⁻
I⁻
I₂
+
Tyrosine
PEROXIDASE
IODINASE
MIT
DIT
T₃
T₄
DEHALOGENASE
COUPLING ENZYME
MIT
DIT
T₃
T₄
MIT
DIT
T₃
T₄
PROTEASE
BLOOD
CELL
? CELL
COLLOID

Fig.5

Biosynthesis and secretion of the thyroid hormone.

Fig.6

4 McP. siblings (cases 1-4) with goitrous cretinism in 1951.


T
McP
I
II
III
IV
V
McP
EXPLANATION OF SYMBOLS
MALE
FEMALE
PARENTS
CHILDREN
NO. OF INDIVIDUALS
CONSANGUINEOUS MARRIAGE
CRETIN
WERDNIG – HOFFMANN PARALYSIS

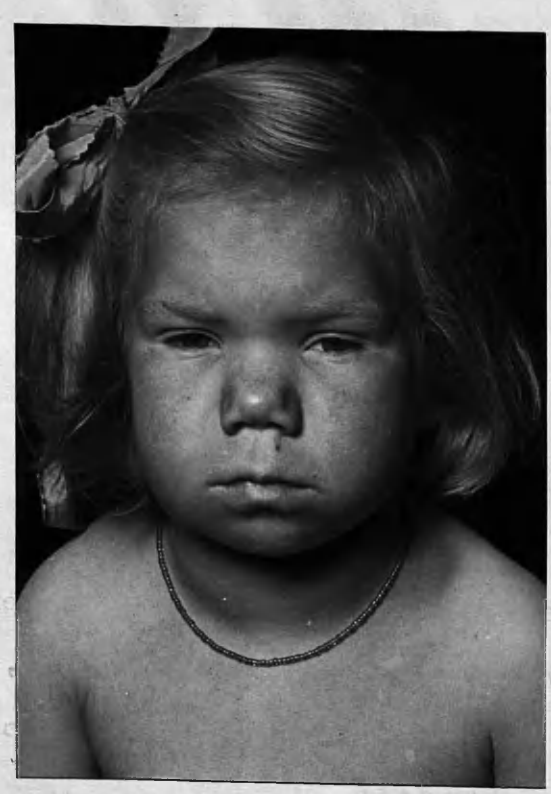


Fig.8

M.McP. (case 1), aged 8 years 5 months,
in 1951, showing dull myxoedematous facies.

Fig.9

M.McP. (case 1), aged 8 years 5 months,
in 1951, with child of same age,
illustrating stunting of growth.

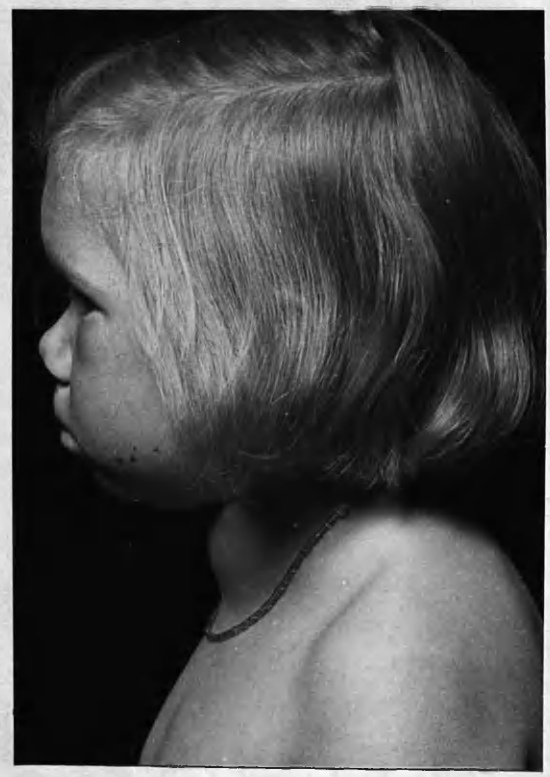


Fig.10

M.T. (case 8), aged 12½ years, in whom evidence of hypothyroidism was least obvious.

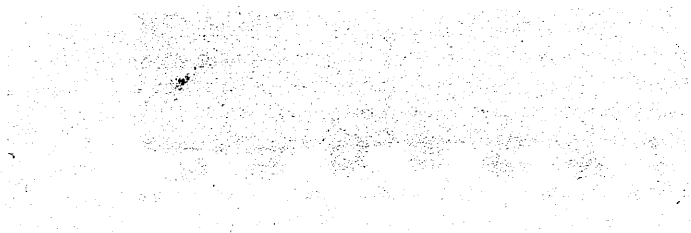


Fig.11

M.McP. (case 1), demonstrating goitre.

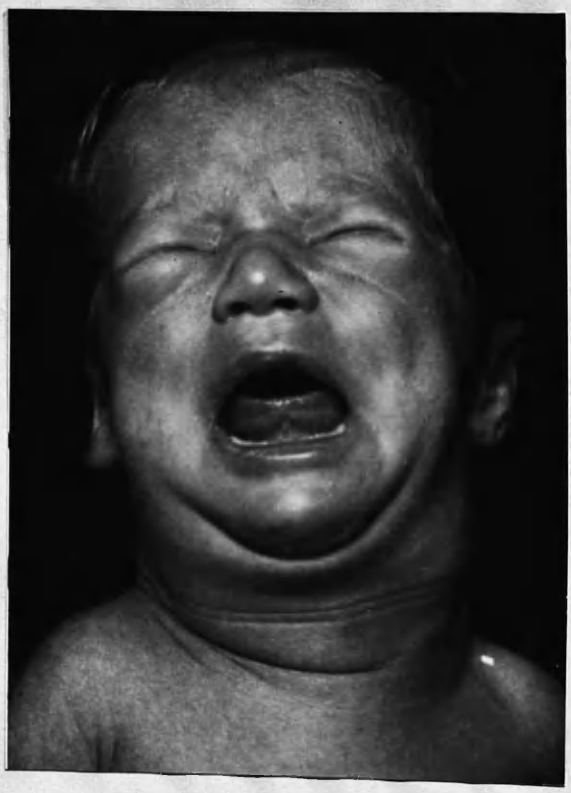


Fig.12

A.T. (case 9), aged 4 weeks, when presence of goitre was confirmed, but there was no evidence of hypothyroidism.

Fig.13

A.T. (case 9), aged 2 years 5 months, with child of same age. Goitre persisted and he was now hypothyroid.

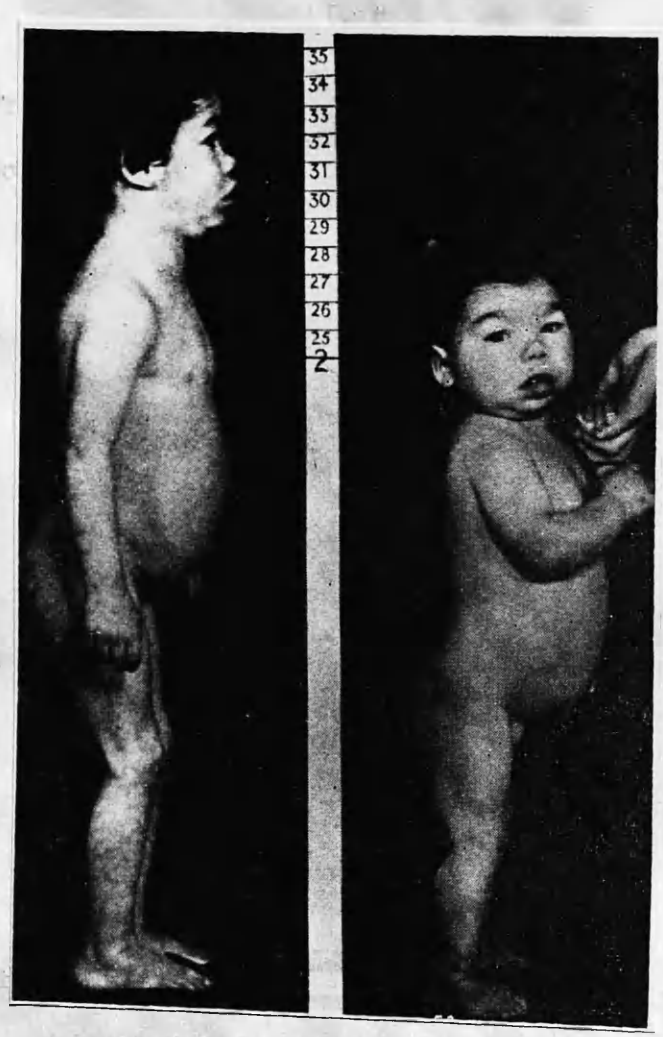

35
34
33
32
31
30
29
28
27
26
25
2

Fig.14

J.N. and M.A.W. (cases 13 and 14), aged $6\frac{1}{2}$ and $3\frac{1}{2}$ years respectively, showing typically cretinous appearance.

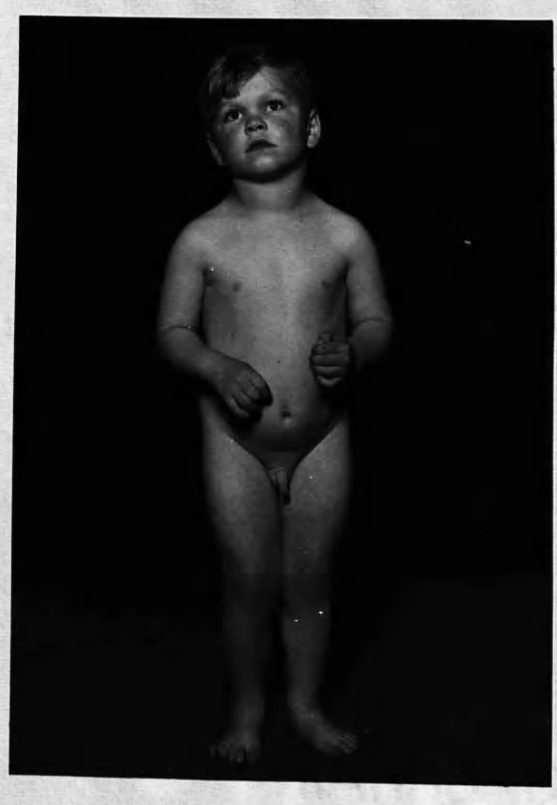


Fig.15

A.D. (case 15), aged 6 years 9 months. He had had thyroid therapy for 2 years until 2 months ago. He was hypothyroid and had a large goitre.

Fig.16

4 McP. siblings (cases 1-4) in 1955, after intermittent thyroid therapy since 1951. Comparison with fig.6 shows obvious improvement (see below).

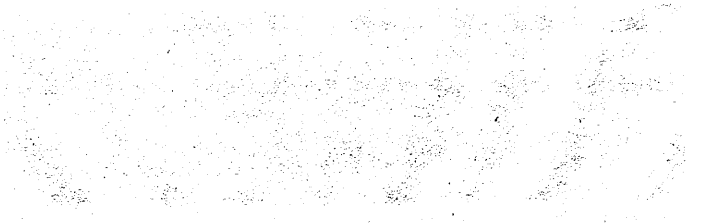
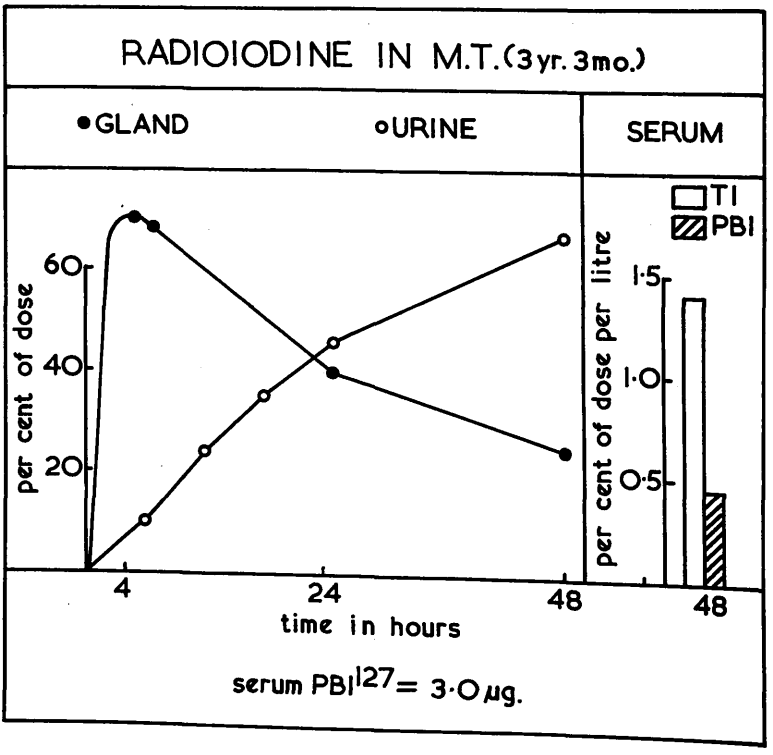


Fig.6

4 McP. siblings (cases 1-4) in 1951.


RADIOIODINE IN M.T.(3yr. 3mo.)
●GLAND
○URINE
SERUM
TI
PBI
per cent of dose
60
40
20
per cent of dose per litre
1·5
1·0
0·5
4
24
48
48
time in hours
serum PBI^{127}= 3·0 µg.

Figs. 17 a b and c

Figures illustrate characteristic findings in routine ^{131}I tests in the 3 groups of tinkers. They show the unusually rapid accumulation of ^{131}I by the thyroid as well as the rapid loss of gland ^{131}I; the slow initial but unusually prolonged urinary excretion of ^{131}I, the elevated serum $PB^{131}I$.

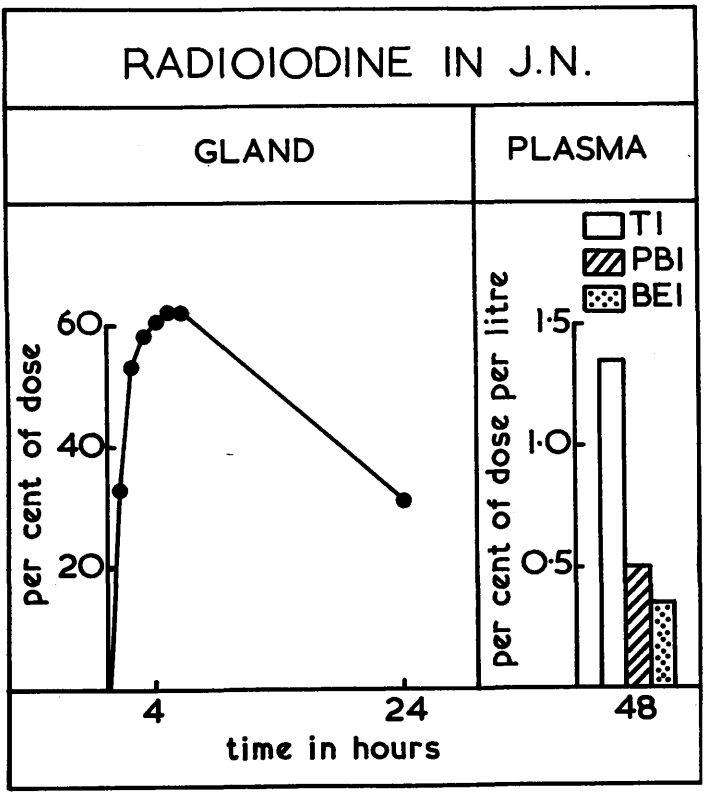
TI = total plasma or serum ^{131}I

$PB^{131}I$ = plasma or serum protein-bound ^{131}I

$BE^{131}I$ = thyroxine-like fraction of total butanol-extractable ^{131}I.

Fig.17a

M.T. (case 12, Group I)


RADIOIODINE IN J.N.
GLAND
PLASMA
TI
PBI
BEI
per cent of dose
60
40
20
per cent of dose per litre
1·5
1·0
0·5
4
24
48
time in hours

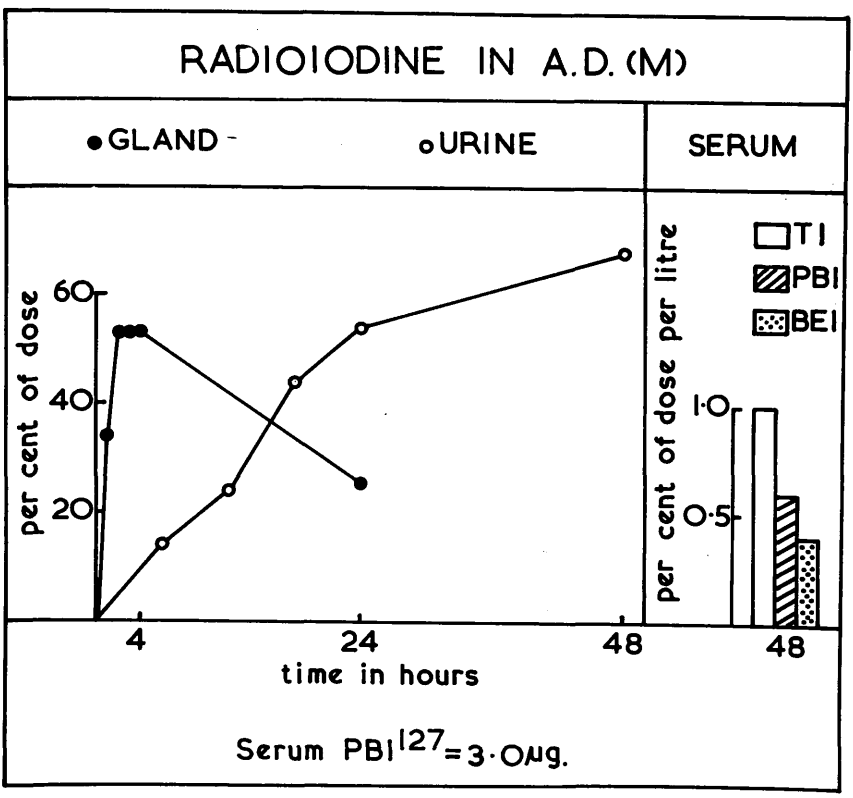
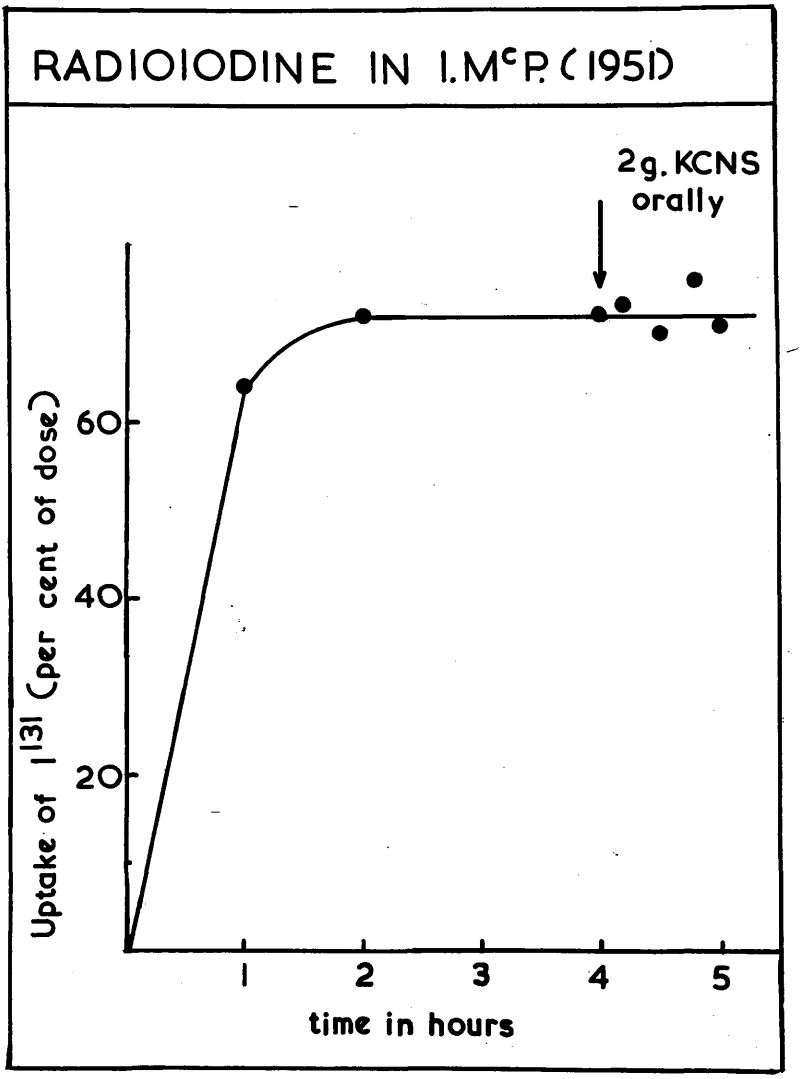

RADIOIODINE IN A.D. (M)
● GLAND
o URINE
SERUM
TI
PBI
BEI
per cent of dose
60
40
20
per cent of dose per litre
1·0
0·5
4
24
48
48
time in hours
Serum PBI^{127} = 3·0µg.

Fig.17b

J.N. (case 13, Group II).

Fig.17c

A.D. (case 15, Group III).


RADIOIODINE IN I.McP. (1951)
2g. KCNS orally
Uptake of I^{131} (per cent of dose)
60
40
20
1
2
3
4
5
time in hours

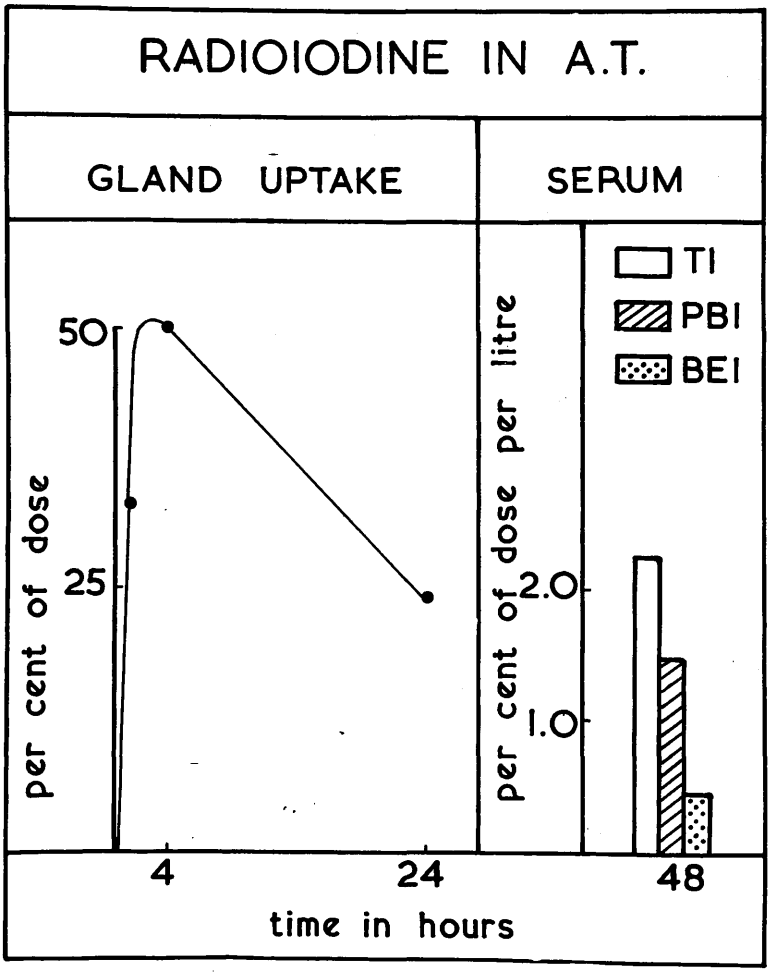

RADIOIODINE IN A.T.
GLAND UPTAKE
SERUM
50
25
per cent of dose
per cent of dose per litre
2.0
1.0
TI
PBI
BEI
4
24
48
time in hours

Fig.19

A.T. (case 9). Figure illustrates typical rapid accumulation of ^{131}I by the thyroid gland, as well as rapid loss of radioactivity. Serum $PB^{131}I$ is raised.

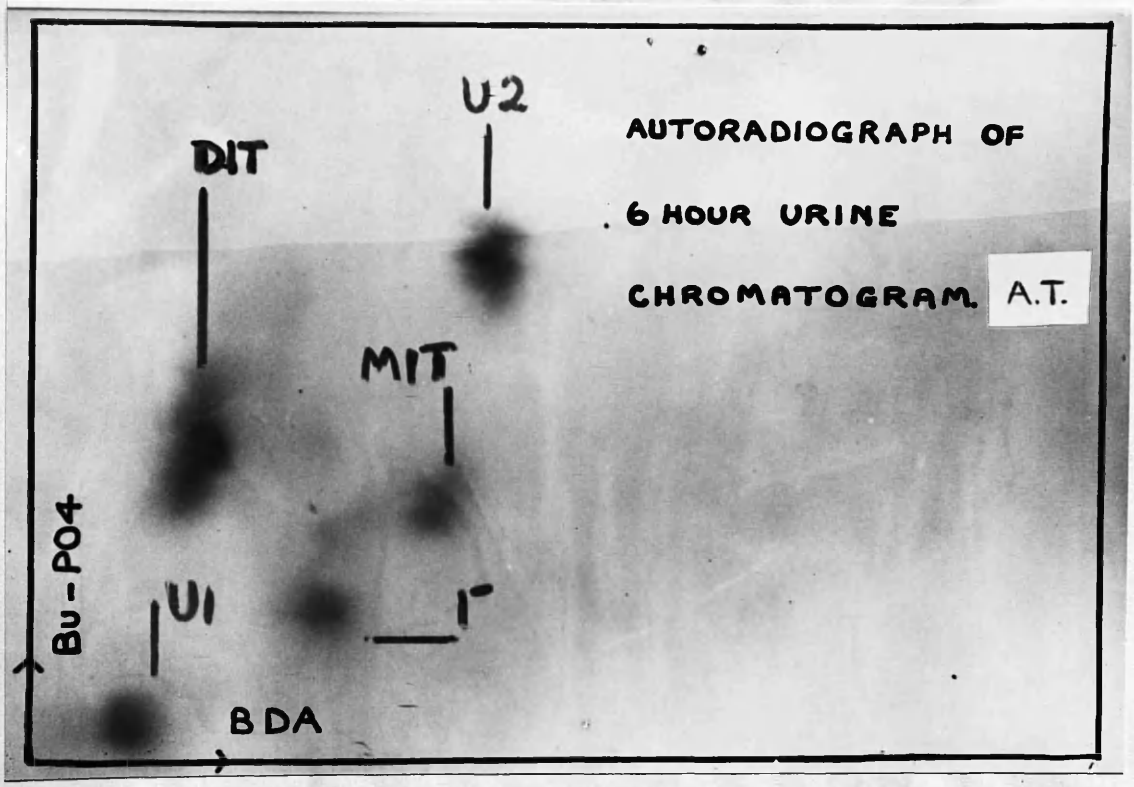

U2
DIT
AUTORADIOGRAPH OF
6 HOUR URINE
CHROMATOGRAM. A.T.
MIT
Bu-PO4
U1
I⁻
BDA

Fig.20

A.T. (case 9). Autoradiograph of two-way ascending chromatogram prepared from butanol extracts of urine excreted 0-6 hours after 100 μc ^{131}I. First solvent, butanol phosphate buffer; second solvent, butanol dioxane ammonia. Iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified, and there are 2 unknown spots of radioactivity (U1 and U2).

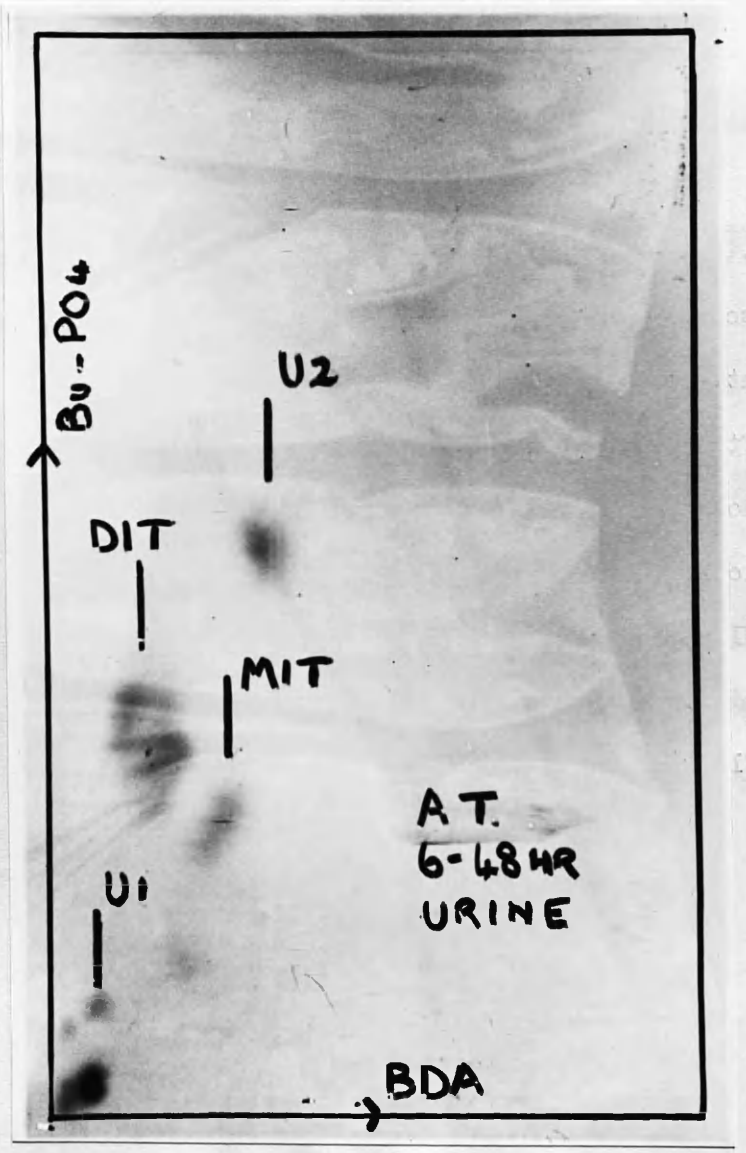

Bu-PO4
U2
DIT
MIT
A.T.
6-48 HR
URINE
U1
BDA

Fig.21

A.T. (case 9). Similar study to that in fig.20 but with urine of period 6-48 hours. Findings also are similar. Black spot at origin probably due to overloading.

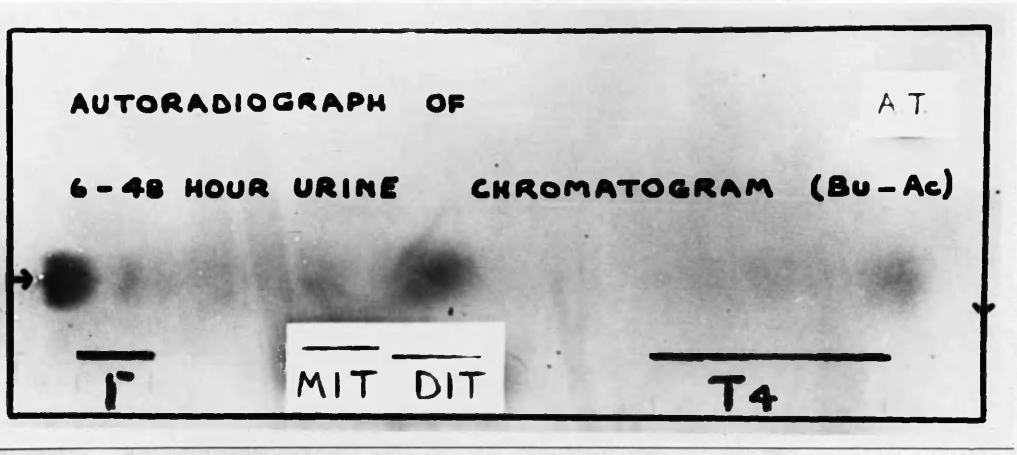

AUTORADIOGRAPH OF
AT
6-48 HOUR URINE CHROMATOGRAM (Bu-Ac)
I⁻
MIT DIT
T4

Fig.22

A.T. (case 9). Autoradiograph of ascending chromatogram prepared from butanol extract of urine excreted 6-48 hours after 100 μc ^{131}I. Solvent, butanol acetic acid. Diiodotyrosine (DIT) is readily identified. Iodide (I^-) and monoiodotyrosine (MIT) are also present. There is an unidentified black spot near the origin and another zone overlying and overriding the thyroxine (T4) zone.

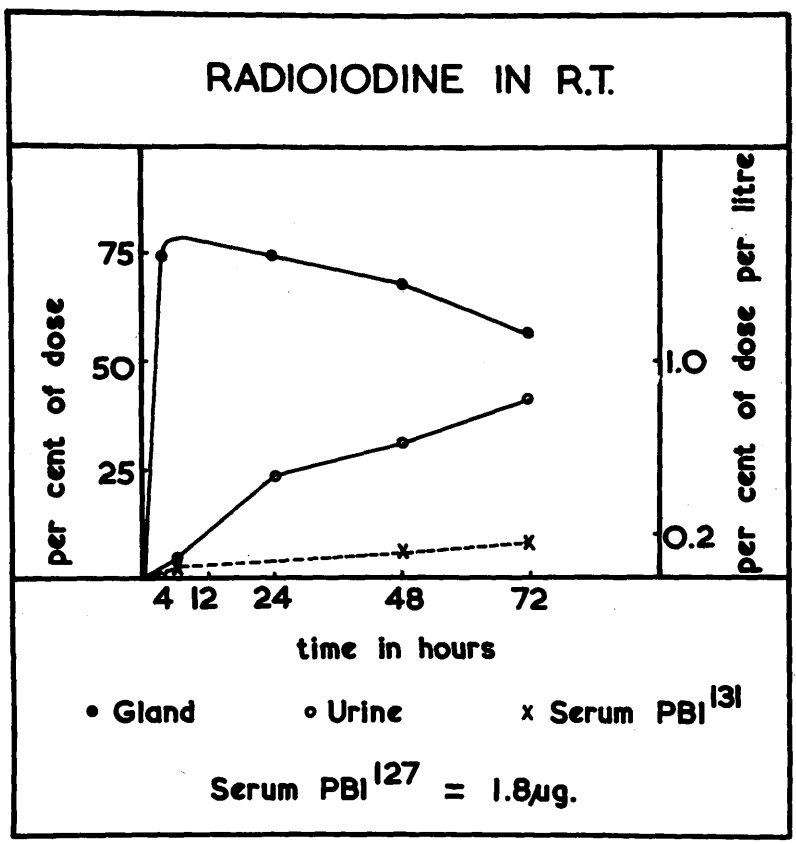
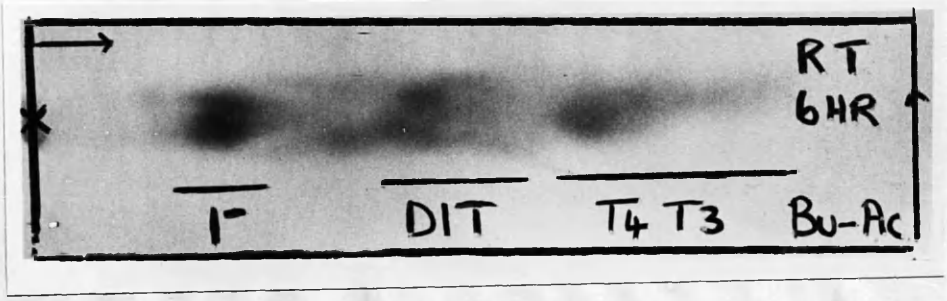

RADIOIODINE IN R.T.
per cent of dose
75
50
25
per cent of dose per litre
1.0
0.2
4
12
24
48
72
time in hours
• Gland
o Urine
x Serum PBI131
Serum PBI127 = 1.8µg.

Fig.23

R.T. (case 10). Accumulation and retention of ^{131}I by the thyroid gland, urinary excretion of radioactivity and serum $PB^{131}I$.


RT
6HR
I⁻
DIT
T4 T3
Bu-Ac

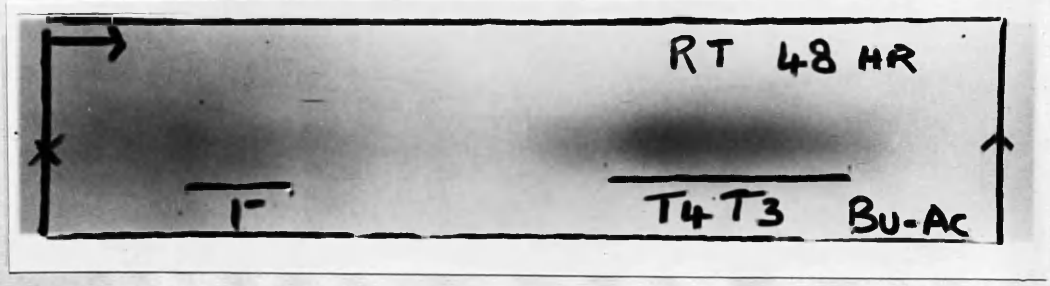
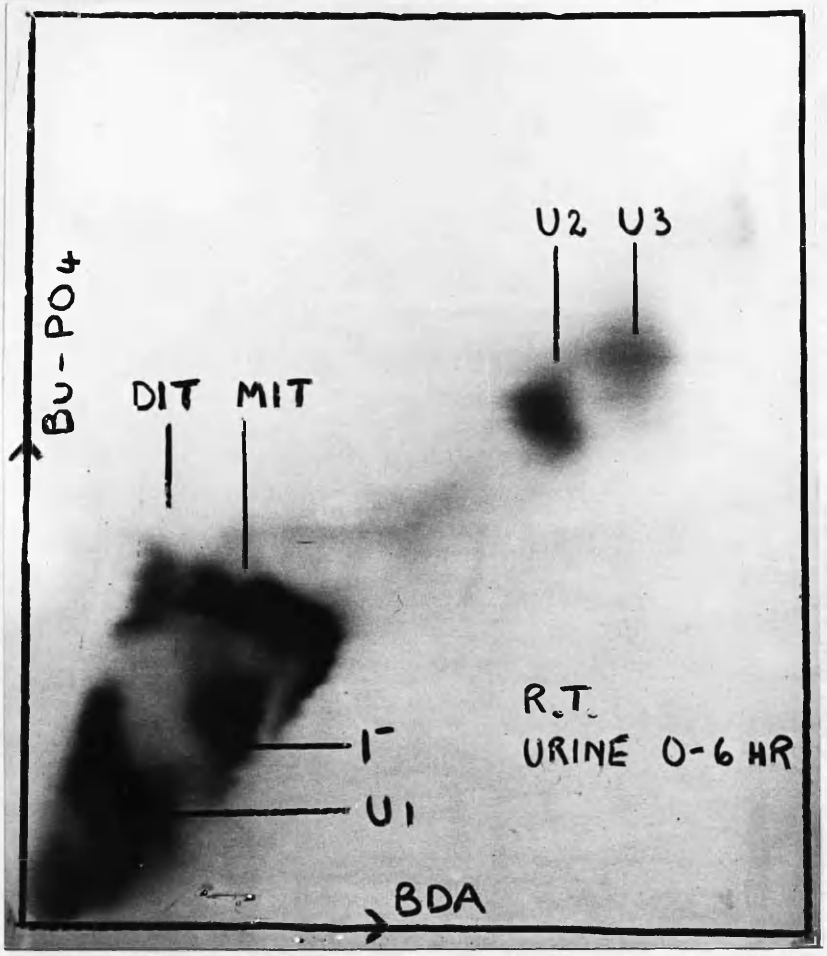

RT 48 HR
I⁻
T4 T3
Bu-Ac

Fig.25

R.T. (case 10). Autoradiograph of ascending chromatogram prepared from butanol extract of serum withdrawn 6 hours after 1 mc ^{131}I. Solvent butanol acetic acid. Iodide (I^-), diiodotyrosine (DIT) and thyroxine and/or triiodothyronine (T4 T3) are identified. The blackening between I^- and DIT is presumably due to monoiodotyrosine.

Fig.26

R.T. (case 10). Similar study to that in fig.25 but with 48 hour serum. By 48 hours the T4 T3 zone shows the greatest radioactivity.


U2 U3
BU-PO4
DIT MIT
R.T.
URINE 0-6 HR
I-
U1
BDA

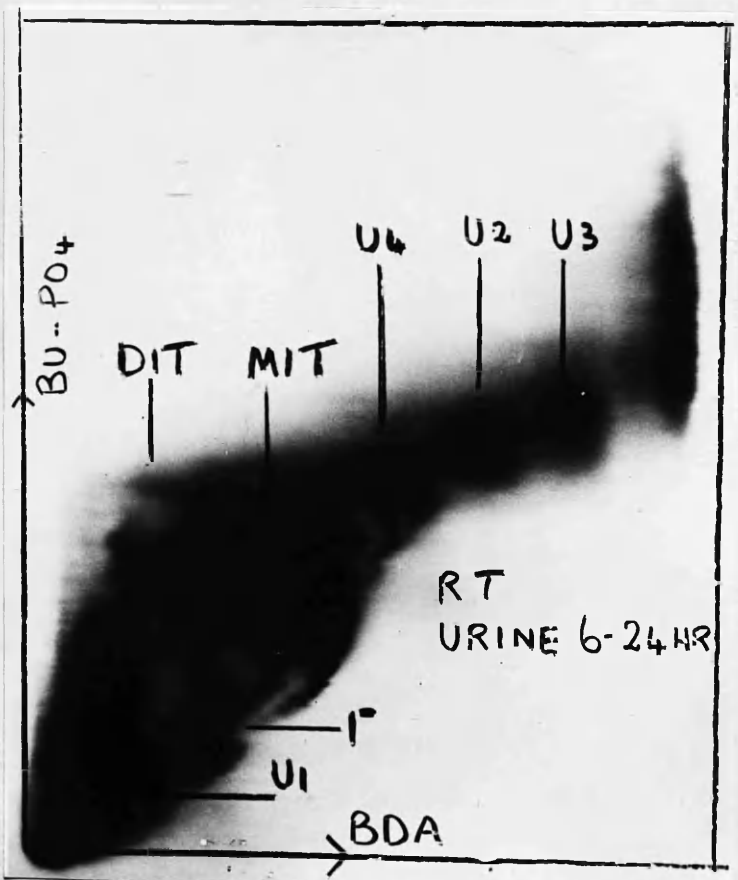
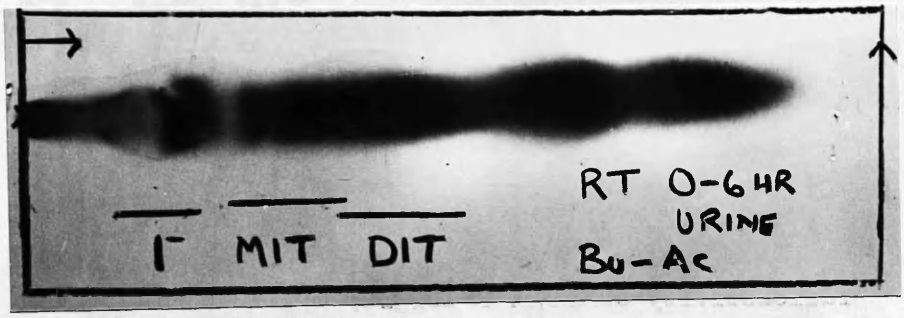

U4 U2 U3
BU-PO4
DIT MIT
RT
URINE 6-24 HR
I-
U1
BDA

Fig.27

R.T. (case 10). Autoradiograph of two-way ascending chromatogram prepared from butanol extract of urine excreted 0-6 hours after 1 mc ^{131}I. First solvent, butanol phosphate buffer; second solvent, butanol dioxane ammonia. Iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified. There are several spots of radioactivity of unknown origin, marked U1 - 3, as well as a spot at the origin which may be due to overloading.

Fig.28

R.T. (case 10). Similar study to that in fig.27 but with urine from 6-24 hour period. A fourth unknown spot is present (U4); it was only vaguely seen in fig.27. There is also a large zone of blackening at the solvent front.


I
MIT
DIT
RT 0-6 HR
URINE
Bu-Ac

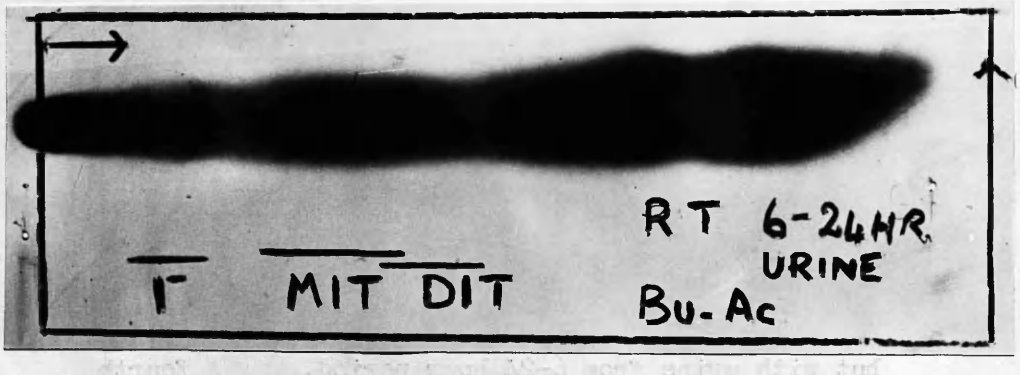
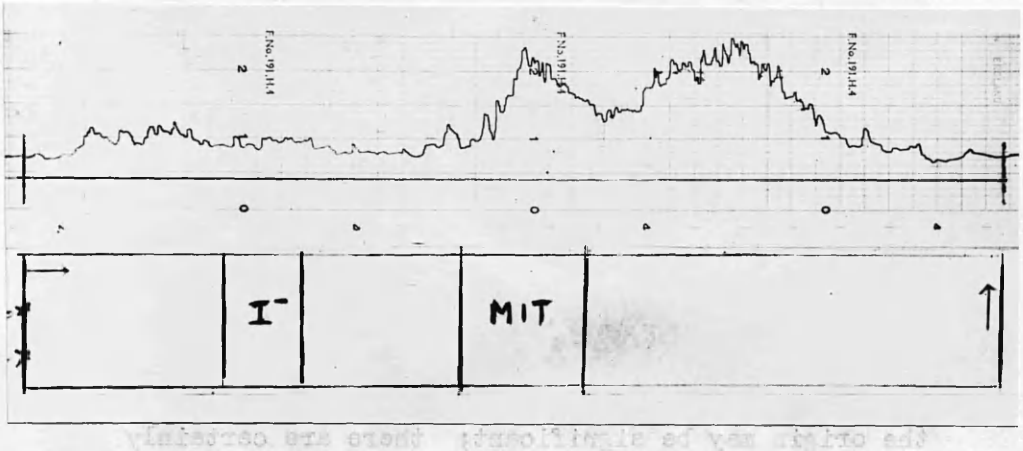

I
MIT
DIT
RT 6-24 HR
URINE
Bu-Ac

Fig.29

R.T. (case 10). Autoradiograph of ascending chromatogram prepared from butanol extract of urine excreted 0-6 hours after 1 mc ^{131}I. Solvent, butanol acetic acid. Iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified. The blackening just beyond the origin may be significant; there are certainly 2 well-marked zones of radioactivity in and beyond the usual thyroxine zone.

Fig.30

R.T. (case 10). Similar study to that in fig.29 but with urine from period 6-24 hours. The intense blackening due to over exposure makes it difficult to define the limits of the radioactive zones; they appear similar to those in fig.29.


I⁻
MIT

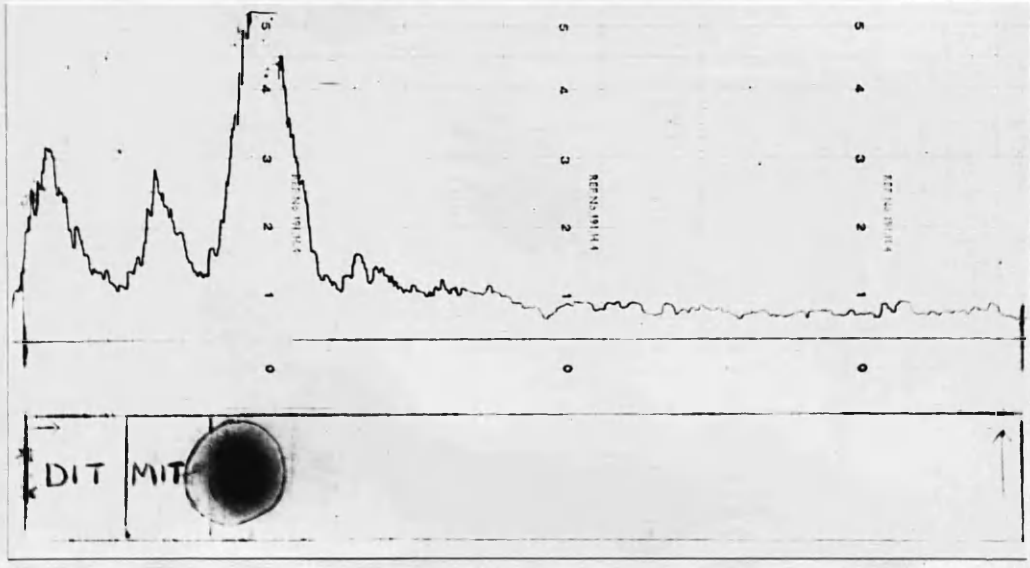

DIT
MIT

Fig.31

A.D. (case 16), 1958. Scan for radioactivity of ascending chromatogram prepared from butanol extract of urine passed 0-2 hours after an oral dose of $M^{131}IT$. Solvent, butanol acetic acid. Virtually all of the radioactivity is in the monoiodotyrosine (MIT) zone, or in another zone corresponding approximately to the thyroxine zone. This latter zone is probably due to monoiodotyrosine conjugates.

Fig.32

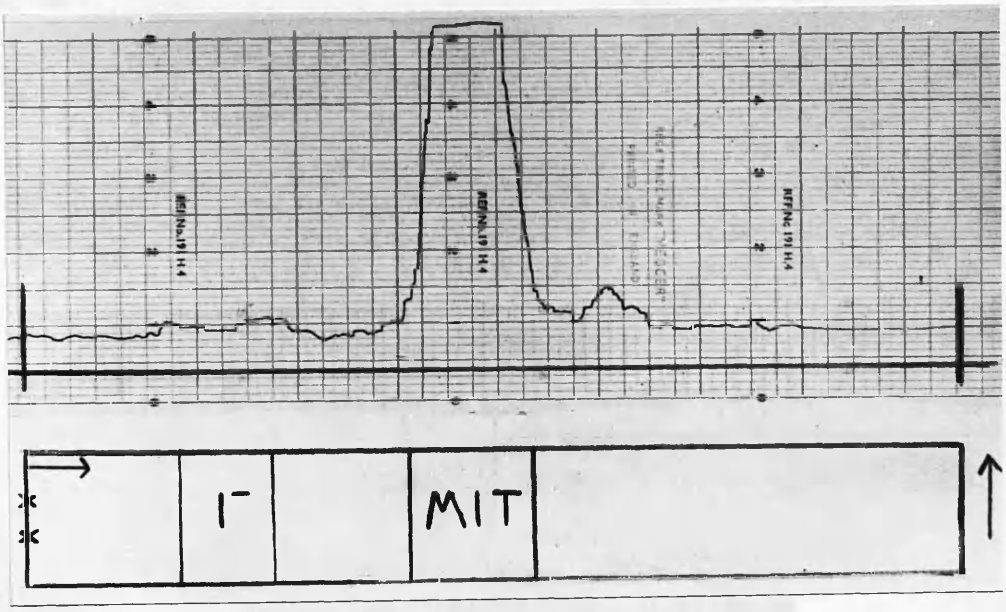
A.D. (case 16), 1958. Similar study to that in fig.31 but solvent, butanol ammonia. The large peak of radioactivity which is just beyond the iodide spot is probably due to a monoiodotyrosine conjugate.

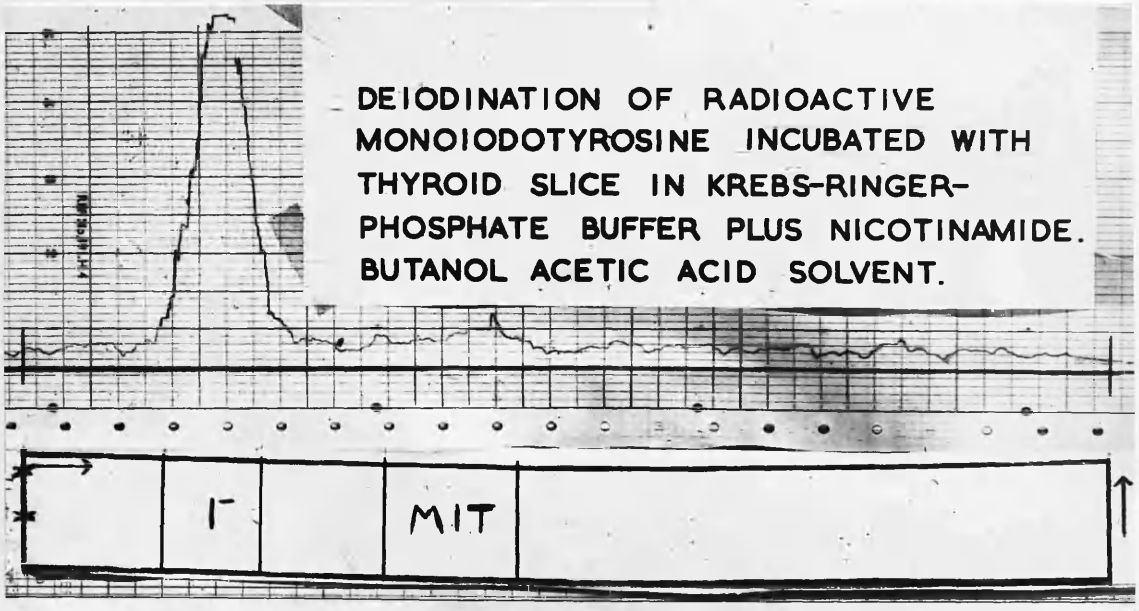
Fig.33

I.McP. (case 2), aged 11 years 7 months, in 1951 with child of 12 years. Photograph illustrates marked stunting of growth and thick neck with enlarged thyroid gland.

Fig.34

I.McP. (case 2), in 1955 after intermittent thyroid therapy since 1951. Photograph demonstrates satisfactory growth and development and alert facies but persistence of large goitre.





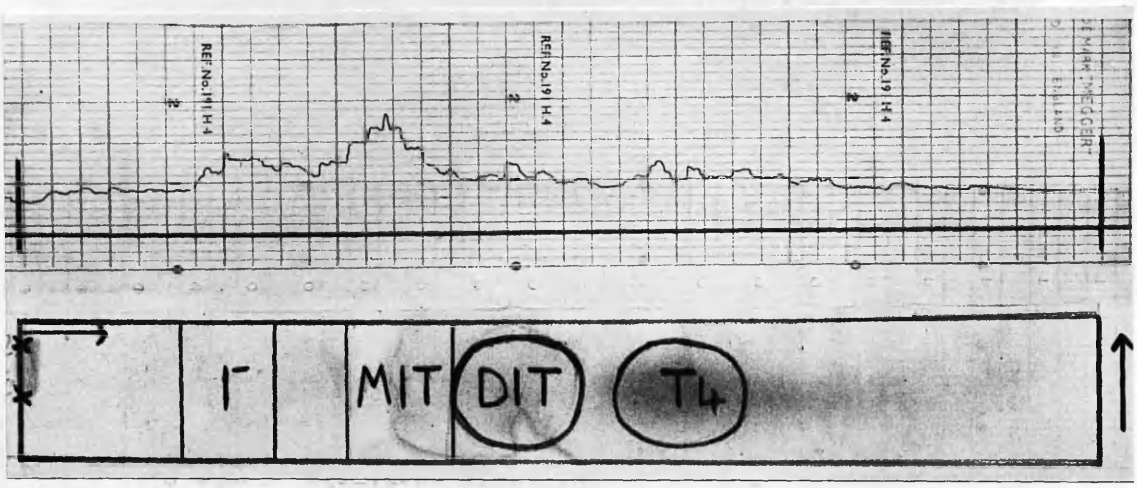
DEIODINATION OF RADIOACTIVE MONOIODOTYROSINE INCUBATED WITH THYROID SLICE IN KREBS-RINGER-PHOSPHATE BUFFER PLUS NICOTINAMIDE. BUTANOL ACETIC ACID SOLVENT.

Fig.35

I. McP. (case 2), aged 18 years. Scan for radioactivity of ascending chromatogram prepared from butanol extract of Krebs-Ringer phosphate buffer (pH 7.4), in which slices of fresh thyroid tissue have been incubated at 37°C. for 18 hours with $M^{131}IT$ and a trace of nicotinamide. Solvent, butanol acetic acid. Radioactivity has persisted in the monoiodotyrosine (MIT) zone, indicating impaired dehalogenase activity. Compare with fig.36.

Fig.36

Similar study to that in fig.35, but with thyroid tissue from adult case of non-toxic nodular goitre. Over 90 per cent of the radioactivity is present in the iodide (I^-) spot, indicating normal dehalogenase activity.


REF.No.191 H4
REF.No.191 H4
REF.No.191 H4
I⁻
MIT
DIT
T4

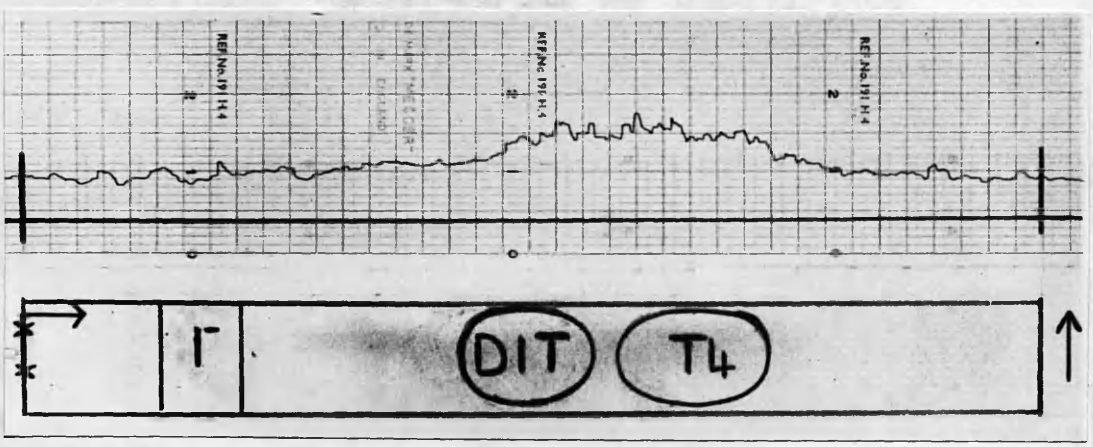

REF.No.191 H4
REF.No.191 H4
REF.No.191 H4
I⁻
DIT
T4

Fig.37

I. McP. (case 2). Scan for radioactivity of ascending chromatogram prepared from butanol extract of serum withdrawn 3 hours after 1 mc ^{131}I. Solvent, butanol acetic acid. Iodide (I^-) and monoiodotyrosine (MIT) are identified and there are probably traces of diiodotyrosine (DIT) and thyroxine (T4).

Fig.38

I. McP. (case 2). Similar study to that in fig.37, but with 48 hour serum. Record is technically poor, but radioactivity corresponds to diiodotyrosine (DIT) and thyroxine (T4) zones.

REF.No.191 H 4
REF.No.191 H 4
REF.No.191 H 4
MIT
DIT

REF.No.191 H 4
REF.No.191 H 4
REF.No.191 H 4
MIT
DIT

REF.No.191 H 4
REF.No.191 H 4
REF.No.191 H 4
MIT
DIT

Fig.39

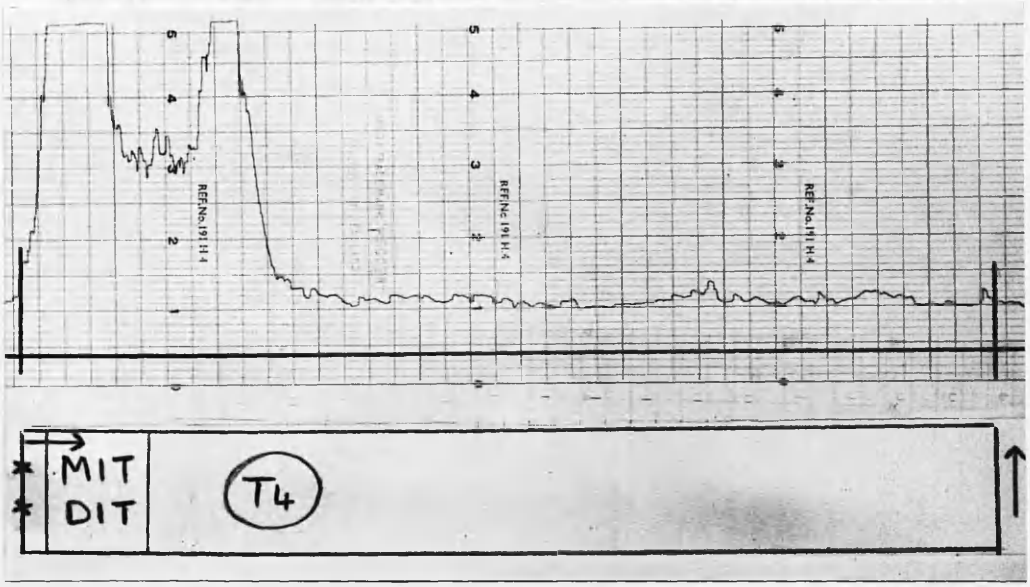
I. McP. (case 2). Scan for radioactivity of ascending chromatogram prepared from butanol extract of urine passed 0-6 hours after 1 mc ^{131}I. Solvent, butanol acetic acid. Iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified. There is radioactivity in and beyond the usual location of thyroxine, presumably due to iodotyrosine conjugates.

Fig.40

I. McP. (case 2). Similar study to that in fig.39 but with urine of 6-24 hour period.

Fig.41

I.McP. (case 2). Similar study to that in figs. 39 and 40 but with urine of 24-48 hour period. Comparison of the 3 records shows that the radioactivity of the so-called conjugates becomes increasingly prominent.


MIT
DIT
T4

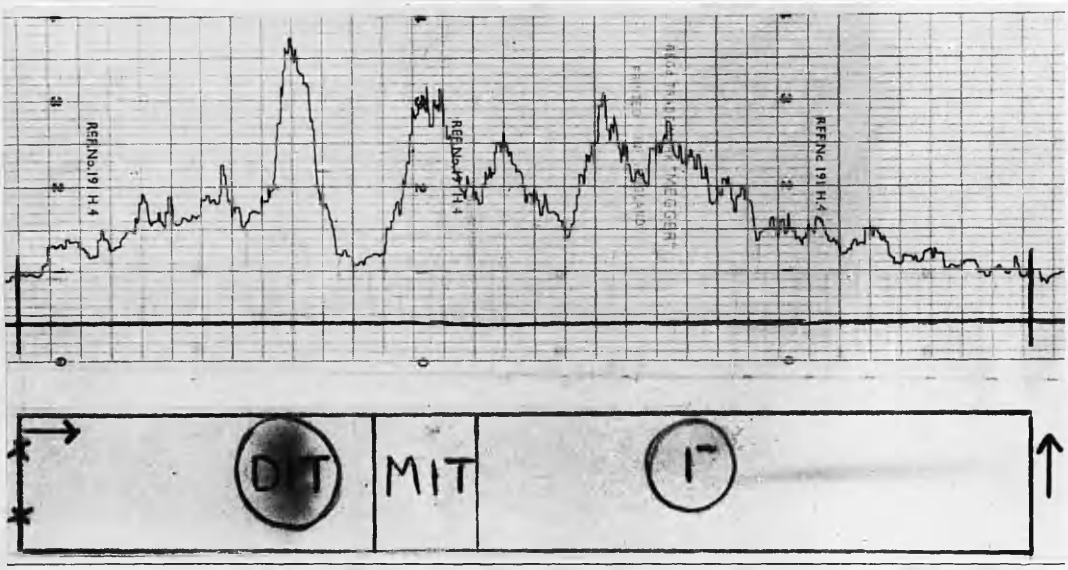
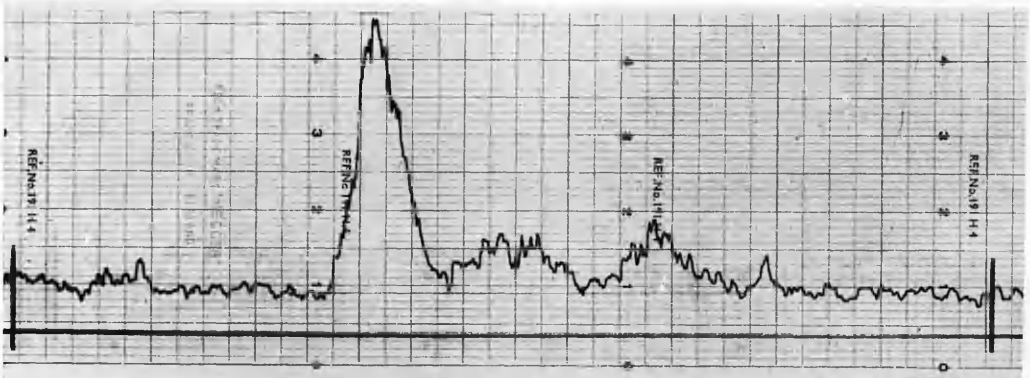

DIT
MIT
1-

Fig.42

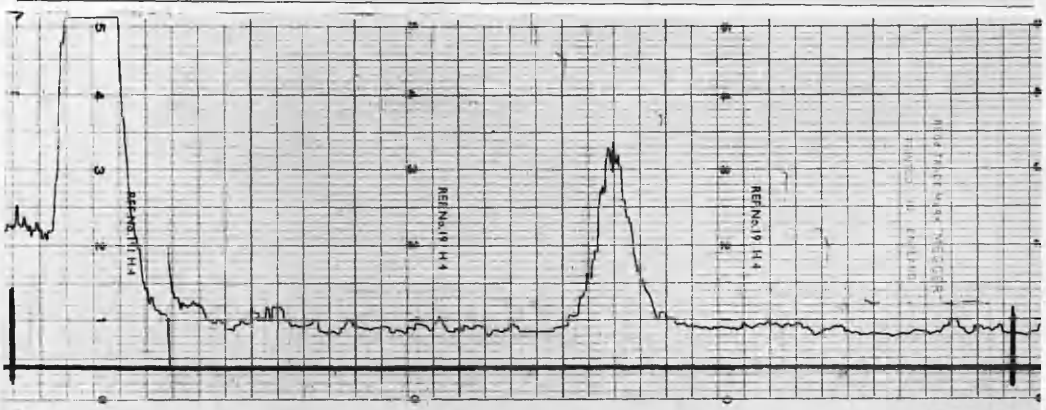
I. McP. (case 2). Scan for radioactivity of ascending chromatogram prepared from butanol extract of urine passed 6-24 hours after 1 mc ^{131}I. Solvent, butanol ammonia. Identification of origin of peaks of radioactivity is difficult, but the interesting feature is a peak of radioactivity just behind the thyroxine (T4) spot, probably due to an iodotyrosine conjugate. Compare fig.32.

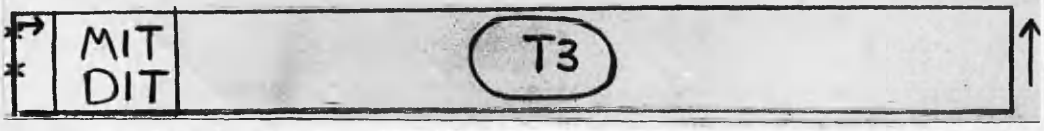
Fig.43

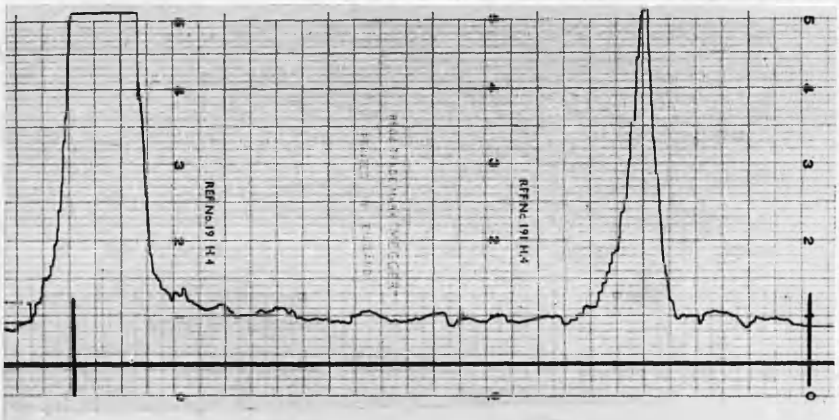
I.McP. (case 2). Similar study as in fig.42, but solvent collidine ammonia. As well as peaks of radioactivity corresponding to iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT), there are at least 4 other peaks, probably due to derivatives of the iodotyrosines.


REF No.19 H4
REF No.19 H4
REF No.19 H4


MIT
DIT
T4


REF No.19 H4
REF No.19 H4
REF No.19 H4


MIT
DIT
T3


REF No.19 H4
REF No.19 H4


MIT
DIT
T4
T2

Fig.44

I. McP. (case 2). Scan for radioactivity of ascending chromatogram prepared from trypsin digest of thyroid gland removed 48 hours after 1 mc ^{131}I. Solvent, butanol acetic acid. Monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified. There is a peak of radioactivity, the origin of which is unknown, between the DIT and T4 zones.

Fig.45

I. McP. (case 2). Similar study to that in fig.44, but solvent butanol ammonia. There is a large peak of radioactivity corresponding to the iodotyrosine markers, and there is an unidentified peak just in advance of triiodothyronine (T3).

Fig.46

I.McP. (case 2). Similar study to that in figs. 44 and 45, but solvent amyl alcohol and ammonia. The unidentified peak of radioactivity lies in advance of the 3-5 diiodothyronine (T2) spot.

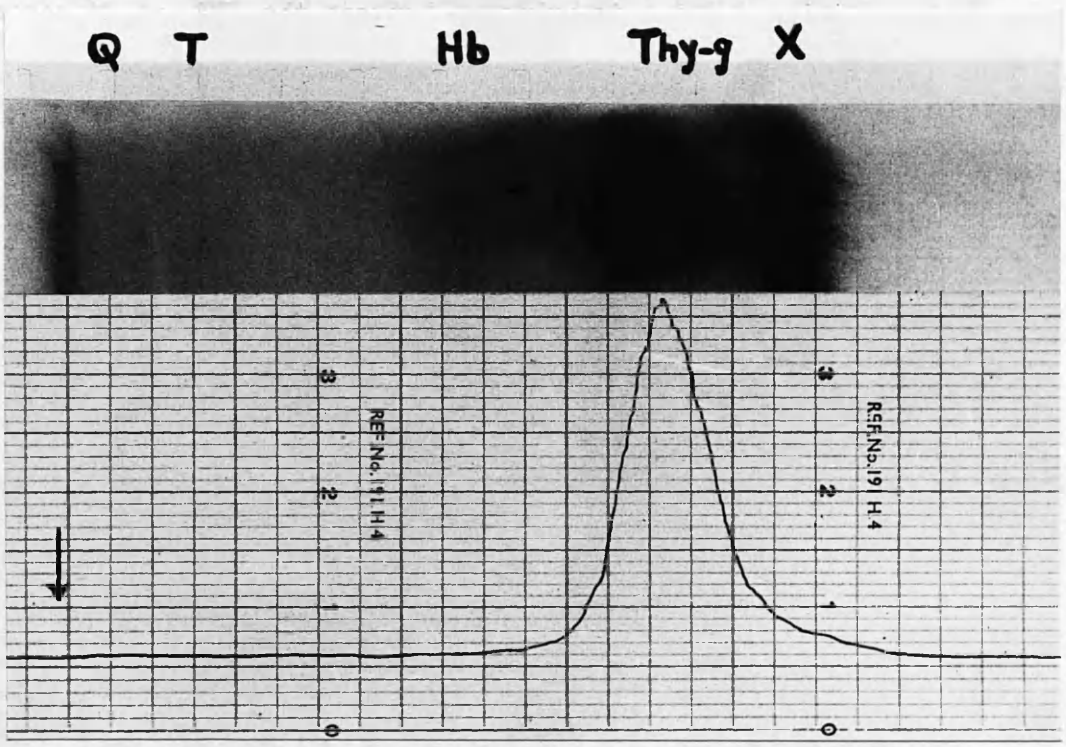
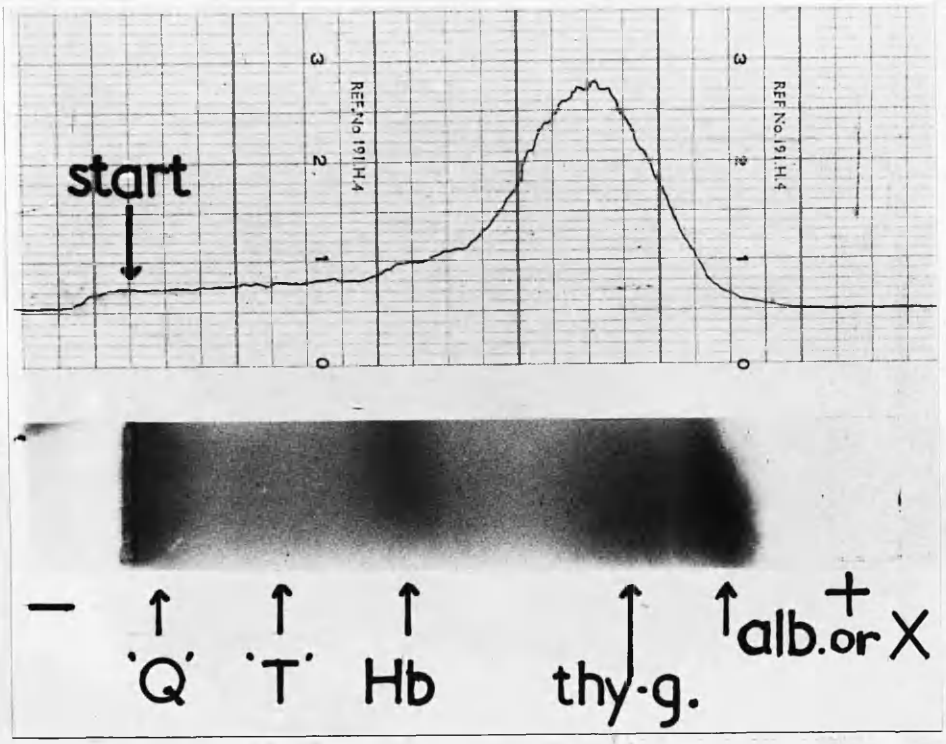

Q
T
Hb
Thy-g
X
REF No. 11 H 4
REF No. 19 H 4
3
2
1
0
3
2
1
0

Fig.47

I. McP. (case 2). Scan for radioactivity of electrophoretogram of thyroid proteins prepared from thyroid tissue removed 48 hours after 1 mc ^{131}I. Proteins stained with aqueous bromphenol blue. Haemoglobin (Hb), thyroglobulin (Thy-g) and albumin (X) bands are easily identified. Faint bands Q and T are present just beyond the start. Radioactivity is associated with the thyroglobulin zone.


start
REF.No 191.H.4
REF.No 191.H.4
—
'Q'
'T'
Hb
thy-g.
alb.
+
or
X

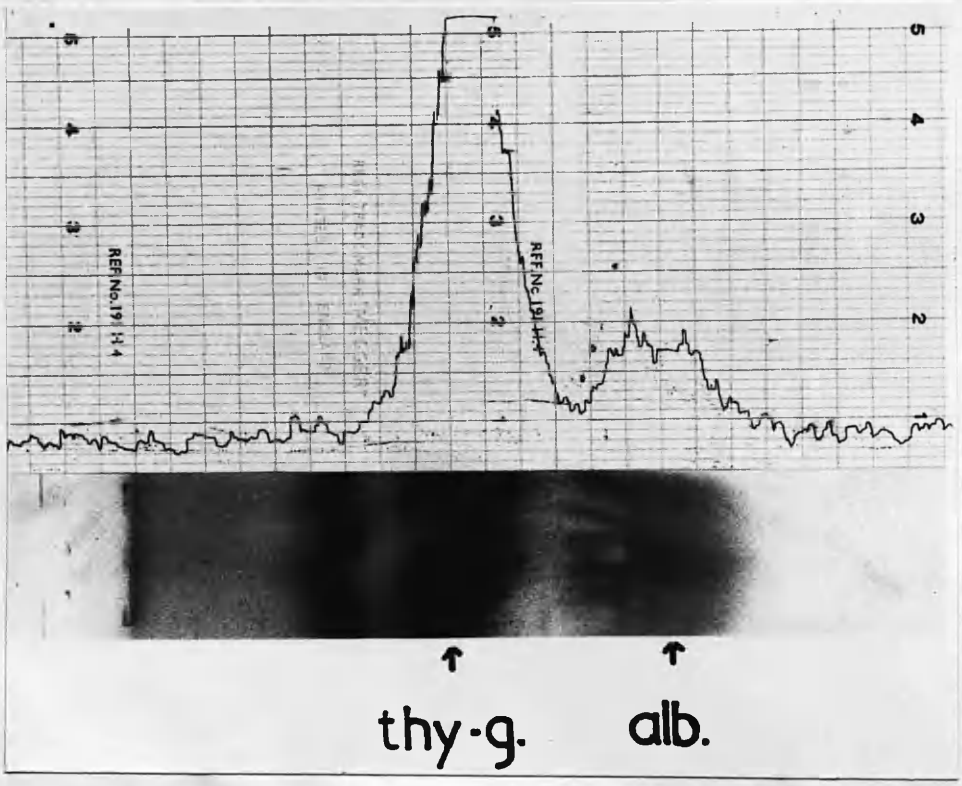

REF.No.191.H.4
REF.No.191.H.4
thy-g.
alb.

Fig.48

Similar study to that in fig.47 but prepared from non-toxic nodular goitre. 5 protein bands are present, designated, in order from the start, Q, T, Hb, thy-g, and alb. Radioactivity is chiefly in the thyroglobulin (thy-g) zone, and to a minor extent only in albumin (alb.).

Fig.49

Similar study to that in figs. 47 and 48, in another case of non-toxic nodular goitre. By chance the thyroglobulin and albumin bands are separated so that the peaks of radioactivity associated with thyroglobulin and albumin are shown to be quite distinct.

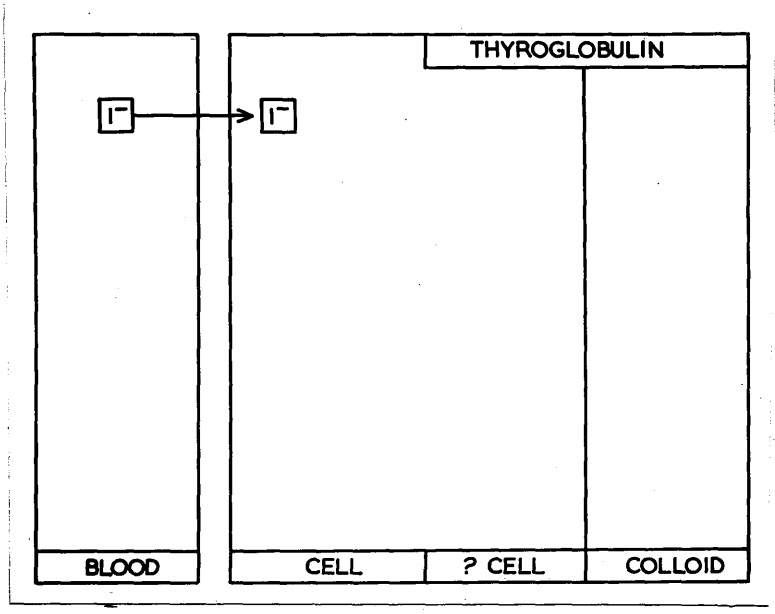

THYROGLOBULIN
I⁻
I⁻
BLOOD
CELL
? CELL
COLLOID

Fig.50

Biosynthesis of the thyroid hormone. Impaired ability to utilize trapped iodide. ?Peroxidase deficiency.

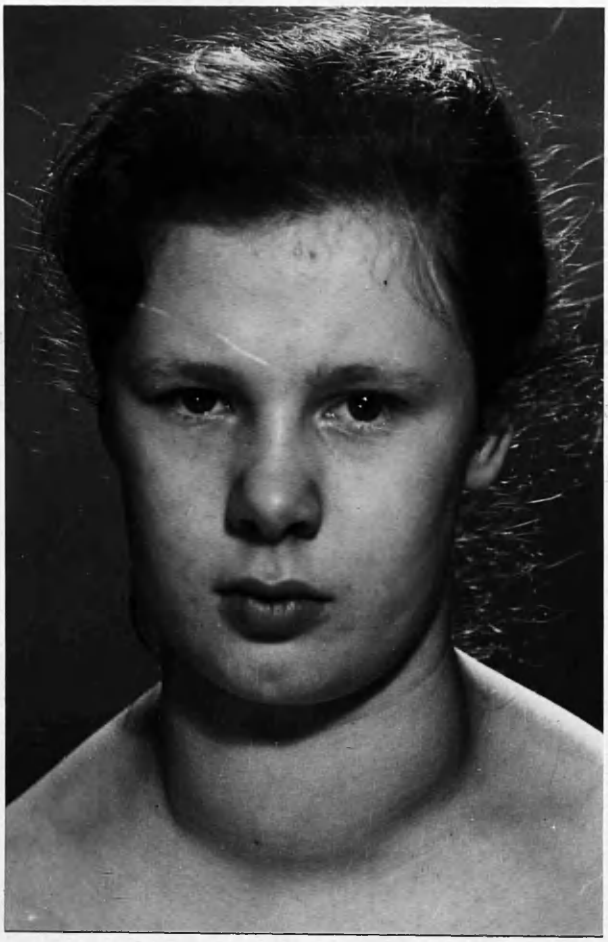


Fig.51

M.McG. (case I), aged 15 years. Photograph demonstrates swelling of neck due to goitre.

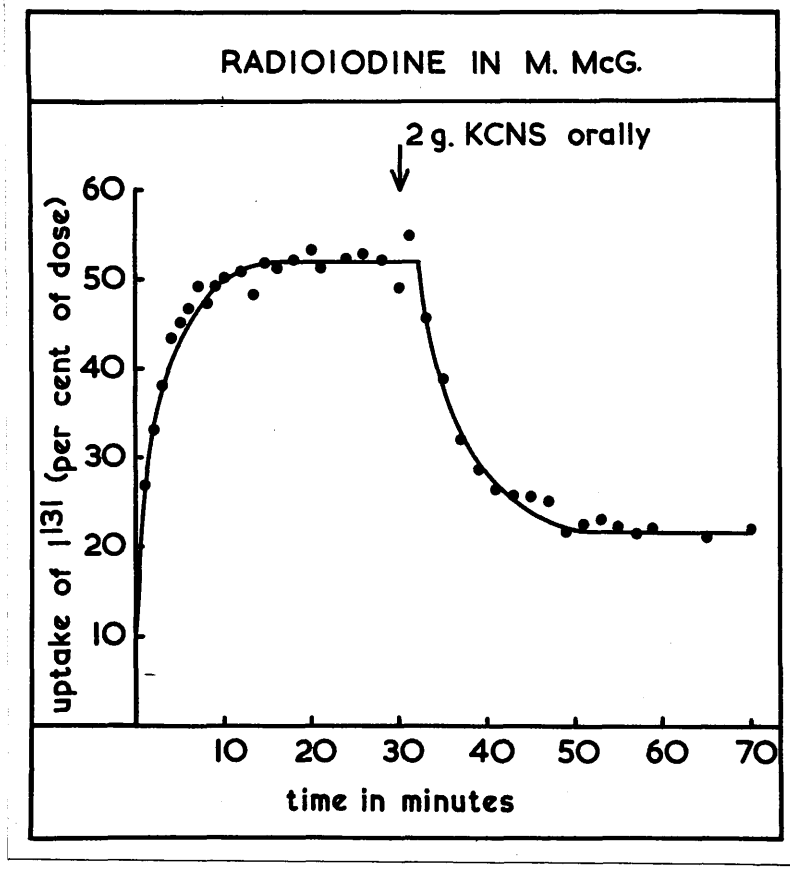
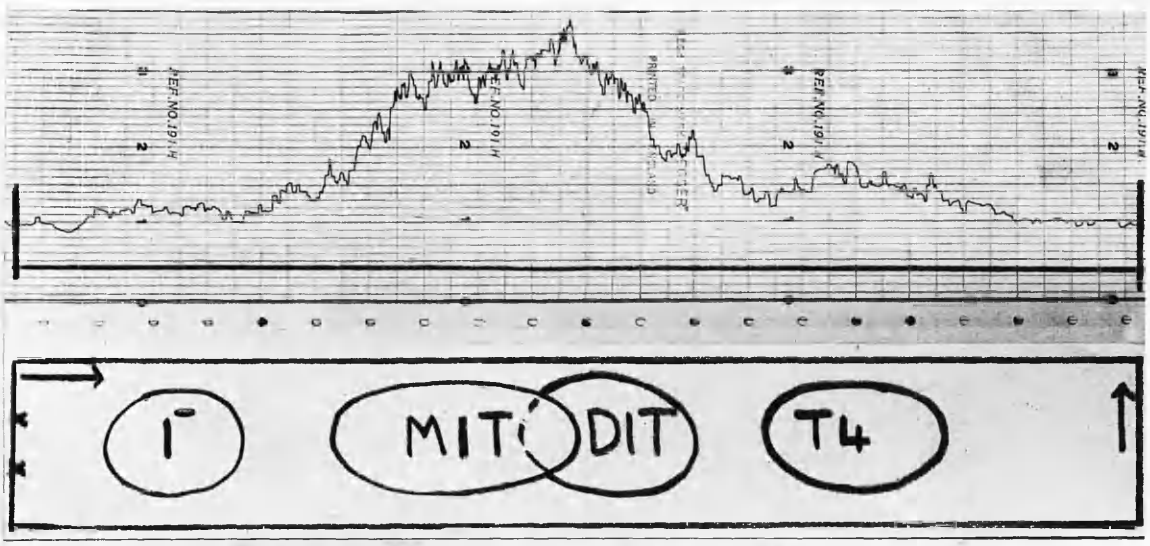

RADIOIODINE IN M. McG.
2 g. KCNS orally
uptake of I131 (per cent of dose)
60
50
40
30
20
10
10
20
30
40
50
60
70
time in minutes

Fig.52

M. McG. (case I). Rapid accumulation of ^{131}I by the thyroid gland after an intravenous dose of 25 μc ^{131}I, with discharge of ^{131}I following an oral dose of 2 g. potassium thiocyanate.


I⁻
MIT
DIT
T4

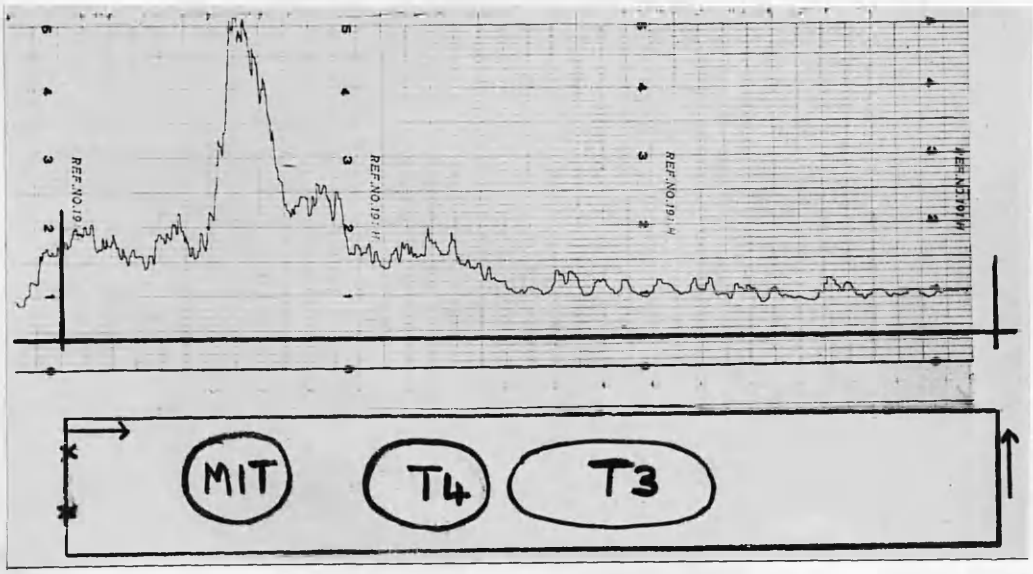

MIT
T4
T3

Fig.53

M.McG. (case I). Scan for radioactivity of ascending chromatogram prepared from butanol extract of thyroid gland, removed 24 hours after 100 μc ^{131}I, and hydrolyzed by 2N-NaOH. Solvent, butanol acetic acid. Iodide (I^-), the iodotyrosines (MIT and DIT) and thyroxine (T4) are identified, but the separation of MIT from DIT is technically poor.

Fig.54.

M. McG. (case I). Similar study to that in fig. 53 but solvent butanol ammonia. Greatest peak of radioactivity corresponds to MIT marker. Radioactivity in T4 zone confirms presence of thyroxine.

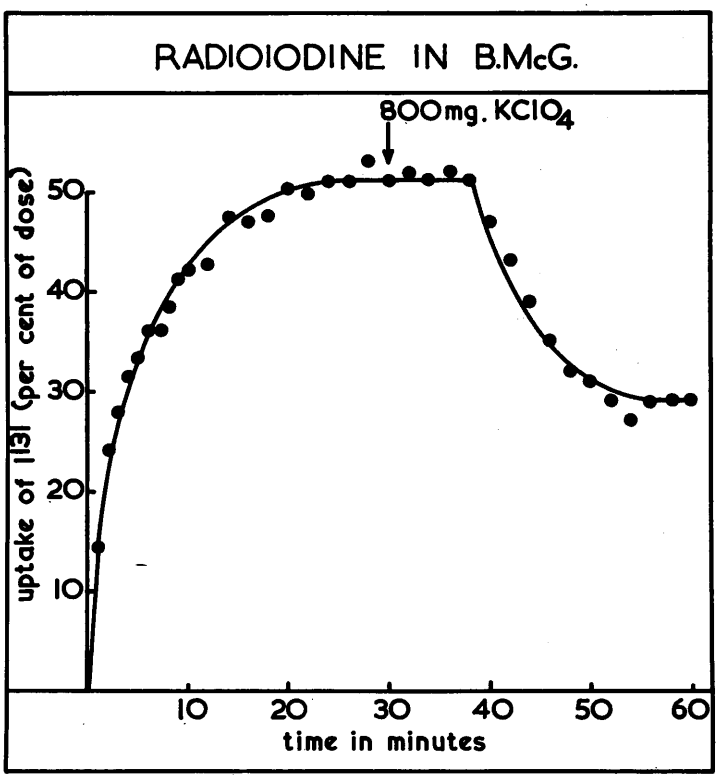
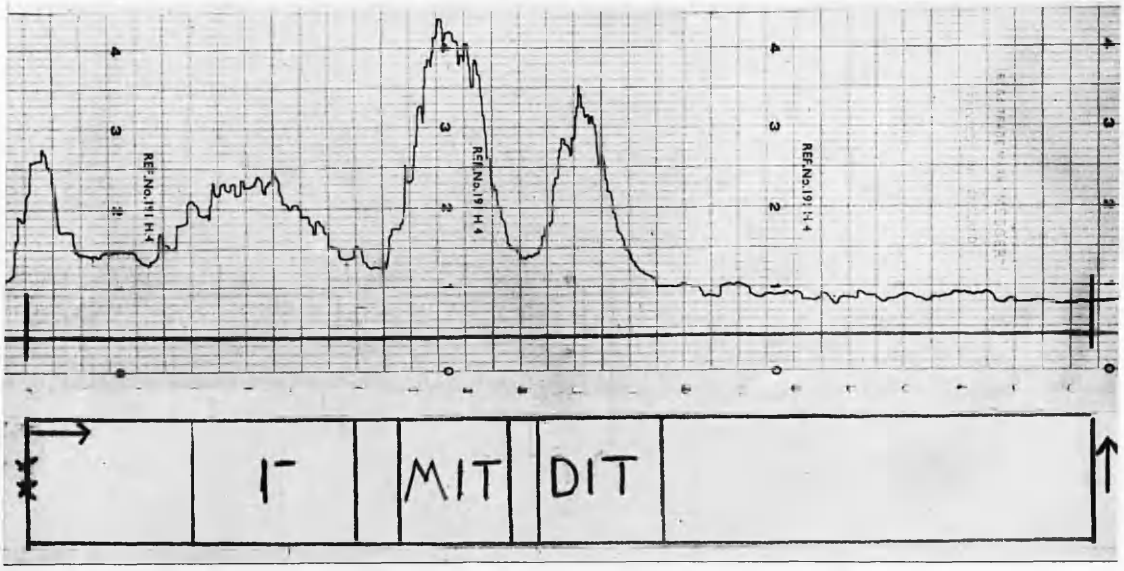

RADIOIODINE IN B.McG.
800mg. KClO4
uptake of 131I (per cent of dose)
50
40
30
20
10
10
20
30
40
50
60
time in minutes

Fig.55

B. McG. (case II). Rapid accumulation of ^{131}I by the thyroid gland after an intravenous dose of 25 μc ^{131}I, with discharge of ^{131}I following an oral dose of 800 mg. potassium perchlorate.


I⁻
MIT
DIT

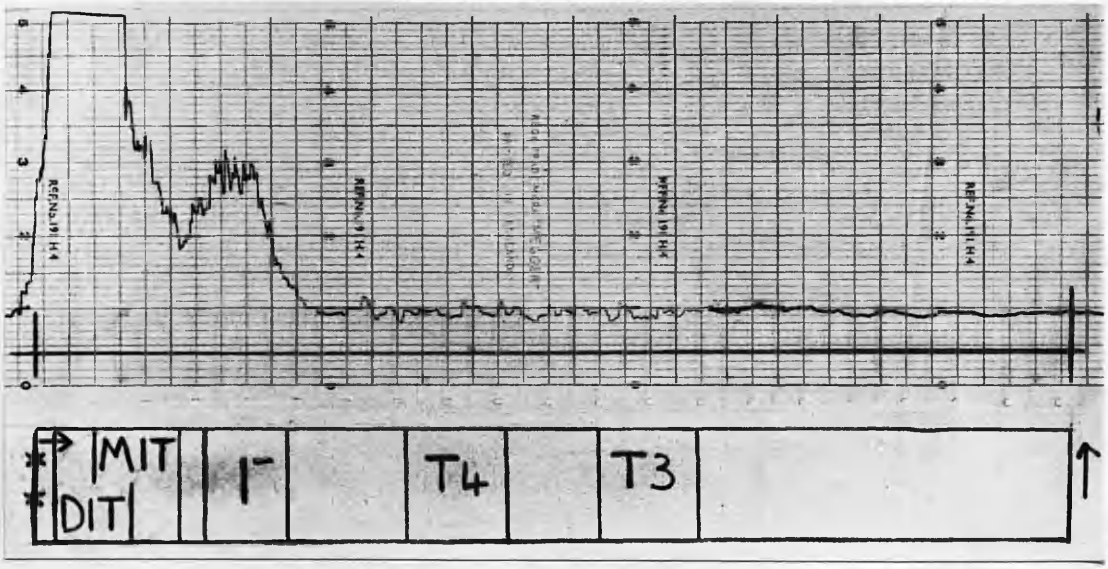

MIT
DIT
I⁻
T4
T3

Fig.56

B. McG. (case II). Scan for radioactivity of ascending chromatogram prepared from trypsin digest of thyroid gland removed 48 hours after 100 μc ^{131}I. Solvent, butanol acetic acid. Iodide (I^-), monoiodotyrosine (MIT), and diiodotyrosine (DIT) are identified. There is no radioactivity in the usual thyroxine zone.

Fig.57

B. McG. (case II). Similar study to that in fig.56 but solvent butanol ammonia. Absence of thyroxine confirmed.

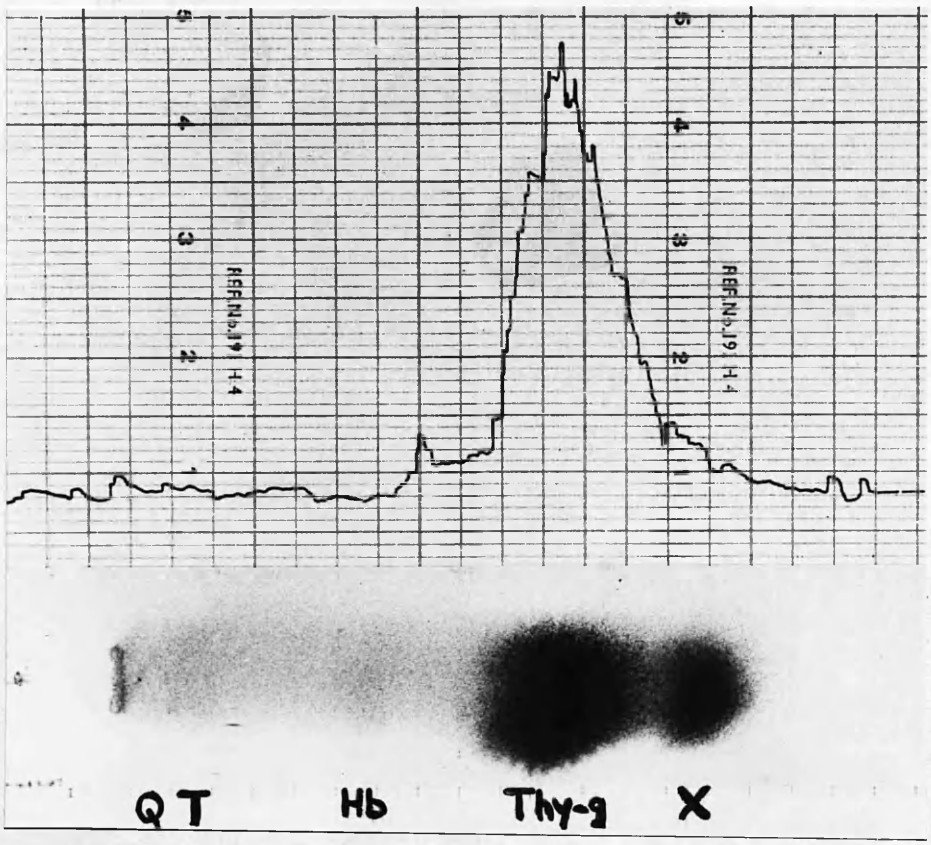

REF.No.J9 H-4
REF.No.J9 H-4
QT
Hb
Thy-g
X

Fig.58

B. McG. (case II). Scan for radioactivity of electrophoretogram prepared from thyroid tissue removed 48 hours after 100 μc ^{131}I. Proteins stained with aqueous bromphenol blue. Thyroglobulin (Thy-g) and albumin (X) are easily identified and there are also bands of Q protein, gamma globulin (T) and haemoglobin (Hb). Radioactivity is associated with thyroglobulin and to a minor degree with albumin.

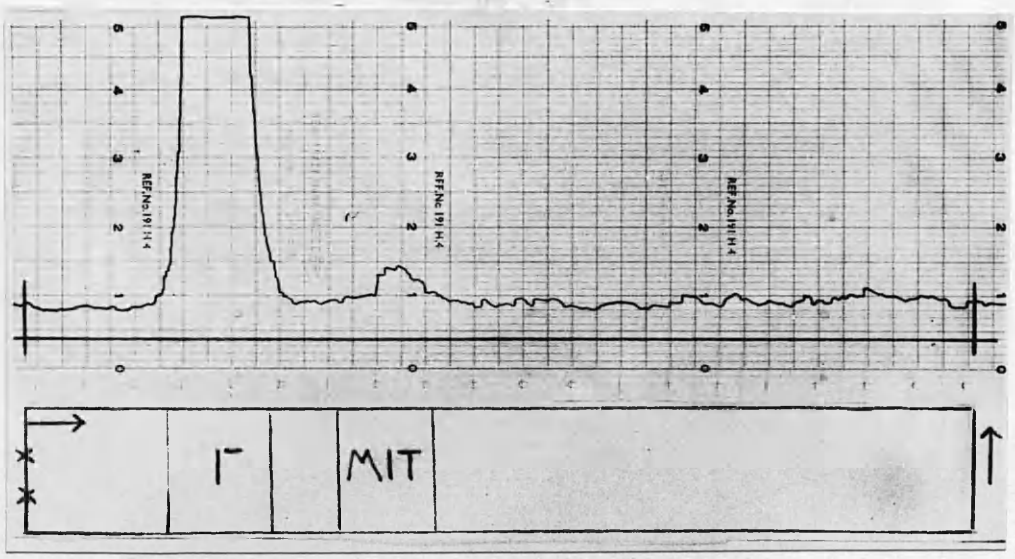

MIT

Fig.59

B. McG. (case II). Scan of ascending chromatogram prepared from Krebs-Ringer phosphate buffer in which fresh thyroid slices have been incubated with $M^{131}IT$ and nicotinamide. Over 90 per cent of the radioactivity is in the iodide (I^-) spot, indicating normal dehalogenase activity.

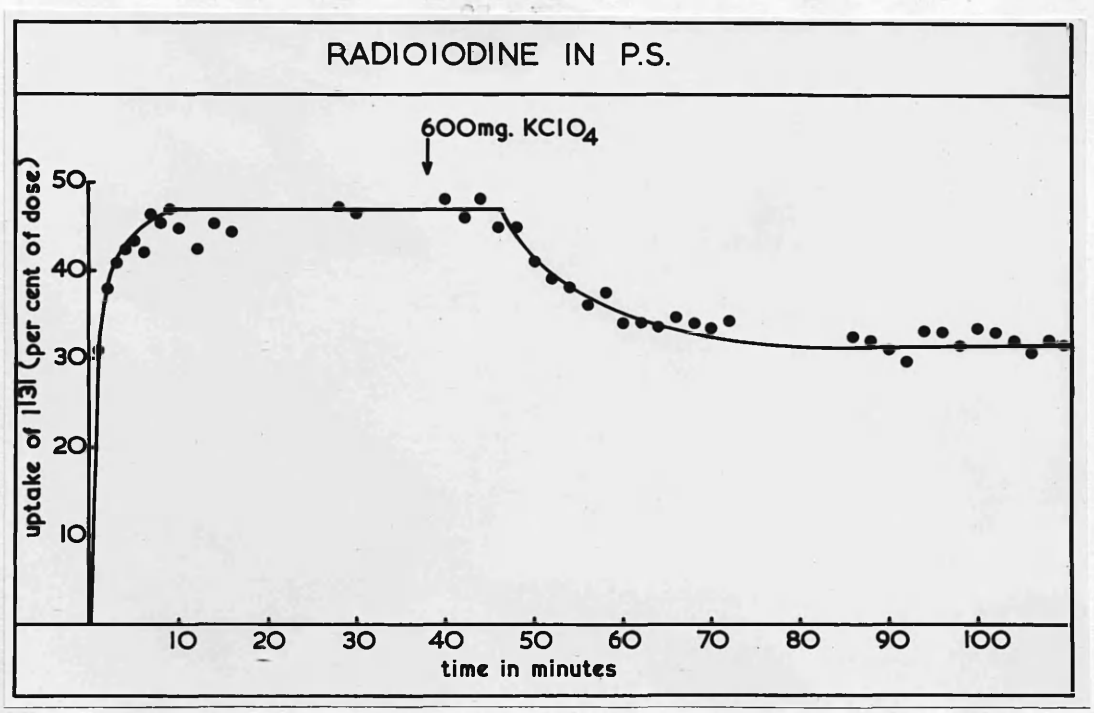

RADIOIODINE IN P.S.
600mg. KClO4
uptake of 131I (per cent of dose)
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100
time in minutes

Fig.60

P.S. (case III). Rapid accumulation of ^{131}I by the thyroid gland after an intravenous dose of 25 μc ^{131}I, with discharge of ^{131}I following 600 mg. potassium perchlorate.

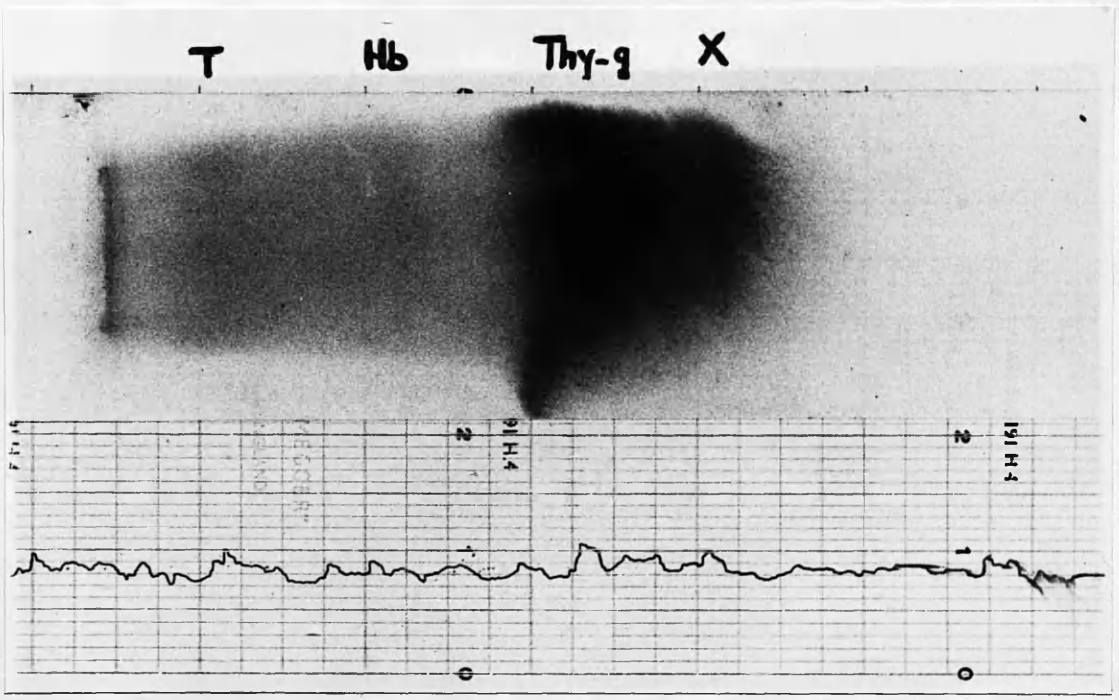

T
Hb
Thy-g
X

Fig.61

A.A. (case IV). Scan for radioactivity of electrophoretogram prepared from thyroid tissue removed 48 hours after 100 μc ^{131}I. Proteins stained with aqueous bromphenol blue. Gamma globulin (T), haemoglobin (Hb), thyroglobulin (Thy-g) and albumin (X) identified. No definite Q band. Because of previous iodine medication there was no uptake of radioactivity.

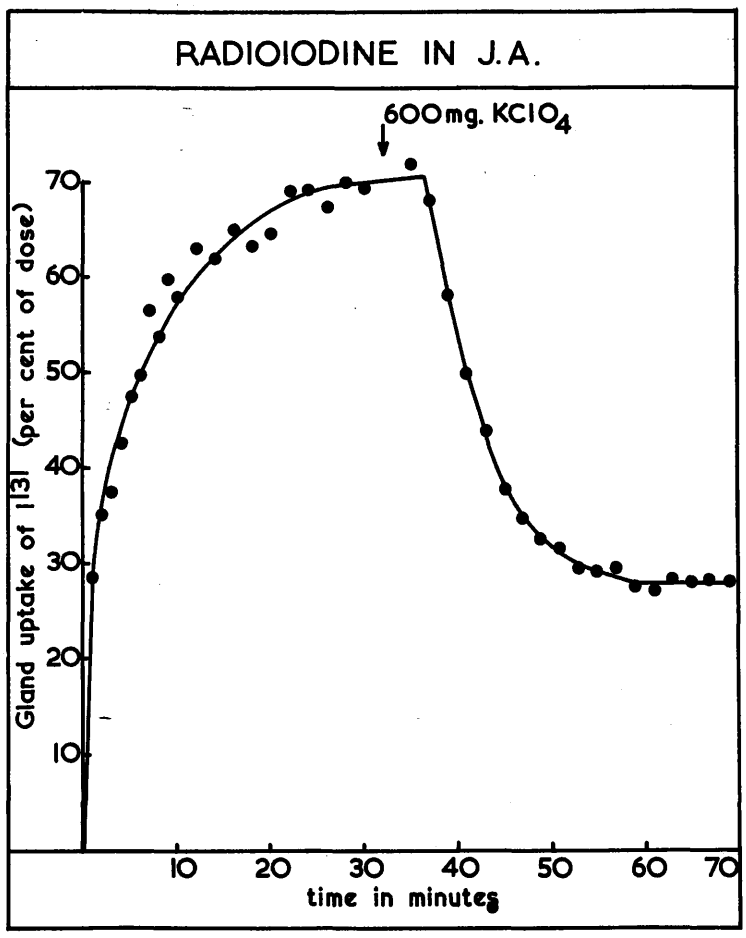

RADIOIODINE IN J.A.
600mg. KClO4
Gland uptake of I131 (per cent of dose)
70
60
50
40
30
20
10
10
20
30
40
50
60
70
time in minutes

Fig.62

J.A. (case V). Rapid accumulation of ^{131}I by the thyroid gland after an intravenous dose of 25 μc ^{131}I, with discharge of ^{131}I following 600 mg. potassium perchlorate.

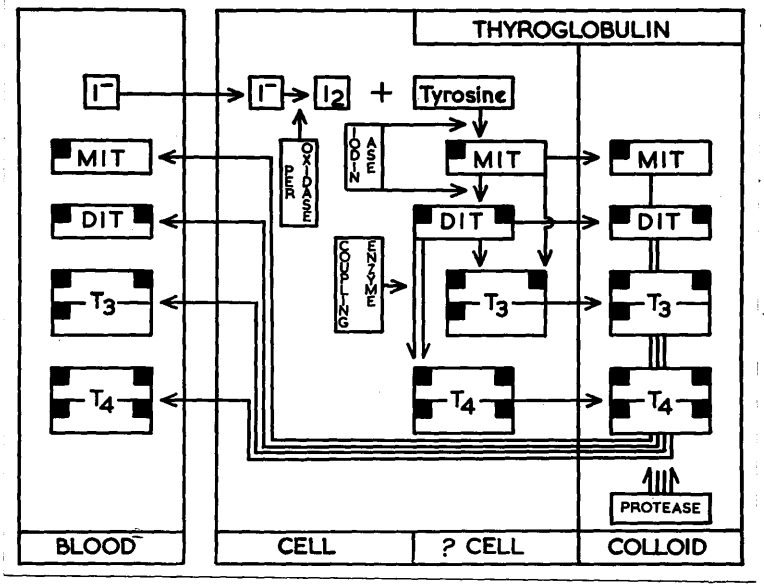

THYROGLOBULIN
I⁻
I⁻
I₂
+
Tyrosine
PEROXIDASE
IODINASE
MIT
DIT
T3
T4
COUPLING ENZYME
MIT
DIT
T3
T4
MIT
DIT
T3
T4
PROTEASE
BLOOD
CELL
? CELL
COLLOID

Fig.63

Biosynthesis of the thyroid hormone. Failure to conserve iodine of iodotyrosines. Lack of dehalogenase.

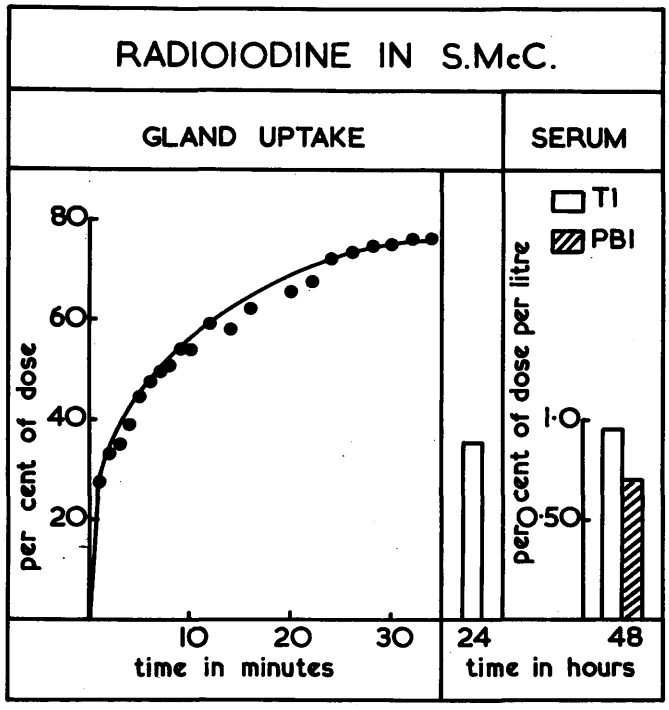

RADIOIODINE IN S.McC.
GLAND UPTAKE
SERUM
per cent of dose
80
60
40
20
10
20
30
time in minutes
24
48
time in hours
per cent of dose per litre
1·0
0·50
TI
PBI

Fig.64

S. McC. (case VI). Rapid accumulation of ^{131}I by the thyroid gland and raised serum radioactivity after an intravenous dose of 25 µc ^{131}I.

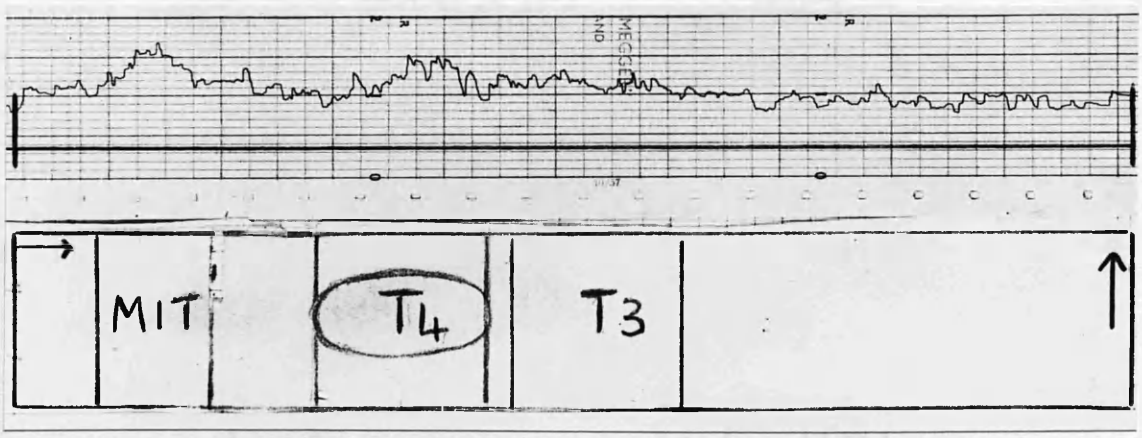

MIT
T4
T3

Fig.65

S. McC.(case VI). Scan for radioactivity of ascending chromatogram prepared from butanol extract of serum withdrawn 48 hours after 100 μc ^{131}I. Solvent, butanol ammonia. Monoiodotyrosine (MIT) and thyroxine (T4) are identified. There is probably a trace of triiodothyronine (T3).

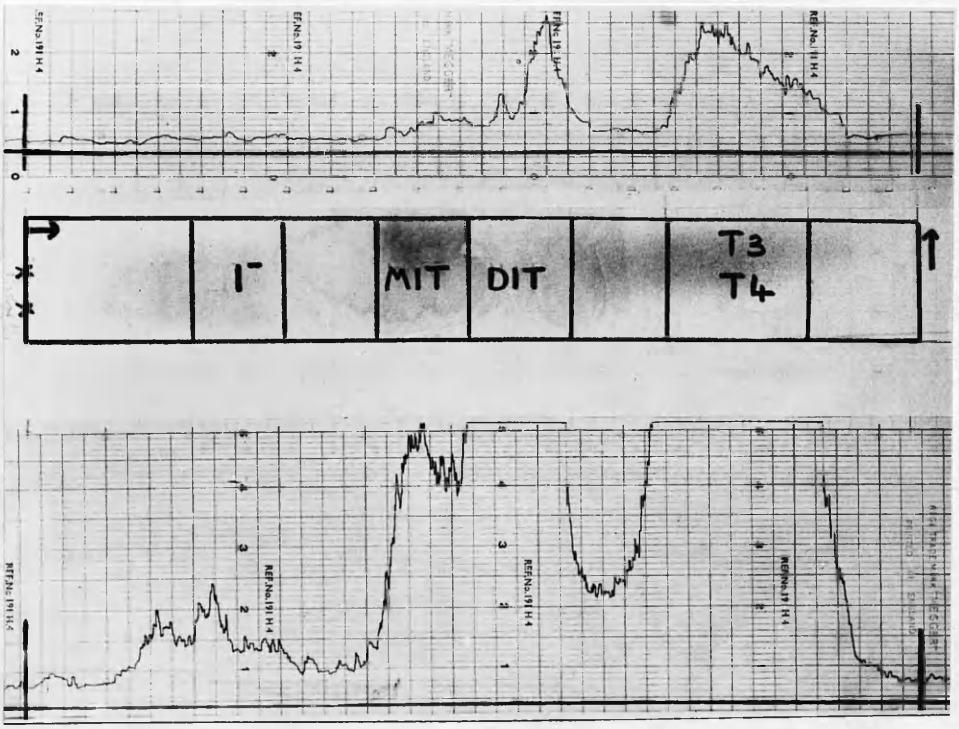
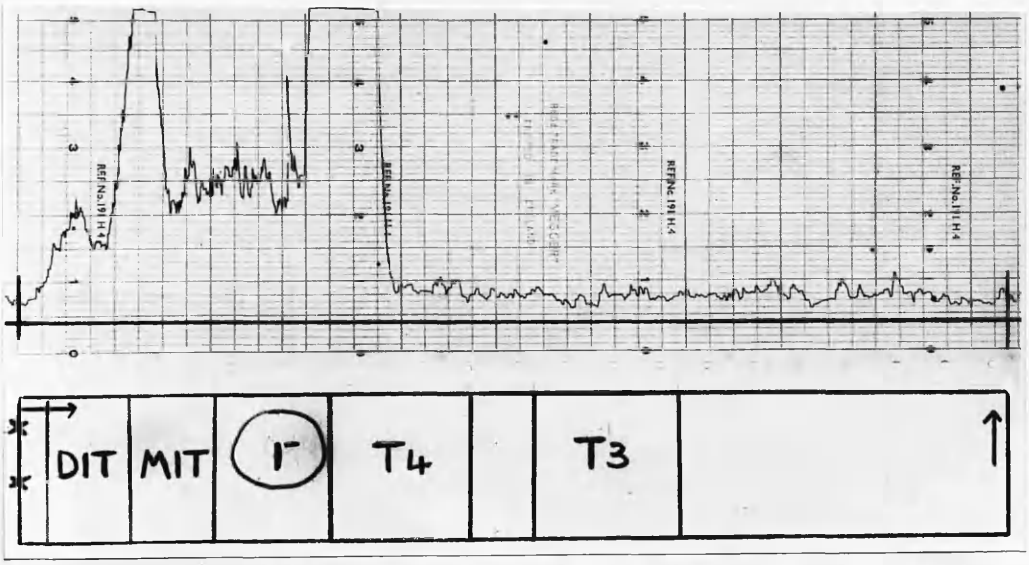

I⁻
MIT
DIT
T3
T4

Fig.66

S. McC. (case VI). Scan for radioactivity of ascending chromatogram prepared from butanol extract of urine passed in period 0-24 hours after 100 μc ^{131}I. Solvent, butanol acetic acid. Iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified. Basis of greatest peak of radioactivity in T4 T3 zone is probably iodotyrosine conjugates. (Lower scale is approximately 10X upper scale).


DIT
MIT
I⁻
T4
T3

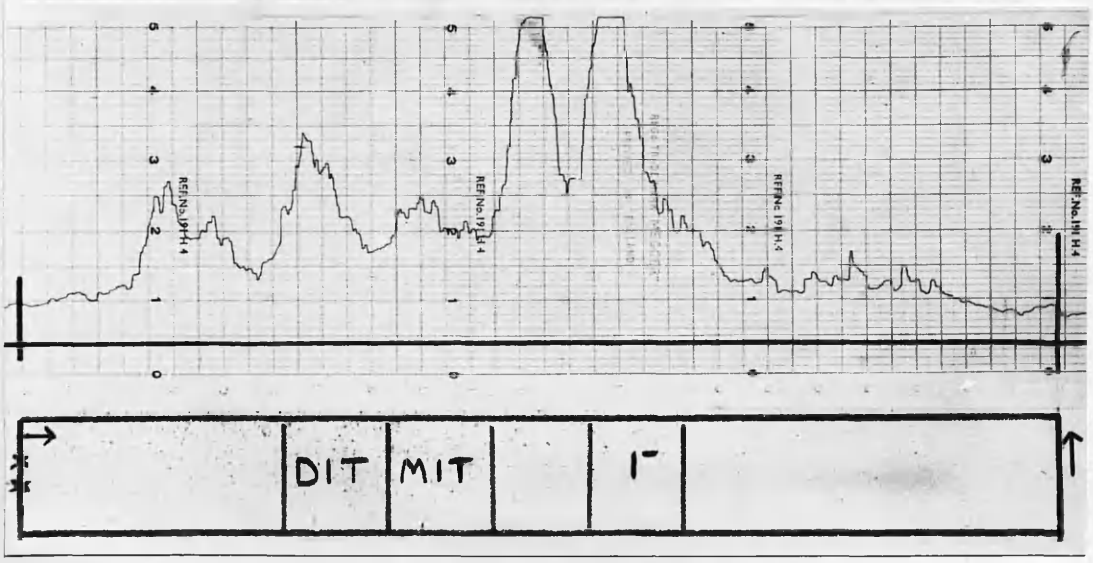
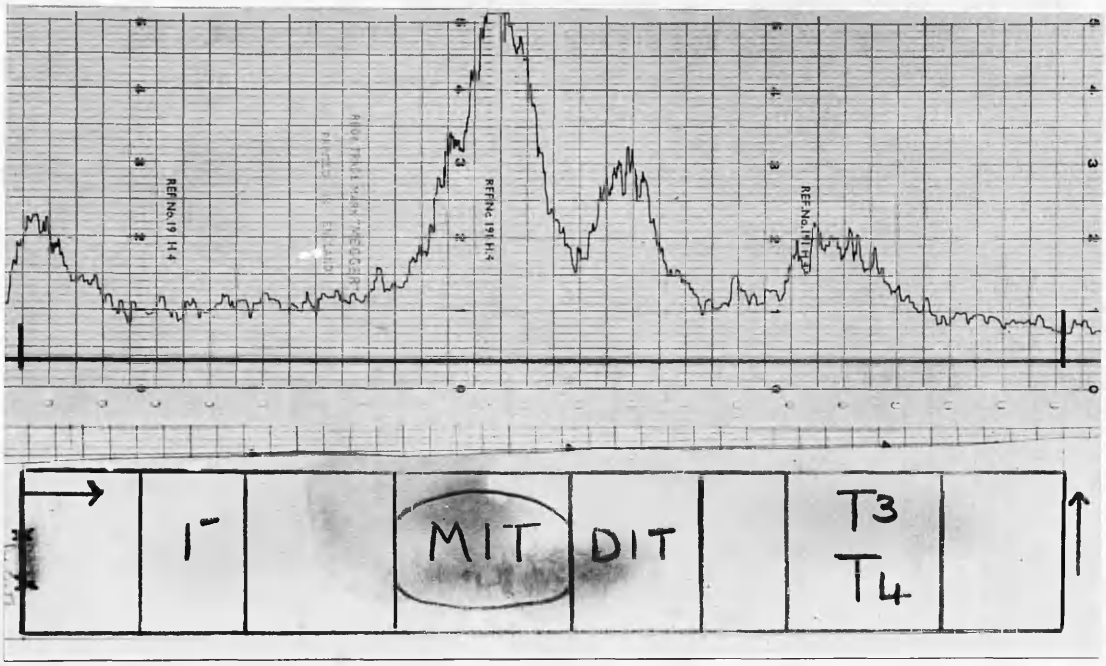

DIT
MIT
I⁻

Fig.67

S. McC. (case VI). Similar study to that in fig.66 but solvent butanol ammonia. Greatest peak of radioactivity, probably due to iodotyrosine conjugates, lies between iodide (I^-) and thyroxine (T4) spots.

Fig.68

S. McC. (case VI). Similar study to that in figs.66 and 67, but solvent collidine ammonia. As well as peaks of radioactivity corresponding to iodide (I^-), monoiodotyrosine (MIT) and diiodotyrosine (DIT), there are at least 4 other peaks, probably due to iodotyrosine derivatives.


REF No.19 H.4
REF No.19 H.4
REF No.19 H.4
1-
MIT
DIT
T3
T4

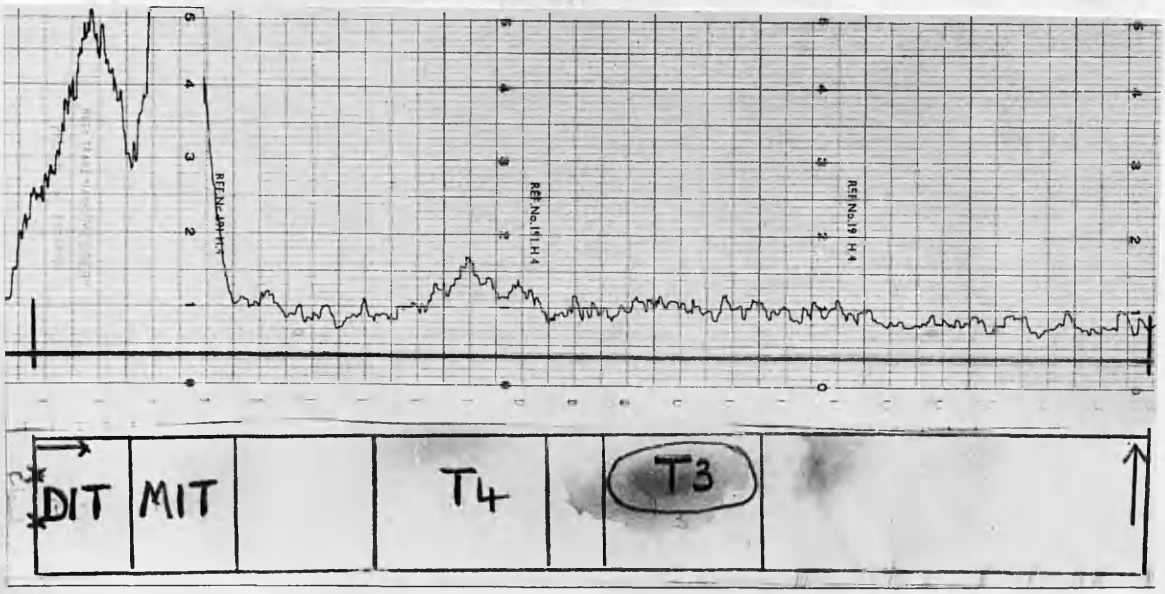
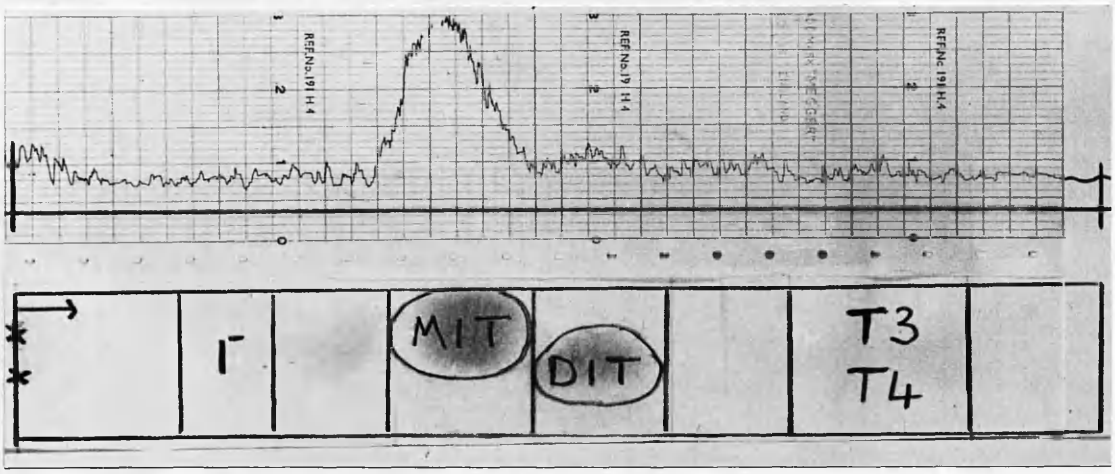

REF No.19 H.4
REF No.19 H.4
REF No.19 H.4
DIT
MIT
T4
T3

Fig.69

S. McC. (case VI). Scan for radioactivity of ascending chromatogram prepared from trypsin digest of paranodular tissue of thyroid removed 48 hours after 100 μc ^{131}I. Solvent, butanol acetic acid. Monoiodotyrosine (MIT), diiodotyrosine (DIT) and thyroxine (T4) and/or triiodothyronine (T3) are identified. MIT/DIT ratio is 2.0.

Fig.70

S. McC. (case VI). Similar study to that in fig.69 but solvent butanol ammonia. Presence of thyroxine (T4) confirmed. It is doubtful if there is any appreciable triiodothyronine (T3).


I
MIT
DIT
T3
T4

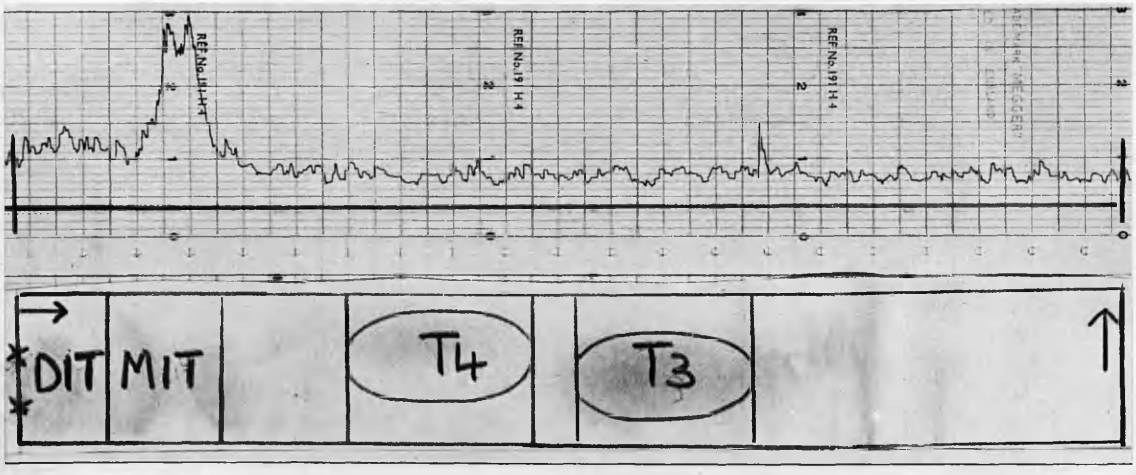

DIT
MIT
T4
T3

Fig.71

S. McC. (case VI). Similar study to that in figs.69 and 70 but with material from nodule. Butanol acetic acid solvent. Monoiodotysosine (MIT) and diiodotyrosine (DIT) are identified. MIT/DIT ratio is 14.4. There is no thyroxine.

Fig.72

S. McC. (case VI). Similar study to that in fig.71 but solvent butanol ammonia. Absence of thyroxine is confirmed.

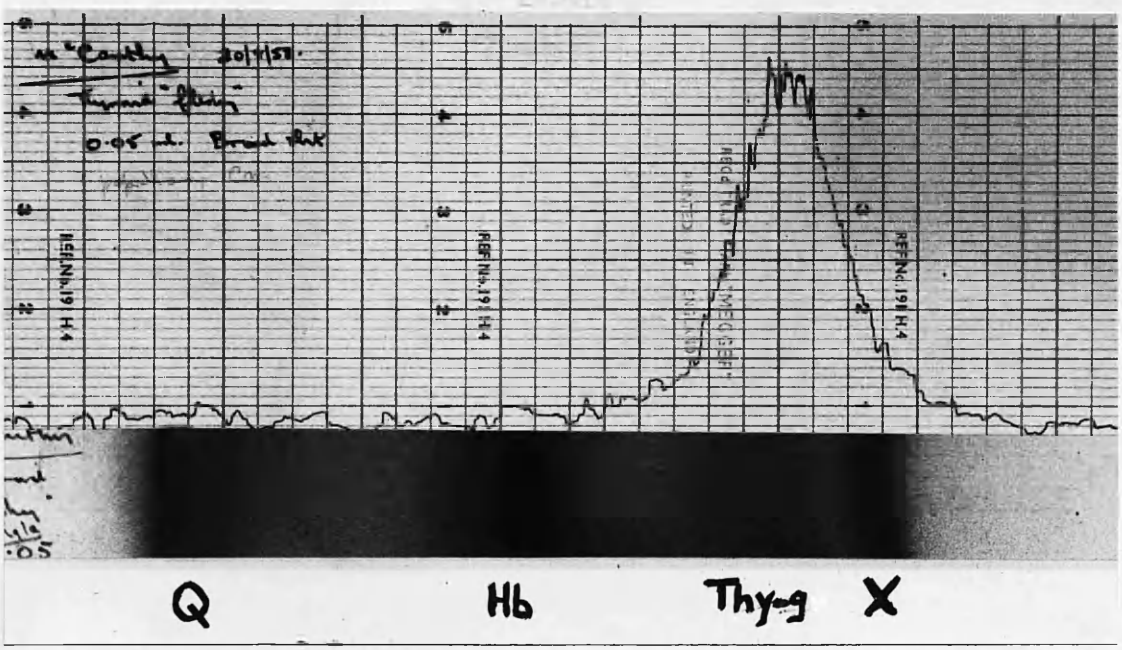

REFNo.191 H-4
REFNo.191 H-4
REFNo.191 H-4
Q
Hb
Thy-g
X

Fig.73

S. McC. (case VI). Scan for radioactivity of electrophoretogram of thyroid proteins prepared from thyroid tissue removed 48 hours after 100 μc ^{131}I. Proteins stained with aqueous bromphenol blue. Q protein, haemoglobin (Hb), thyroglobulin (Thy-g) and albumin (X) are identified. Radioactivity is associated with thyroglobulin and to a lesser extent with albumin.

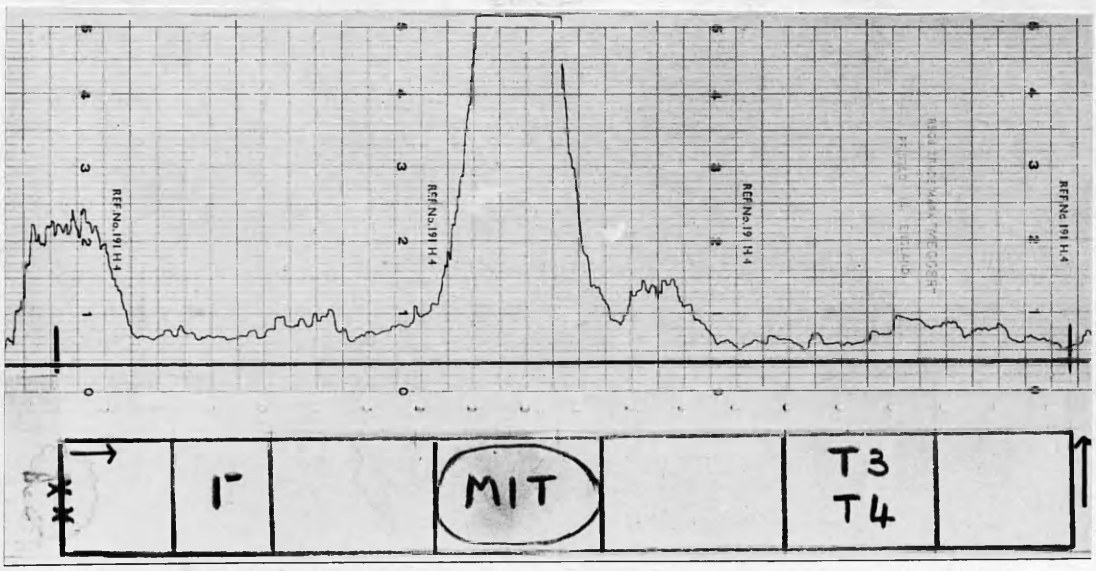
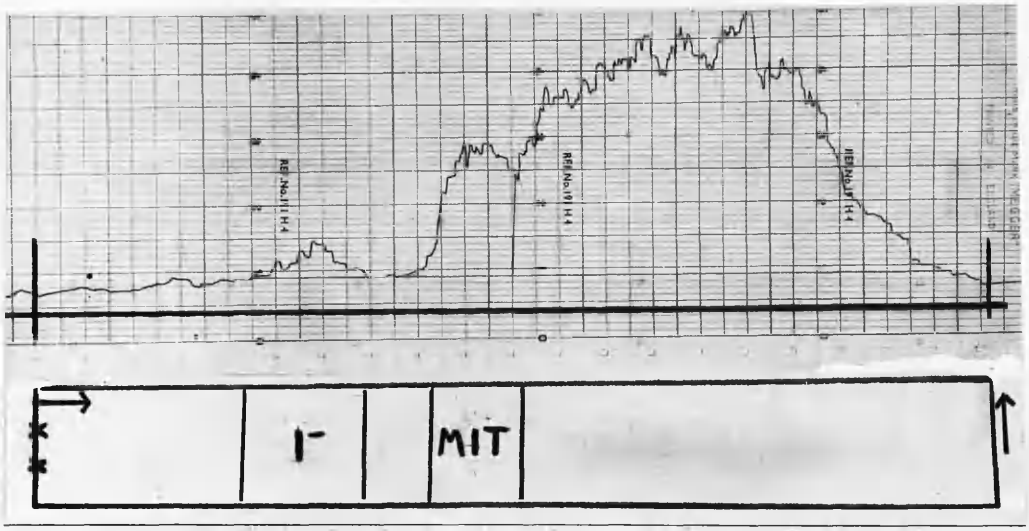
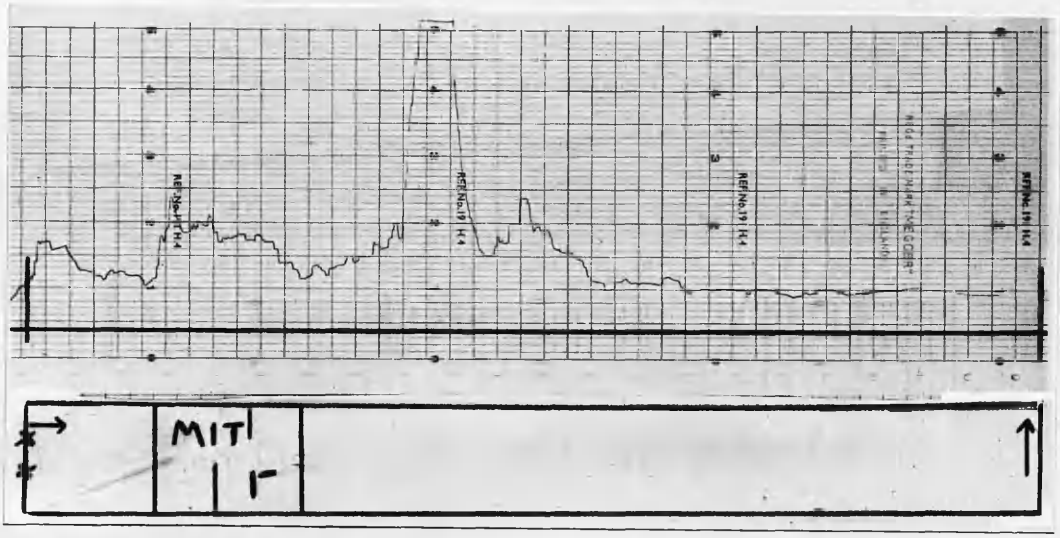

REF No. 191 H-4
REF No. 191 H-4
REF No. 191 H-4
REF No. 191 H-4
1"
MIT
T3
T4

Fig.74

S. McC. (case VI). Scan for radioactivity of ascending chromatogram prepared from Krebs-Ringer phosphate buffer in which fresh slices of thyroid tissue have been incubated with $M^{131}IT$ and nicotinamide. Radioactivity has persisted in the monoiodotyrosine (MIT), indicating impaired dehalogenase activity.


MIT


MIT

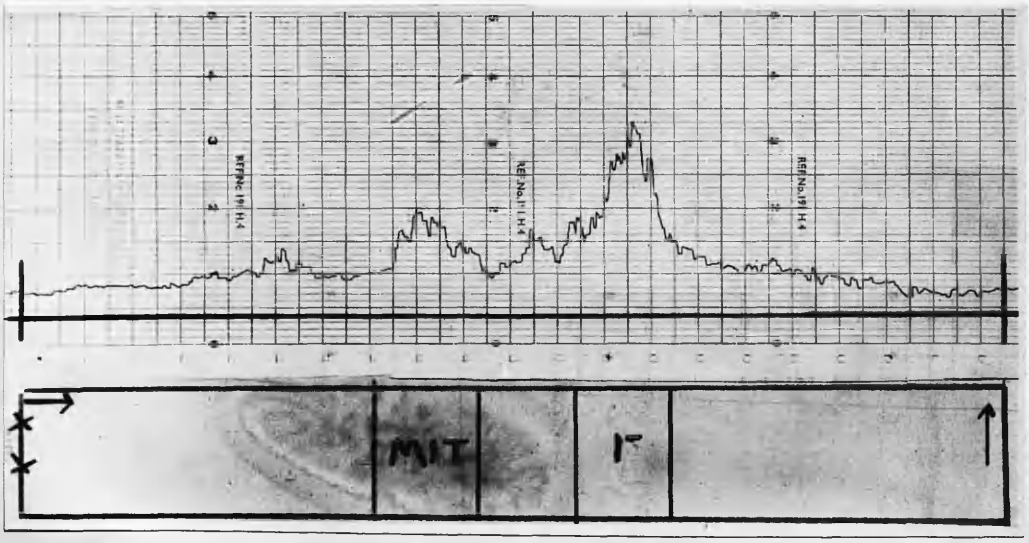

MIT

Fig.75 a

S. McC. (case VI). Scan for radioactivity of ascending chromatogram prepared from butanol extract of urine passed in period 2-6 hours after an oral dose of 20 μc $M^{131}IT$. Solvent, butanol acetic acid. 96.1 per cent of the radioactivity is present in the MIT zone, or in another zone corresponding roughly to the thyroxine zone, and almost certainly due to monoiodotyrosine conjugates.

Fig.75 b

S. McC. (case VI). Similar study to that in fig.75 a but solvent butanol ammonia. The large peak of radioactivity beyond the iodide (I^-) spot is almost certainly due to a monoiodotyrosine conjugate.

Fig.75 c

S. McC. (case VI). Similar study to that in figs.75 a and b but solvent collidine ammonia. As well as peaks of radioactivity corresponding to iodide (I^-) and monoiodotyrosine (MIT) there are other unidentified peaks, almost certainly due to monoiodotyrosine conjugates. The apparent iodide (I^-) peak, which is relatively much greater in this chromatogram, almost certainly includes a conjugate as well as iodide.

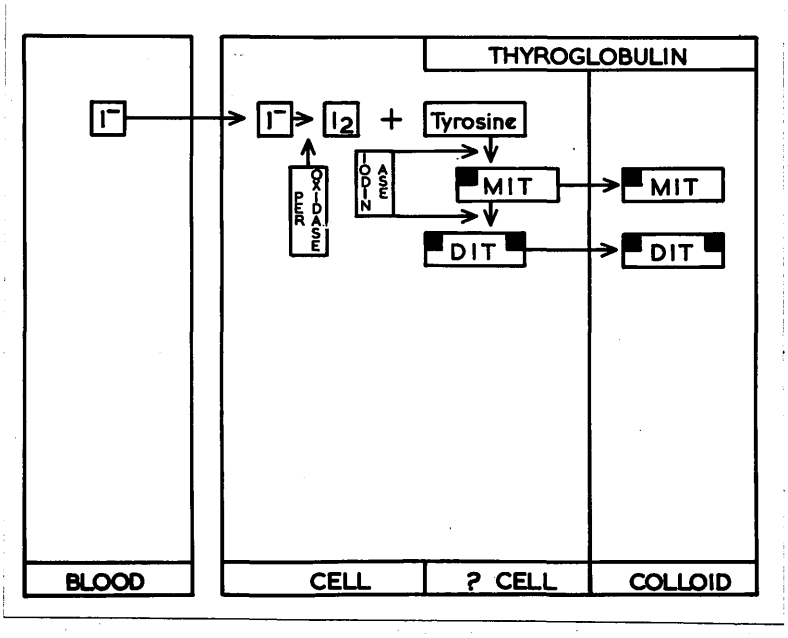

THYROGLOBULIN
I⁻
I⁻
I₂
+
Tyrosine
PEROXIDASE
IODINASE
MIT
MIT
DIT
DIT
BLOOD
CELL
? CELL
COLLOID

Fig.76

Biosynthesis of the thyroid hormone. Failure to couple iodotyrosines. ?Coupling enzyme deficiency.

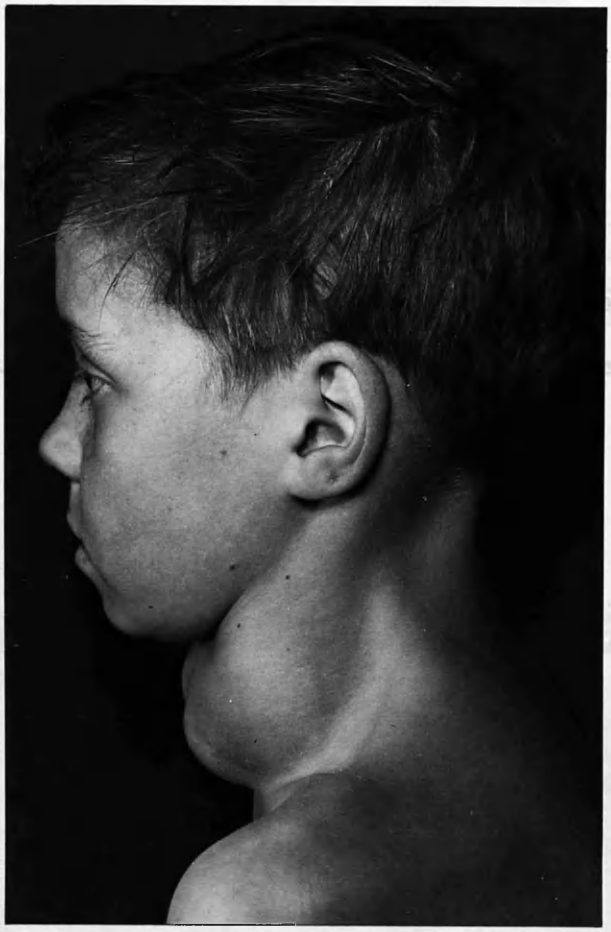


Fig.77

S.S. (case VII), aged 10 years; demonstrating goitre.

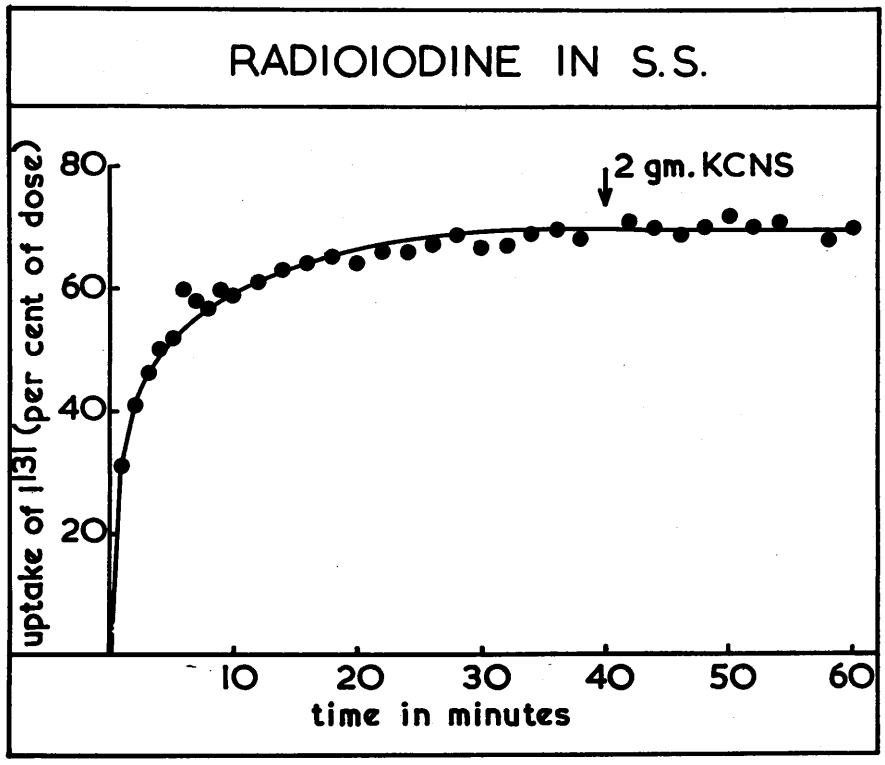
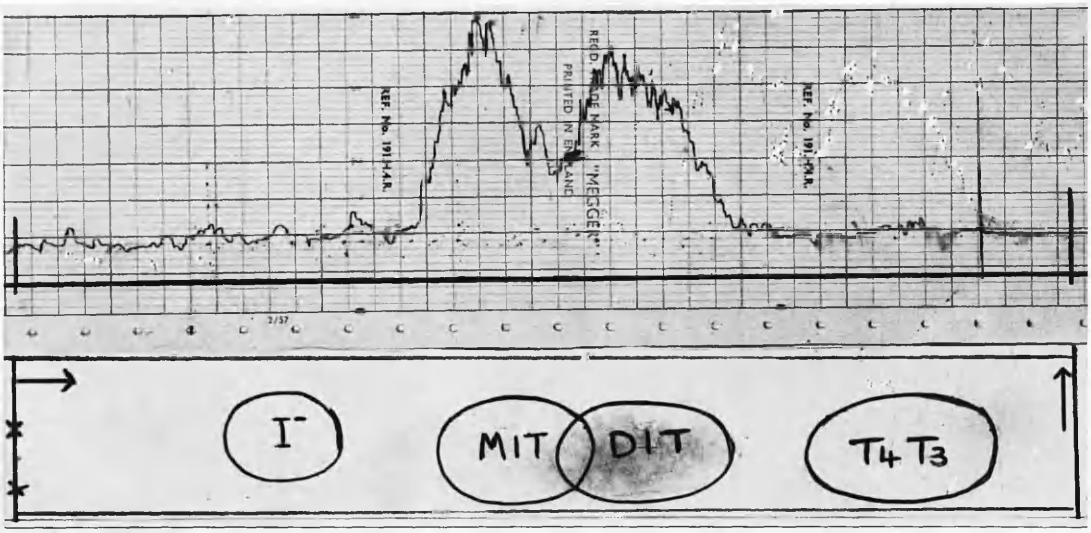

RADIOIODINE IN S.S.
uptake of I131 (per cent of dose)
80
60
40
20
2 gm. KCNS
10
20
30
40
50
60
time in minutes

Fig.78

S.S. (case VI). Rapid accumulation of ^{131}I by the thyroid gland after an intravenous dose of 25 μc ^{131}I, and absence of effect of an oral dose of 2 g. potassium thiocyanate on the ^{131}I content of thyroid.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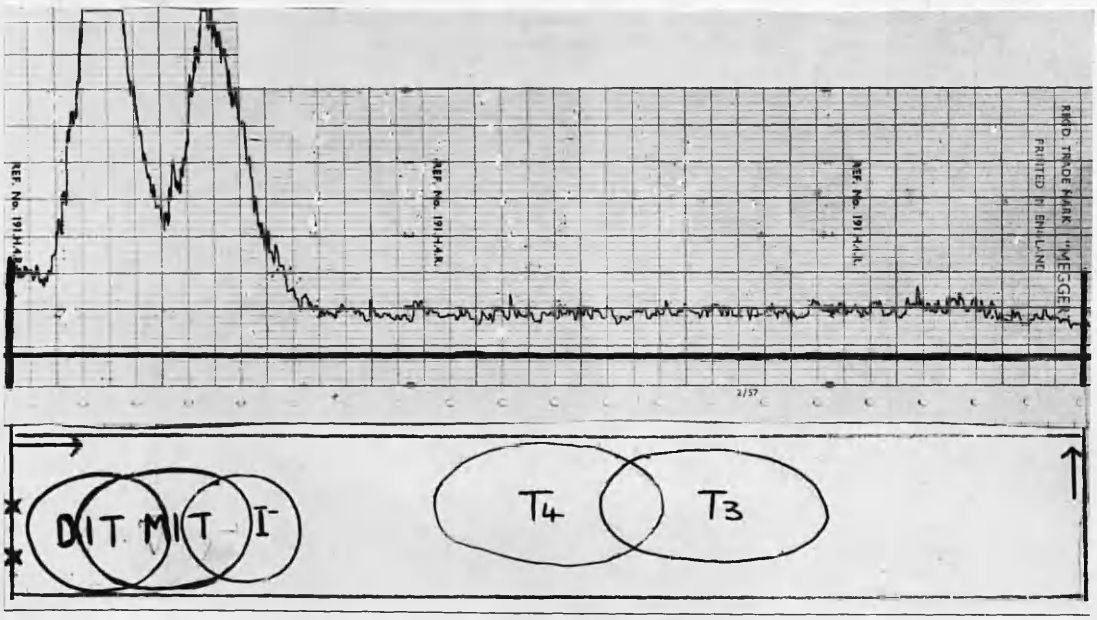
REF. No. 191-P.B.A.
REID'S TRADE MARK "MEGGEN"
PRINTED IN ENGLAND
I⁻
MIT
DIT
T4 T3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REF. No. 191-H.A.R.
REF. No. 191-H.A.R.
REID'S TRADE MARK "MEGGEN"
PRINTED IN ENGLAND
DIT MIT I⁻
T4
T3

Fig.79

S.S. (case VII). Scan for radioactivity of ascending chromatogram prepared from butanol extract of trypsin digest of thyroid gland removed 24 hours after 100 μc ^{131}I. Solvent, butanol acetic acid. Monoiodotyrosine (MIT) and diiodotyrosine (DIT) are identified. There is no thyroxine.

Fig.80

S.S. (case VII). Similar study to that in fig.79 but butanol ammonia solvent. Absence of thyroxine is confirmed.

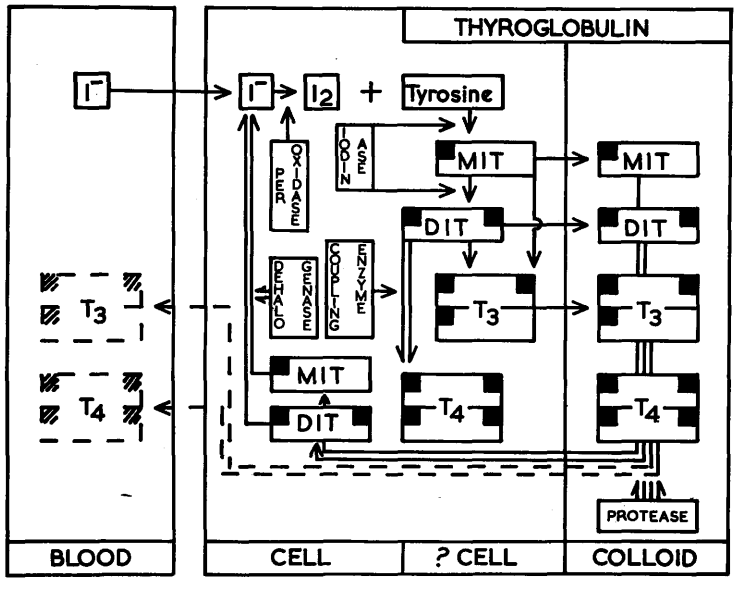

THYROGLOBULIN
I⁻
I⁻
I₂
+
Tyrosine
PEROXIDASE
IODINASE
MIT
DIT
T₃
T₄
DEHALOGENASE
COUPLING ENZYME
MIT
DIT
T₃
T₄
MIT
DIT
T₃
T₄
PROTEASE
BLOOD
CELL
? CELL
COLLOID

Fig.81

Biosynthesis of the thyroid hormone. Production of an abnormal iodinated thyroid protein.

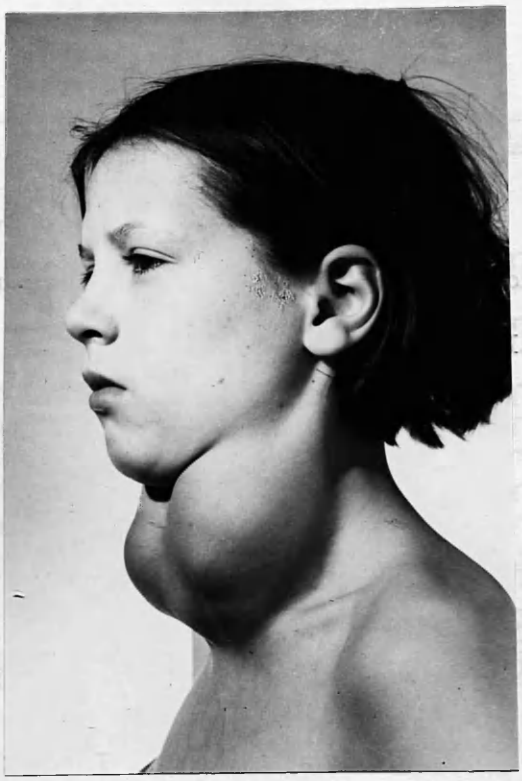
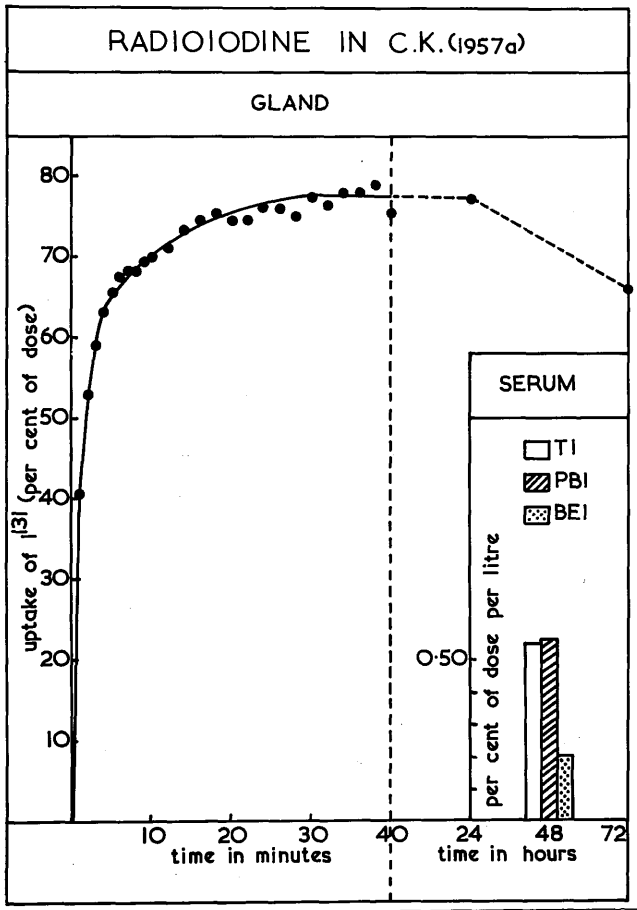


Fig.82

C.K. (case VIII), 19 years, in 1958, showing enormous goitre weighing 560 g.


RADIOIODINE IN C.K.(1957a)
GLAND
uptake of I^{131} (per cent of dose)
80
70
60
50
40
30
20
10
10 20 30 40
time in minutes
24 48 72
time in hours
SERUM
TI
PBI
BEI
per cent of dose per litre
0·50

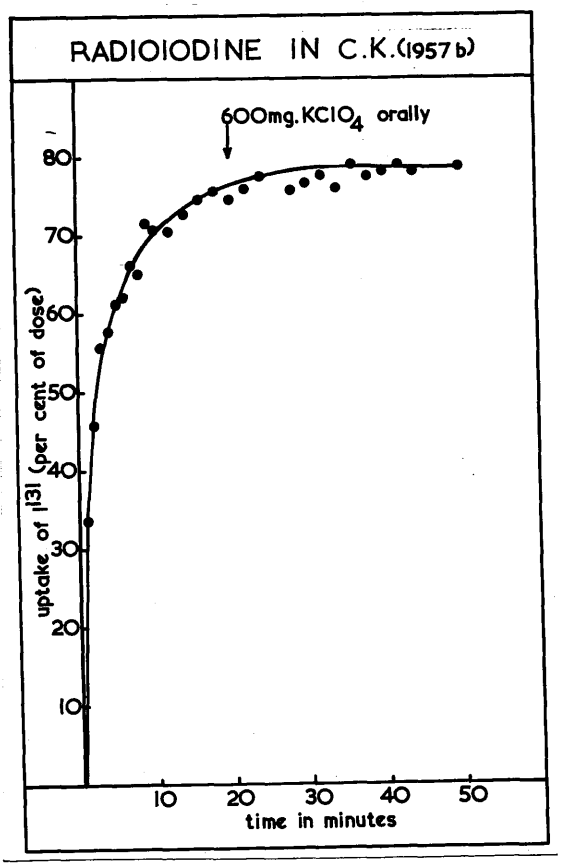
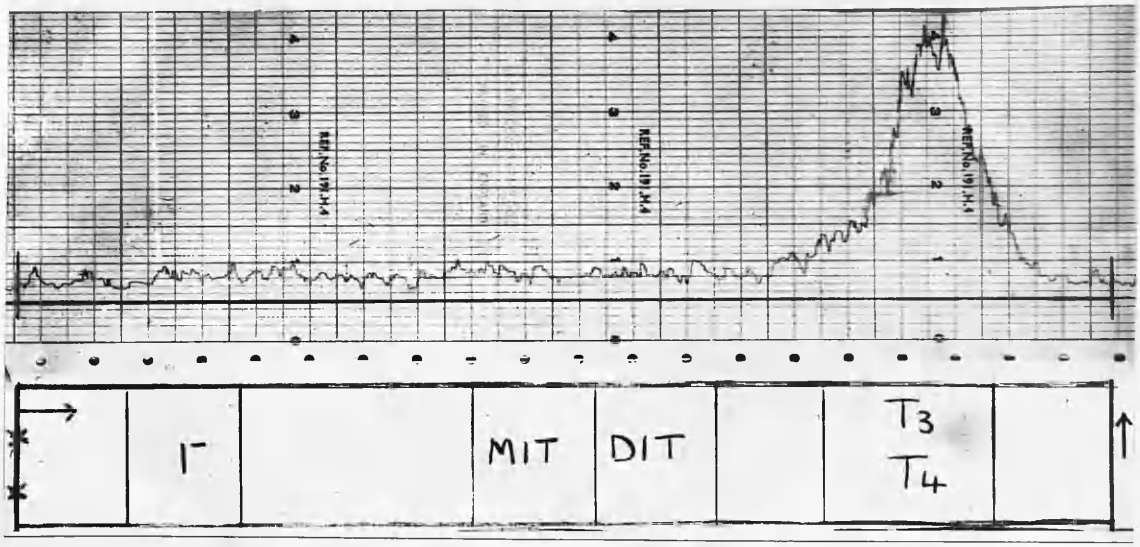

RADIOIODINE IN C.K.(1957b)
600mg. $KClO_4$ orally
uptake of I^{131} (per cent of dose)
80
70
60
50
40
30
20
10
10 20 30 40 50
time in minutes

Fig.83

C.K. (case VIII). Accumulation of ^{131}I by the thyroid gland and serum radioactivity after an intravenous dose of 25 μc ^{131}I. Figure illustrates remarkable avidity for ^{131}I of thyroid and shows marked discrepancy between serum protein-bound (PBI) and butanol-extractable (thyroxine-like) ^{131}I (BEI) values. The total butanol-extractable ^{131}I did not differ significantly from the latter value.

Fig.84

C.K. (case VIII). Accumulation of ^{131}I by the thyroid gland after an intravenous dose of 25 μc ^{131}I, and absence of effect of an oral dose of 600 mg. potassium perchlorate on ^{131}I content of thyroid.


REF.No.19.H.4
I⁻
MIT
DIT
T3
T4

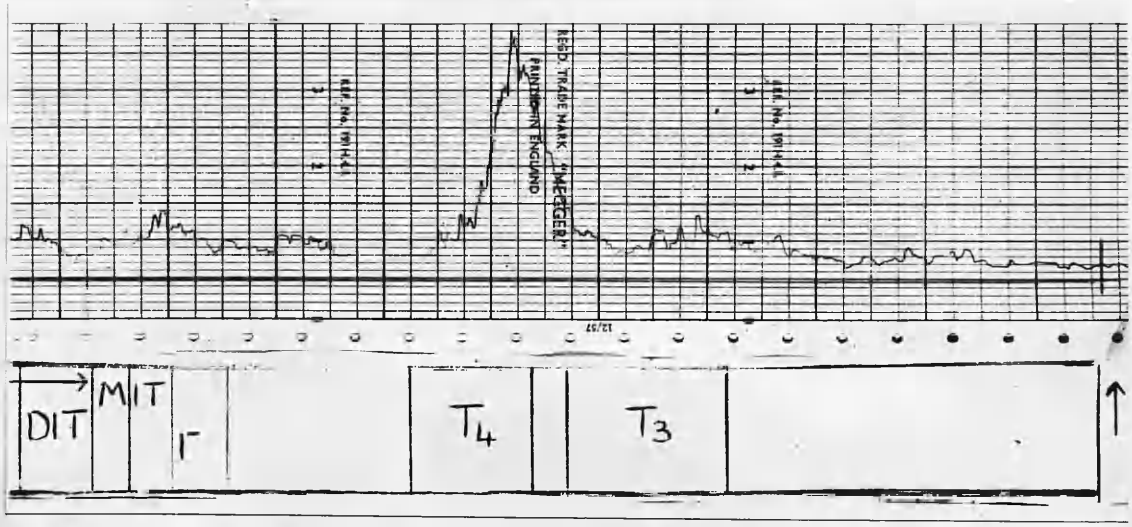
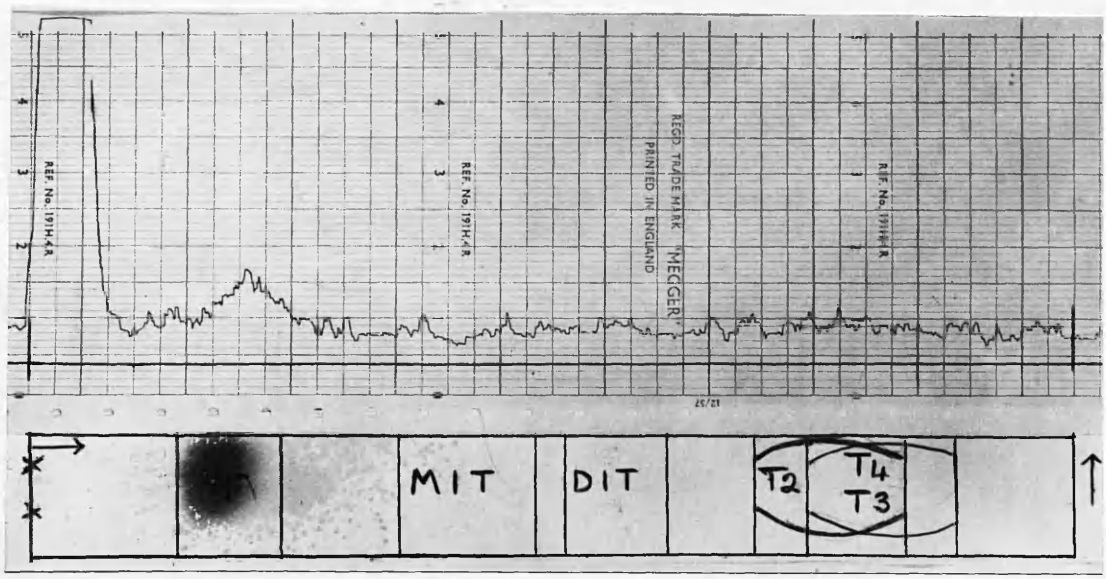

REF.No.19.H.4
REGD. TRADE MARK "MECCANO"
MADE IN ENGLAND
DIT
MIT
I⁻
T4
T3

Fig.85

C.K. (case VIII). Scan for radioactivity of ascending chromatogram prepared from butanol extract of serum withdrawn 48 hours after 1 mc ^{131}I. Solvent, butanol acetic acid. Radioactivity identified with thyroxine (T4) - triiodothyronine (T3) zone.

Fig.86

C.K. (case VIII). Similar study to that in fig.85, but solvent butanol ammonia. Radioactivity chiefly identified with thyroxine (T4); probably a trace of iodide (I^-) and triiodothyronine (T3) also present.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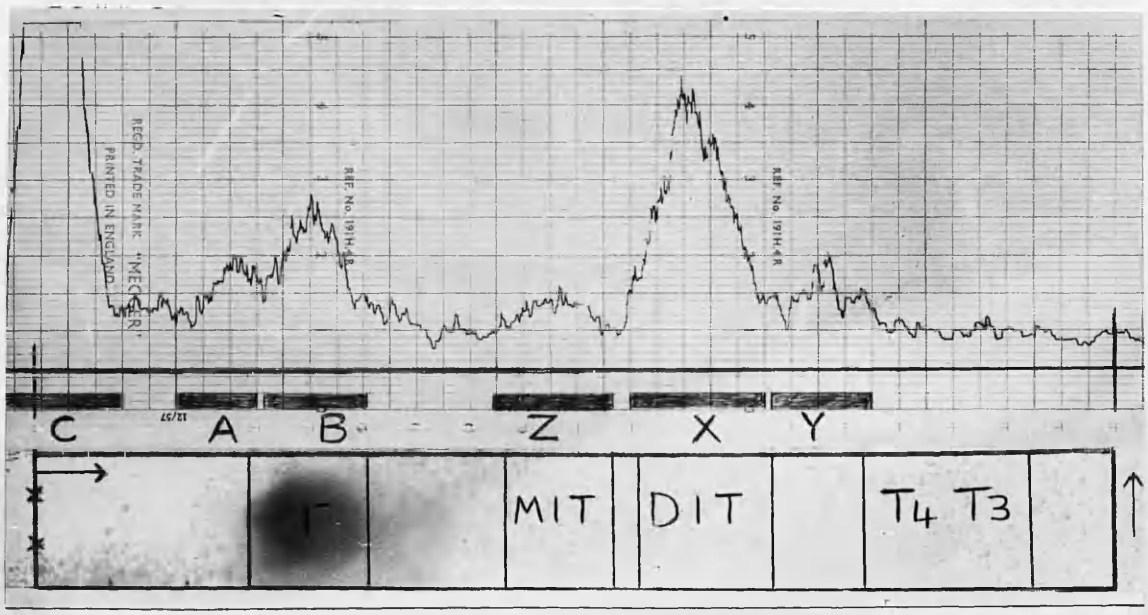
PRINTED IN ENGLAND
12/57
MIT
DIT
T2
T4
T3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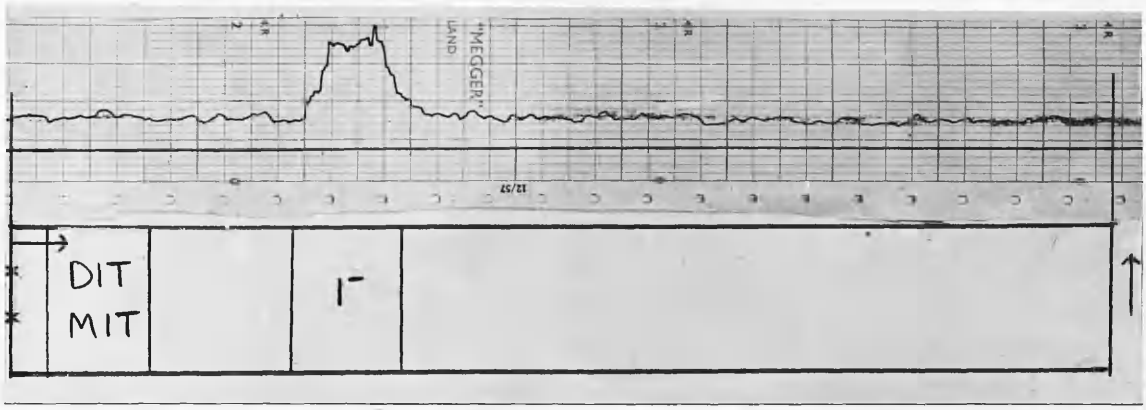
12/57
REF. No. 191H.4R.
REF. No. 191H.4R.
C
A
B
Z
X
Y
MIT
DIT
T4 T3

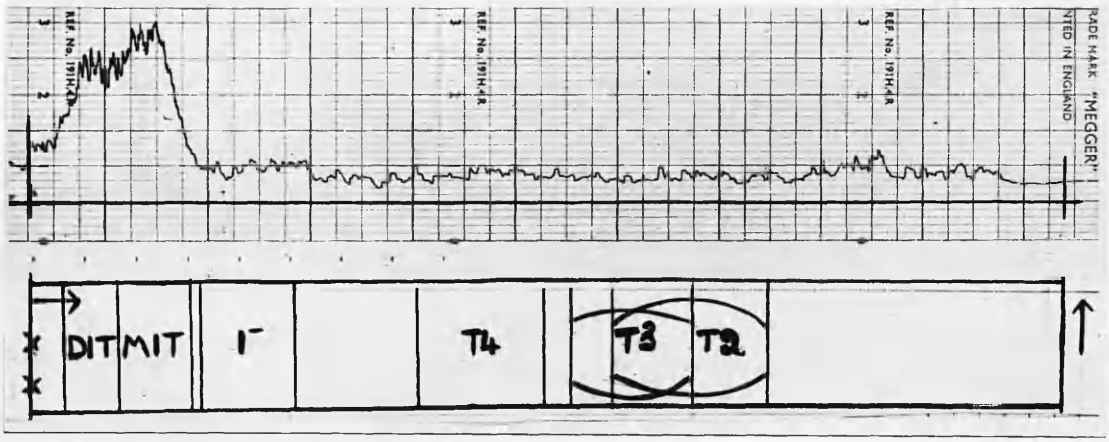
Fig.87

C.K. (case VIII). Scan for radioactivity of ascending chromatogram prepared from undigested thyroid gland homogenate. 1 mc ^{131}I given 48 hours before thyroidectomy. Solvent, butanol acetic acid. Radioactivity persists at origin, apart from a trace of iodide (I^-).

Fig.88

C.K. (case VIII). Scan for radioactivity of ascending chromatogram prepared from trypsin digest of thyroid gland removed 48 hours after 1 mc ^{131}I. Solvent, butanol acetic acid. Iodide (I^-, zone B), monoiodotyrosine (MIT, zone Z), and diiodotyrosine (DIT, zone X) are identified peaks of radioactivity. No thyroxine nor triiodothyronine is detected. Peaks C, A and Y are not identified.


DIT
MIT
1-


DIT
MIT
1-
T4
T3
T2

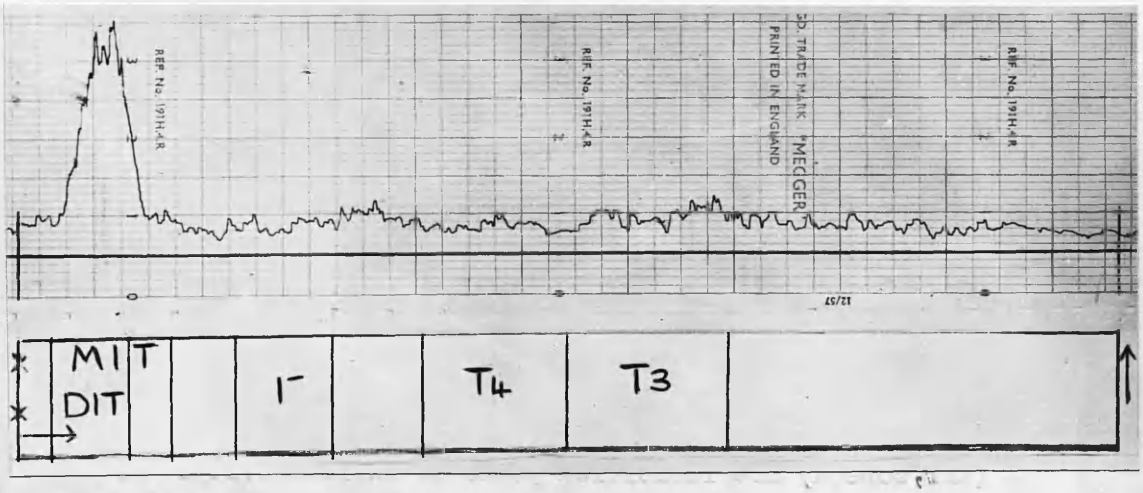

MIT
DIT
1-
T4
T3

Fig.89

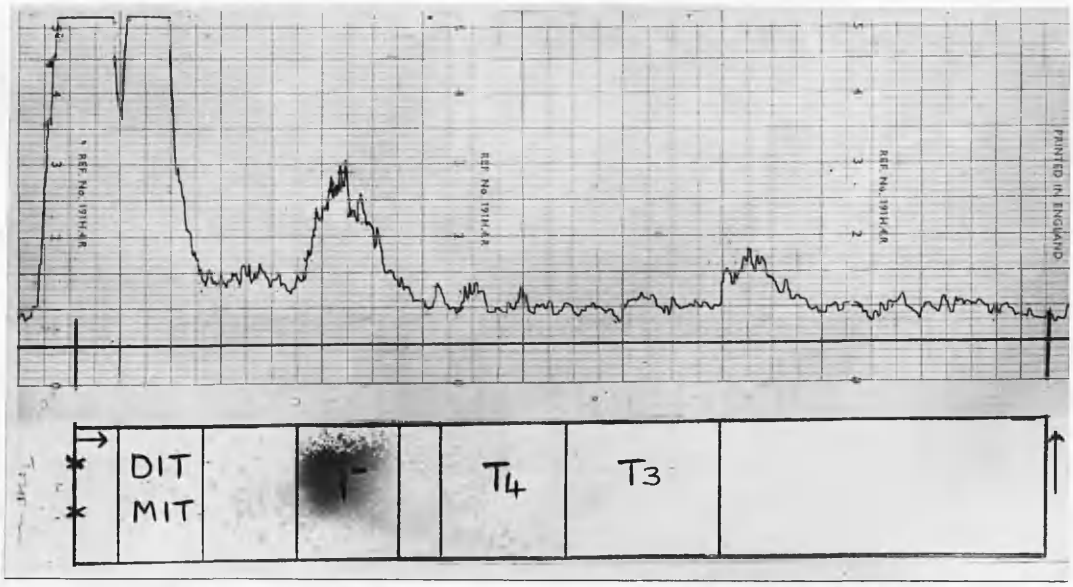
C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone B (fig.88) re-run in butanol ammonia solvent. Identity of iodide (I^-) is confirmed.

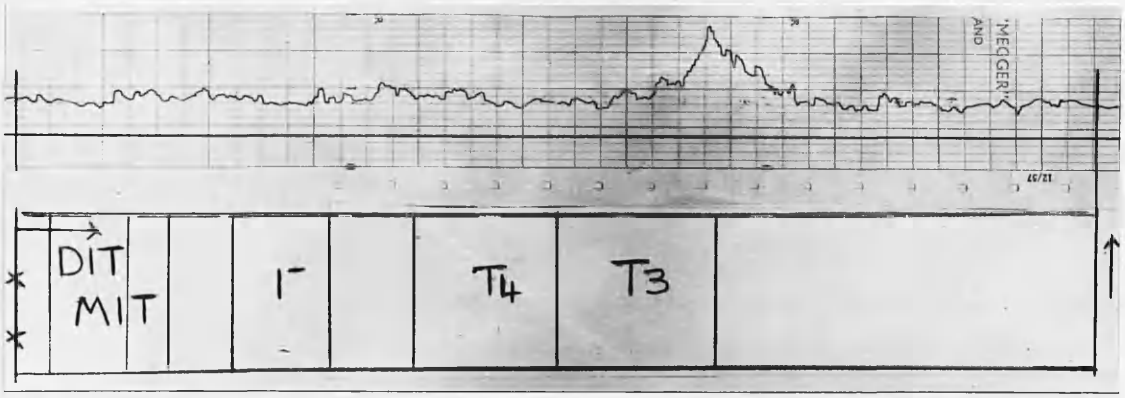
Fig.90

C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone Z (fig.88) re-run in butanol ammonia solvent. Radioactivity persists in MIT zone, confirming its chemical identity.

Fig.91

C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone X (fig.88) re-run in butanol ammonia solvent. Radioactivity persists in DIT zone, confirming its chemical identity.


DIT
MIT
I⁻
T4
T3


DIT
MIT
I⁻
T4
T3

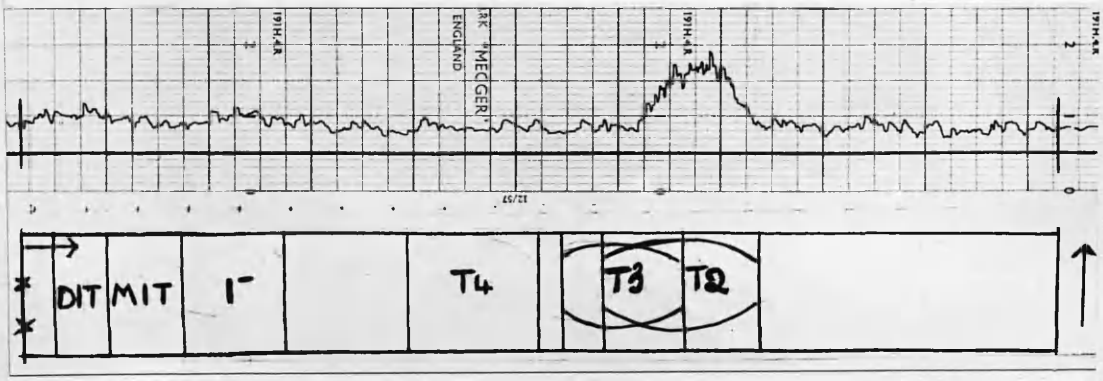

DIT
MIT
I⁻
T4
T3
T2

Fig.92

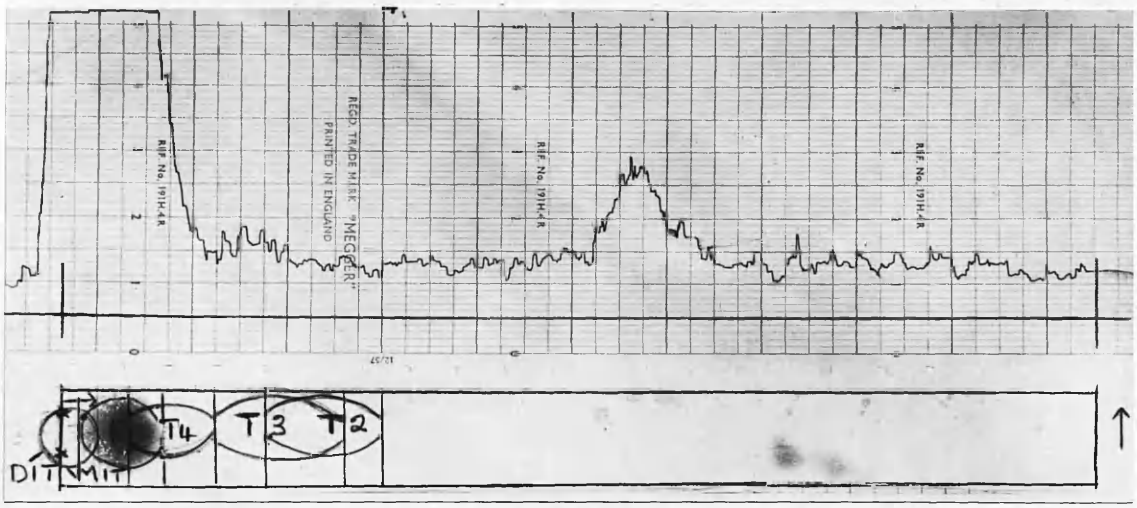
C.K.(case VIII). Scan for radioactivity of ascending chromatogram prepared from trypsin digest of thyroid gland removed 48 hours after 1 mc ^{131}I. Solvent, butanol ammonia. Peaks of radioactivity corresponding to the iodotyrosines (DIT and MIT) and iodide (I^-) are identified. There is a large unidentified peak of radioactivity at the origin, and a second unidentified peak at the end of and beyond the T3 marker.

Fig.93

C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone Y (fig.88) re-run in butanol ammonia solvent. Radioactivity is now sited at the end of and beyond the T3 marker suggesting a common identity with a similar peak in fig.92.

Fig.94

C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone Y (fig.88) which has been boiled in 2 N-NaOH for 1 hour and re-run in butanol ammonia solvent after acidification and butanol extraction. Radioactivity, as in fig.93, is at the end of and beyond the T3 marker; it overlies 3, 5-diiodothyronine (T2).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REGD. TRADE MARK "MEGGER"
PRINTED IN ENGLAND
REF. No. 191H-4R
REF. No. 191H-4R
T4
T3
T2
DIT
MIT

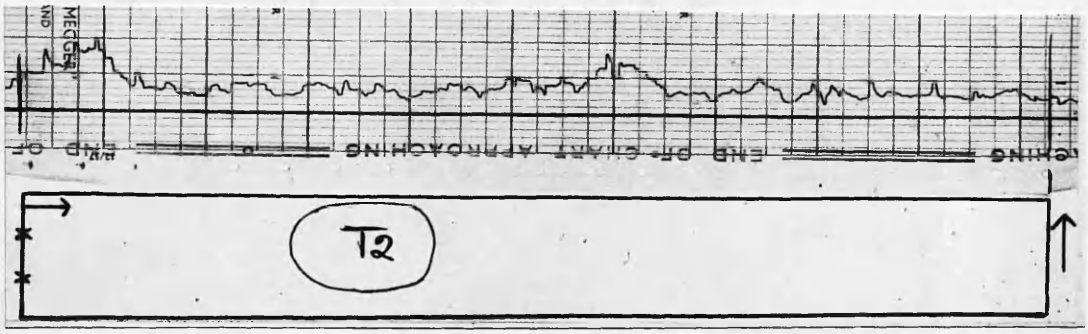

MEGGER
T2

Fig.95

C.K.(case VIII). Scan for radioactivity of ascending chromatogram prepared from trypsin digest of thyroid gland removed 48 hours after 1 mc ^{131}I. Solvent, amyl alcohol and ammonia. There is a small unidentified peak of radioactivity beyond the markers, which include 3,5-diiodothyronine.

Fig.96

C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone Y (fig.88) which has been re-run in amyl alcohol and ammonia solvent. Radioactivity, as in fig.95, is well beyond the T2 marker.
(The radioactivity at the origin is considered to be diiodotyrosine which has been inadvertently washed out with Y).

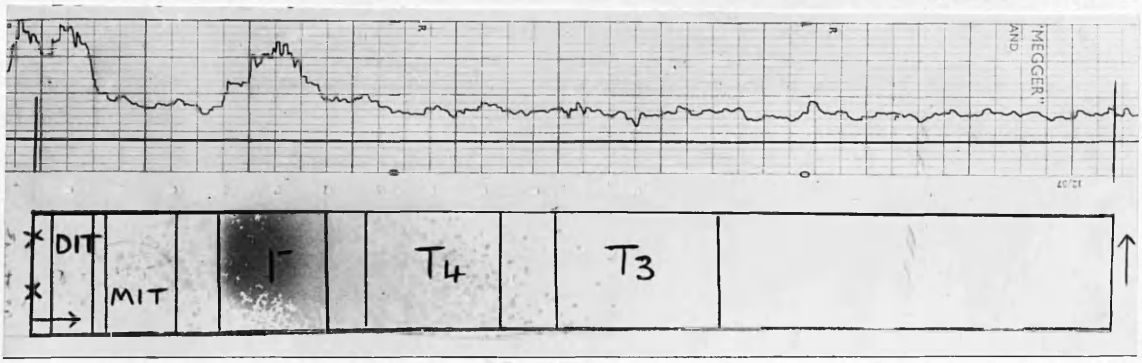
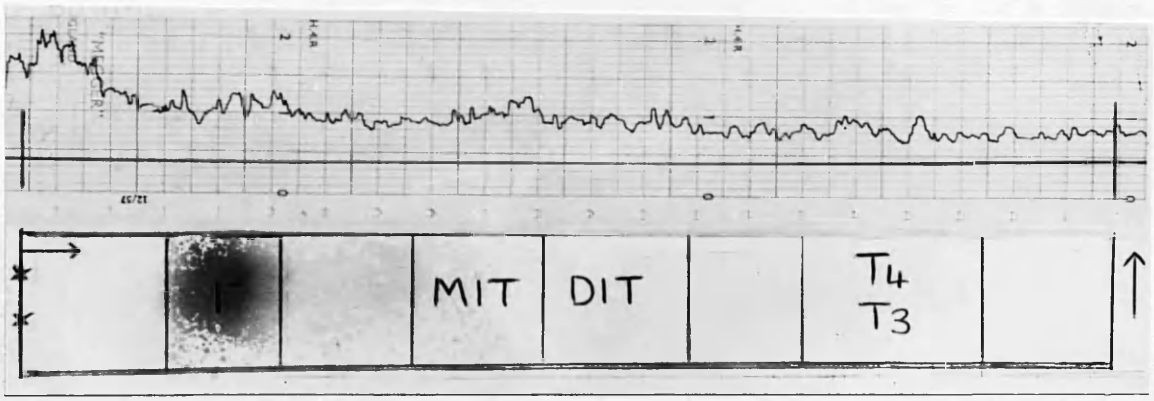

"MEGGER"
AND
DIT
MIT
I⁻
T4
T3

Fig.97

C.K. (case VIII). Scan of ascending chromatogram prepared from eluate of zone A (fig.88) which has been re-run in butanol ammonia solvent. Radioactivity is now divided between the iodide (I^-) zone and an unidentified peak at the origin of the chromatogram.


MIT
DIT
T4
T3

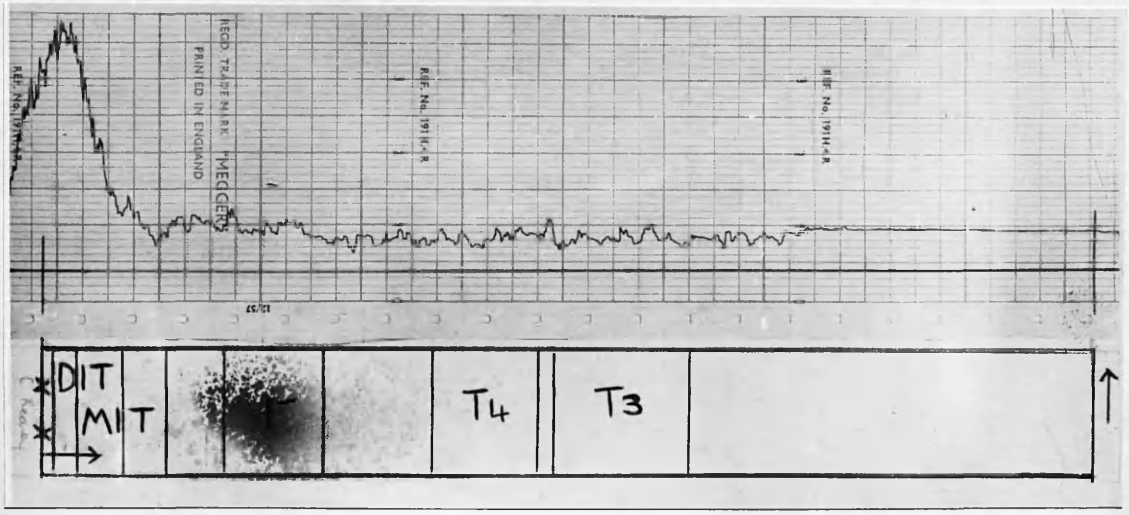
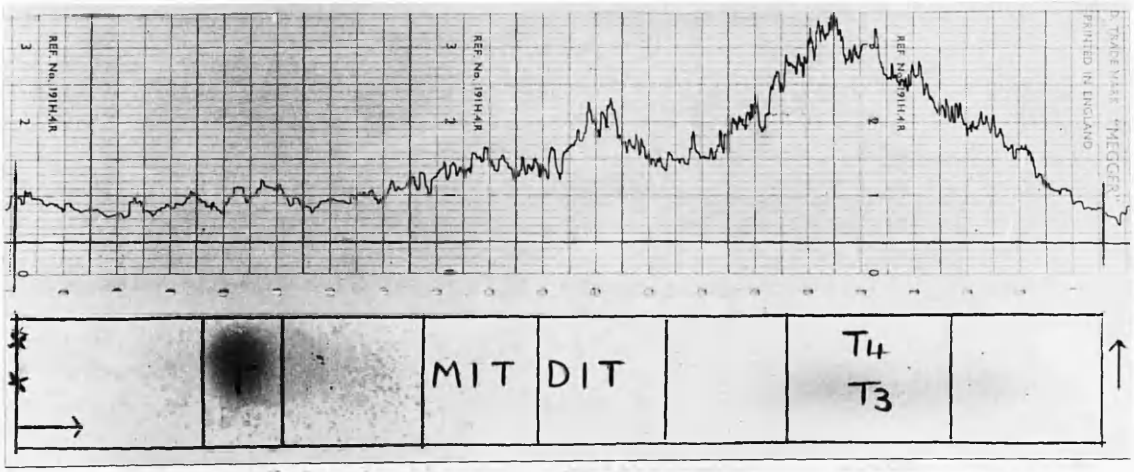

DIT
MIT
T4
T3

Fig.98

C.K. (case VIII). Scan for radioactivity of ascending chromatogram prepared from barbitone buffer in which zone C (fig.88) has been redigested by trypsin. Solvent, butanol acetic acid. Radioactivity persists at origin.

Fig.99

C.K. (case VIII). Similar study to that in fig.98 but solvent butanol ammonia. Radioactivity persists at origin.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MIT
DIT
T4
T3

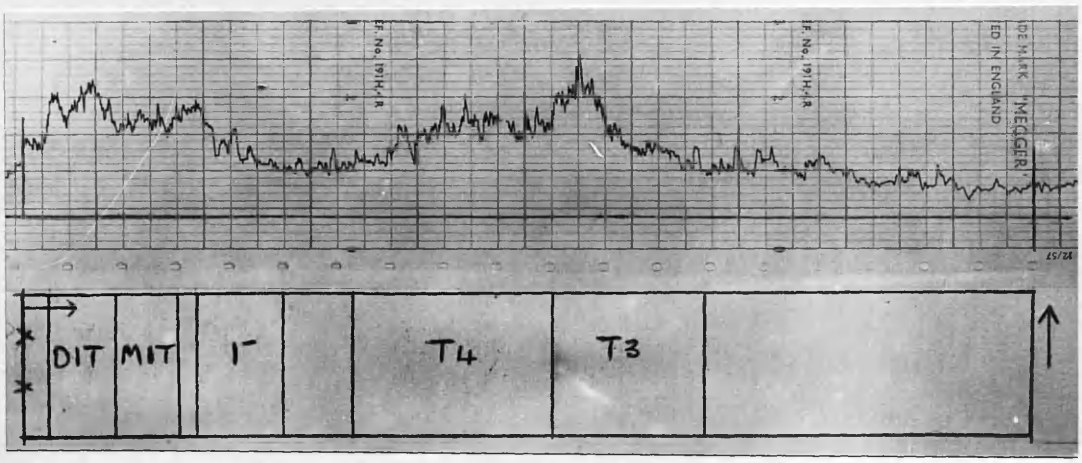
REF. No. 191H-4R
DIT
MIT
I⁻
T4
T3

Fig.100

C.K. (case VIII). Scan for radioactivity of ascending chromatogram prepared from zone C (fig.88), which has been boiled in 2N. NaOH for 1 hour and re-run in butanol acetic acid after acidification and butanol extraction. Radioactivity is now present chiefly in thyroxine (T4) - triiodothyronine (T3) zone but monoiodotyrosine (MIT) and diiodotyrosine (DIT) are also present.

Fig.101

C.K. (case VIII). Similar study to that in fig.100 but butanol ammonia solvent. Result technically poor. There may be some triiodothyronine (T3) as well as thyroxine (T4) and the iodotyrosines (DIT, MIT).

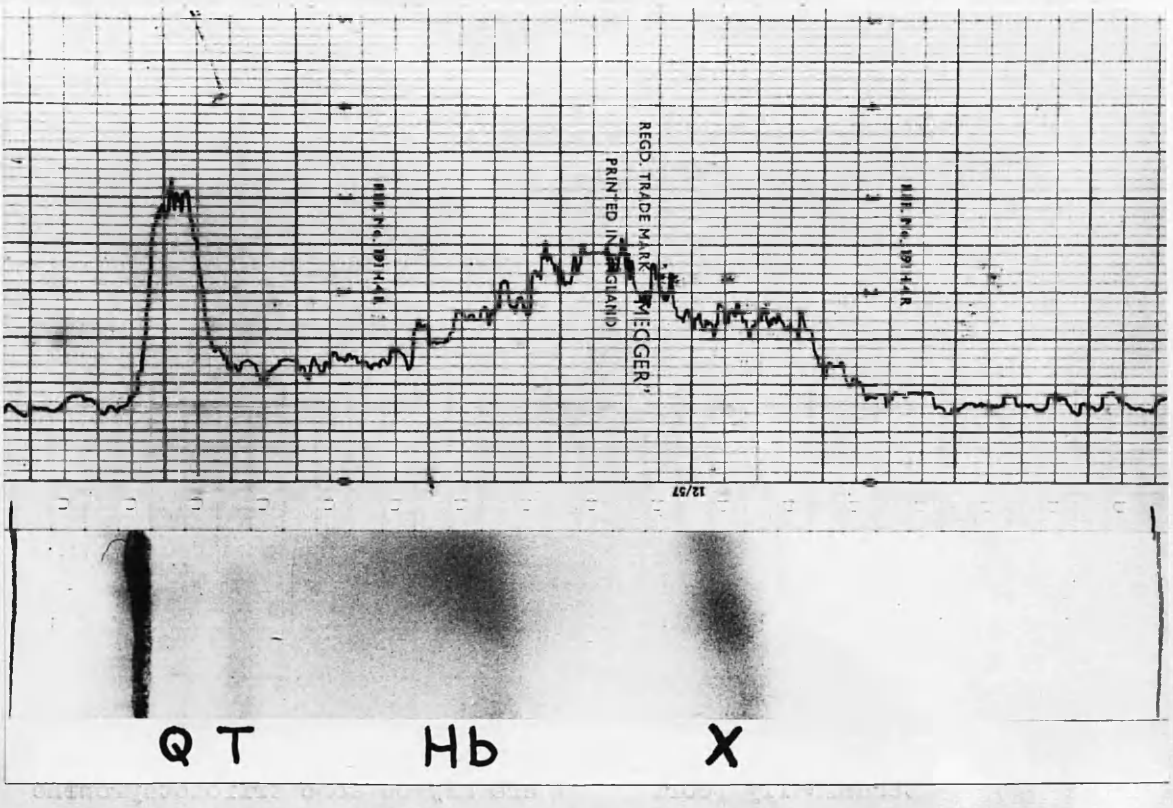
REGD. TRADE MARK "MECGER"
PRINTED IN ENGLAND
12/57
Q T
Hb
X

Fig.102

C.K. (case VIII). Scan for radioactivity of electrophoretogram prepared from thyroid tissue removed 48 hours after 1 mc ^{131}I. Proteins stained with aqueous bromphenol blue. 4 protein bands identified, designated in order from origin, Q (unknown), T (gamma globulin), Hb (haemoglobin) and X (albumin). Most of the radioactivity is present in a broad band in the zones normally occupied by thyroglobulin and albumin. A peak of radioactivity is apparently related to the Q band.

Fig.103

C.K. (case VIII). Scan for radioactivity of ascending chromatogram prepared from Krebs-Ringer phosphate buffer in which fresh slices of thyroid tissue have been incubated with $M^{131}IT$ and nicotinamide. Over 90 per cent of the radioactivity is in the iodide (I^-) spot, indicating normal dehalogenase activity.

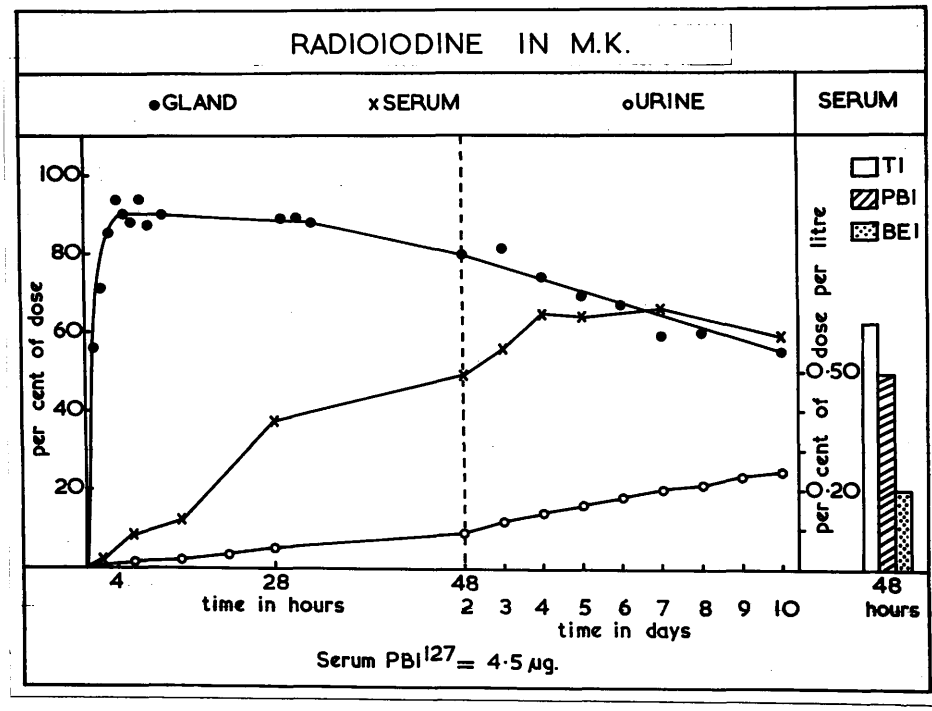
RADIOIODINE IN M.K.
●GLAND
xSERUM
oURINE
SERUM
TI
PBI
BEI
per cent of dose
100
80
60
40
20
per cent of dose per litre
0·50
0·20
4
28
48
time in hours
2 3 4 5 6 7 8 9 10
time in days
48
hours
Serum PBI^{127} = 4·5 µg.

Fig.104

M.K. (case IX). Accumulation of ^{131}I by the thyroid, urinary excretion of ^{131}I and serum radioactivity after an oral dose of 50 μc ^{131}I.

TI = total serum ^{131}I.

PBI = protein-bound serum ^{131}I.

BEI = butanol-extractable (thyroxine-like) ^{131}I.

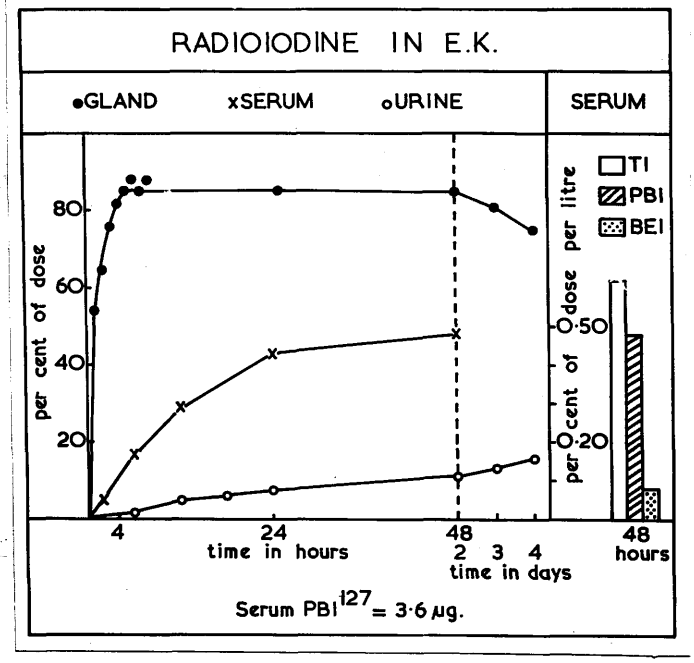
RADIOIODINE IN E.K.
•GLAND
xSERUM
oURINE
SERUM
per cent of dose
80
60
40
20
4
24
48
time in hours
2 3 4
time in days
TI
PBI
BEI
per cent of dose per litre
0·50
0·20
48
hours
Serum PBI^{127} = 3·6 µg.

Fig.105

E.K. (case X). Accumulation of ^{131}I by the thyroid gland, urinary excretion of ^{131}I and serum radioactivity after an oral dose of 50 μc ^{131}I.

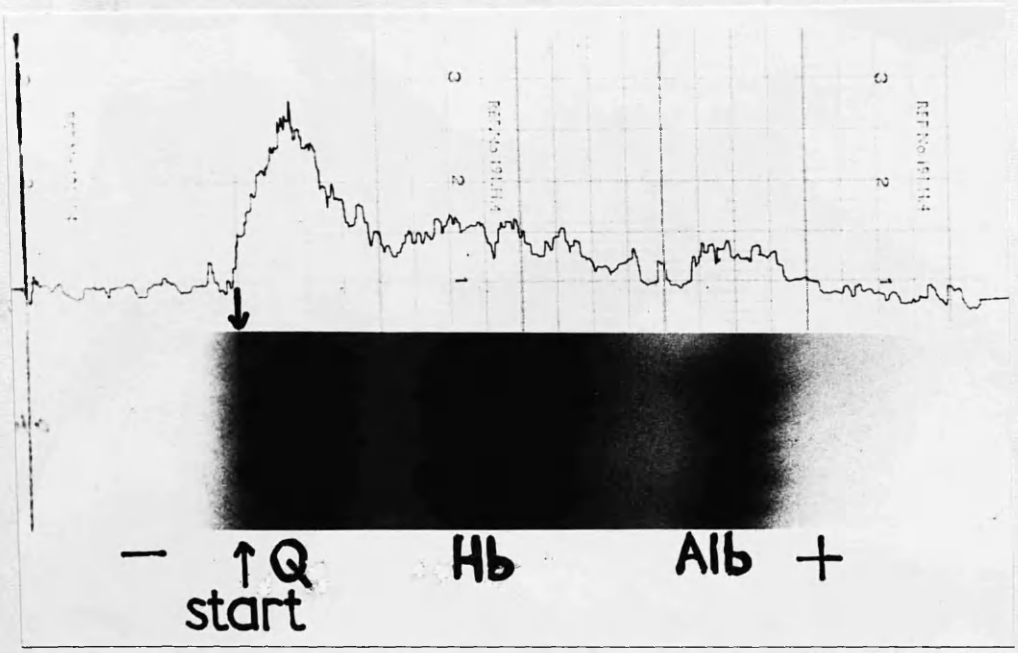
—
↑Q
start
Hb
Alb
+

Fig.106

Scan for radioactivity of electrophoretogram prepared from thyroid gland removed from a case of Hashimoto's thyroiditis 48 hours after 100 μc ^{131}I. Proteins stained with aqueous bromphenol blue. The technical result appears unsatisfactory because of smearing. Q protein, haemoglobin (Hb) and albumin (Alb) are present. There is a well-marked peak of radioactivity close to the origin.

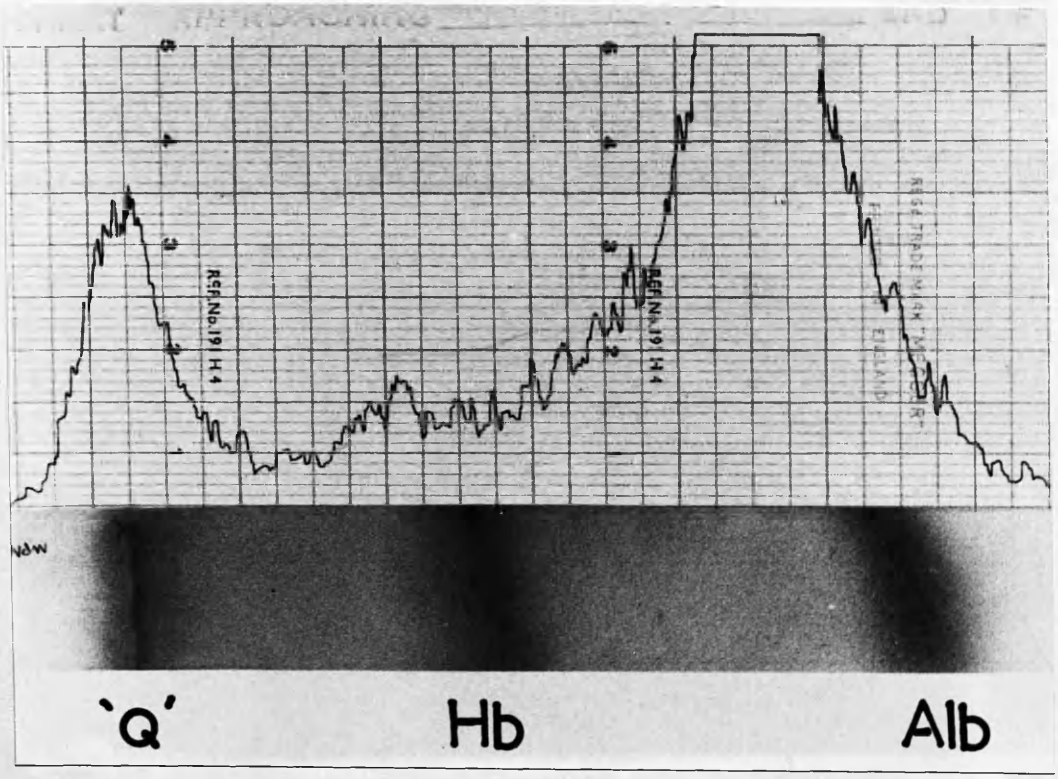
Ref.No.19 H 4
Ref.No.19 H 4
'Q'
Hb
Alb

Fig.107

Similar study to that in fig.106 but with thyroid tissue from a case of thyrotoxicosis prepared for operation with methyl thiouracil. Q protein, haemoglobin (Hb) and albumin (Alb.) are identified. There is virtually no stainable thyroglobulin, but most of the radioactivity is in the thyroglobulin zone, and there is also a peak apparently related to Q protein.

Q
T
Hb
Thy-g
X or Alb.
5
4
3
2
1
REF.No.191.H.4
REF.No.191.H.4

Fig.108

Similar study to that in figs. 106 and 107 but with material from thyrotoxic patient who died 5 days after 10 mc ^{131}I. Q, haemoglobin (Hb), thyroglobulin (Thy-g) and albumin (X or Alb.) are easily identified. There is a faint T band, which has not photographed well. The radioactivity is in the thyroglobulin zone.

TABLES

TABLE I

Sporadic goitrous cretinism

Summary of 16 cases, showing sex incidence, age at time of initial investigation, with cross reference to position in family tree.

Group No.	Case No.	Family tree reference	Name	Sex	Age in years
I	1	V.10	M.McP	F	8 5/12
	2	V.8	I.McP	F	11 7/12
	3	V.9	A.McP	M	10 6/12
	4	V.7	A.McP	M	15 7/12
	5	V.12	D.T.	F	7 7/12
	6	IV.13	M.T.	F	20
	7	IV.15	J.T.	M	16
	8	IV.10	M.T.	F	12 6/12
	9	V.13	A.T.	M	2 5/12
	10	IV.12	R.T.	F	24
	11	V.6	A.T.	M	5 4/12
	12	V.14	M.T.	F	3 3/12
II	13	-	J.N.	F	6 6/12
	14	-	M.A.W.	F	3 6/12
III	15	-	A.D.	M	6 9/12
	16	-	A.D.	F	5 5/12

TABLE II

Sporadic goitrous cretinism.

Accumulation of ^{131}I by the thyroid gland

Case No.	Year	Percentage of dose in thyroid at hours after administration										
		1	2	3	4	5	6	7	8	24	48	72
1	1951	74	-	74	74	-	-	-	-	31	-	-
	1955	48	67	67	66	67	62	55	51	24	18	8
2	1951	64	72	-	72	-	-	-	-	-	-	-
	1955	10	24	22	23	23	25	-	-	23	25	-
3	1951	52	58	-	64	-	65	-	-	63	49	41
	1955	33	60	61	72	-	72	-	-	72	51	-
4	1951	21	57	65	-	-	-	-	-	20	11	7
5	1952	-	-	-	-	-	-	-	-	40	-	-
6	1952	-	-	-	-	-	-	-	-	36	-	-
7	1952	20	-	-	33	-	-	-	-	29	-	-
8	1952	12	10	17	22	23	20	-	-	21	-	-
9	1955	32	-	-	50	-	-	-	-	24	-	-
10	1956	-	-	-	75	-	-	-	-	75	-	56
11	1957	-	-	-	-	-	-	-	-	-	-	-
12	1957	-	-	-	70	-	68	-	-	41	25	-
13	1952	33	54	58	60	62	62	-	-	31	-	-
14	1952	30	42	46	51	61	57	-	-	25	-	-
15	1954	34	53	52	52	-	-	-	-	25	-	-
16	1954	19	28	28	30	-	-	-	-	18		

TABLE III

Sporadic goitrous cretinism

Effect of potassium thiocyanate on the ^{131}I content of thyroid gland

Case No.	Potassium thiocyanate		
	Time (hrs.)	Amount (g.)	Intermediate effect
1	4	2	Nil
2	4	2	Nil
3	24	2	Nil
4	48	2	Nil

TABLE IV

Sporadic goitrous cretinism

Urinary excretion of ^{131}I

Case No.	Year	Percentage of ^{131}I excreted in various periods (hours)								
		0-6	6-12	12-18	18-24	24-48	0-24	0-48	6-24	T factor
1	1951	-	-	-	-	-	-	-	-	-
	1955	17.8	15.1	25.5	10.0	20.0	68.4	88.4	50.6	0.4
2	1951	-	-	-	-	-	-	-	-	-
	1955	28.6	-	-	-	6.8	58.7	65.5	30.1	1.5
3	1951	13.0	-	-	-	14.0	27.0	41.0	14.0	2.3
	1955	12.6	-	-	-	10.2	31.0	41.2	18.4	1.7
4	1951	9.3	-	-	-	27.0	59.6	86.6	50.3	0.2
5	1952	5.5		25.7	14.8	19.8	46.0	65.8	40.5	0.2
6	1952	8.5	10.0	5.8	11.3	23.0	35.6	58.6	27.1	0.5
7	1952	11.9	8.9	12.3	17.2	17.6	50.3	67.9	38.4	0.5
8	1952	3.3		13.3	7.9	9.8	24.5	34.3	21.2	0.5
9	1955	-	-	-	-	-	-	-	-	-
10	1956	4.8	-	-	-	6.7	24.0	30.7	19.2	0.8
11	1957	-	-	-	-	-	-	-	-	-
12	1957	10.4	13.7	10.9	10.9	21.0	45.9	66.9	35.5	0.4
13	1952	-	-	-	-	-	-	-	-	-
14	1952	-	-	-	-	-	-	-	-	-
15	1954	14.0	10.4	20.6	9.6	13.7	54.6	68.3	40.6	0.5
16	1954	11.6	7.8	26.7	14.4	17.8	60.5	78.3	48.9	0.3

TABLE V

Sporadic goitrous cretinism.

Percentage of ^{131}I present in plasma or serum at 48 hours, as total iodine ($T^{131}I$), protein-bound iodine ($PB^{131}I$), and butanol extractable (thyroxine-like) iodine ($BE^{131}I$). Results expressed as per cent of dose.

Case No.	Year	$T^{131}I$	$PB^{131}I$	$\frac{PB^{131}I \times 100}{T^{131}I}$	$BE^{131}I$
1	1951	-	-	-	-
	1955	0.29	0.08	27%	< 0.05
2	1951	-	-	-	-
	1955	0.26	0.08	31%	< 0.05
3	1951	-	-	-	-
	1955	0.90	0.83	92%	0.19
4	1951	0.95	0.50	52%	-
5	1952	1.02	0.49	48%	-
6	1952	0.45	0.10	21%	-
7	1952	1.78	1.12	62%	-
8	1952	0.32	0.08	26%	0.09
9	1955	2.22	1.45	65%	0.48
10	1955	0.25	0.12	48%	-
11	1957	-	-	-	-
12	1957	1.39	0.43	31%	-
13	1952	1.35	0.49	36%	0.36
14	1952	1.32	0.40	30%	0.25
15	1954	1.00	0.61	61%	0.40
16	1954	0.92	0.28	30%	-

TABLE VI

Sporadic goitrous cretinism.

Comparison of the relative proportions of the various iodine fractions in the serum of a tinker patient and a patient with congenital goitre who had an abnormal iodinated compound in her serum.

Fraction of serum	Tinker patient. Case 2 1958.	Patient with congenital goitre. Case VIII, Chapter 19
Total serum ^{131}I ($T^{131}I$)	100%	100%
Protein-bound ^{131}I ($PB^{131}I$)	89%	100%
Total butanol-extractable ^{131}I ($TBE^{131}I$)	86%	43%
Thyroxine-like ^{131}I ($BE^{131}I$)	57%	37%

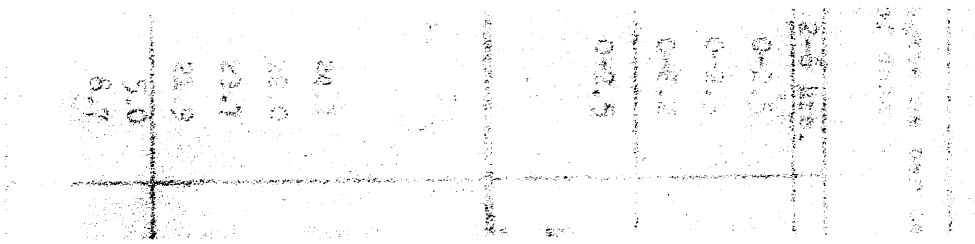

TABLE VII

Sporadic goitrous cretinism

Urinary excretion of radioactive (^{131}I) monoiodotyrosine after an oral dose of ^{131}I-labelled L-monoiodotyrosine

Control cases		MIT + MIT conjugate as per cent of ^{131}I in urine		MIT + MIT conjugate as per cent of dose		
Diagnosis	No. of cases	0-2 hrs.	2-6 hrs.	0-2 hrs.	2-6 hrs.	0-6 hrs
'Normal'	5	0-17.6	0-10.2	0-3.3	0-2.5	0-5.7
Primary myxoedema	3	0- 9.5	0-10.2	0-1.1	0-2.3	0-3.4
Non-toxic goitre	5	0-14.1	0- 7.7	0-1.1	0-2.2	0-3.3
	13	0-17.6	0-10.2	0-3.3	0-2.5	0-5.7 Mean $\pm 2\sigma$ $=1.2 \pm 4.0$

Tinker cases							
Case No.	Patient	Sex	Age Years				
12	M.T.	F	$3\frac{3}{12}$	75.0	100.0	6.2	24.7	30.9
10	R.T.	F	26	100.0	98.2	19.6	22.0	41.6
15	A.D.	M	10	23.8	67.4	5.1	23.7	28.8
16	A.D.	F	9	100.0	64.6	14.4	21.9	36.3
17	A.T.	M	19	14.2	37.0	1.7	5.0	6.7
18	W.T.	M	28	13.9	34.8	1.0	8.7	9.7
19	J.McP.	F	$10\frac{10}{52}$	31.7	24.7	-	-	-
20	A.B.	F	$17\frac{17}{52}$	42.8	13.3	-	-	-

TABLE VIII

Chromatographic analysis of ^{131}I compounds in rat thyroids removed at varying times after an intraperitoneal injection of ^{131}I.

Rat No.	Time killed after intra-peritoneal injection of ^{131}I	Uptake of ^{131}I in thyroid		Zonal distribution of ^{131}I present in chromatogram (per cent)						
		Per cent of dose	Per cent of dose per 10 mg.	Origin	I^-	MIT	DIT	Y	T4	MIT/DIT
1	4 hrs	6.7	3.0	3.0	12.0	37.4	41.3	3.7	2.6	0.91
2	4 hrs	5.0	2.0	8.1	3.1	40.0	43.2	3.1	2.5	0.93
3	4 hrs	5.7	4.7	6.1	3.2	37.7	47.2	-	5.8	0.80
4	4 hrs	5.9	3.9	6.7	2.8	39.9	43.9	-	6.7	0.91
5	24 hrs	10.9	5.6	9.4	2.5	27.7	53.5	-	6.7	0.52
6	24 hrs	10.8	4.6	5.3	1.8	31.9	55.7	-	5.3	0.58
7	48 hrs	8.8	4.5	8.8	7.2	27.3	49.4	2.1	5.2	0.55
8	48 hrs	11.3	5.3	12.1	9.8	21.9	48.8	-	7.4	0.45
9	48 hrs	11.4	10.4	11.5	7.4	19.0	52.1	-	10.0	0.36
10	48 hrs	-	-	11.1	6.8	22.3	53.0	-	6.8	0.42

Key to abbreviations I^- Iodide
MIT Monoiodotyrosine
DIT Diiodotyrosine
T4 Thyroxine
Y Unknown between DIT and T4

TABLE IX

Sporadic goitre

Summary of clinical data.

Group No.	Case No.	Name	Sex	Age in years when: Patient attended hospital	Age in years when: Goitre was noticed	Age in years when: Patient had thyroidectomy	Clinical status	Other clinical features of note	Family history of goitre
I	I	M.McG	F	15	10	15	Euthyroid	Congenital deaf-mutism.	Sister of case II
	II	B.McG	F	19	15	19	Euthyroid	Congenital deaf-mutism.	Sister of case I
	III	P.S.	M	(i) 3/12 elsewhere (ii) 10	earlier than 3/12	3/12 -	Euthyroid	Congenital deaf-mutism. Sensory aphasic disturbance.	Nil
	IV	A.A.	F	18	birth	18	Euthyroid	Congenital deaf-mutism.	Sister of case V; Also paternal great-aunt.
	V	J.A.	M	19	19	-	Euthyroid	Congenital deaf-mutism.	Brother of case IV; Also paternal great-aunt.
II	VI	S.McC	F	9	7	11	Euthyroid	Nil	Maternal aunt.

TABLE IX Ctd.

Sporadic goitre

Summary of clinical data.

Group No.	Case No.	Name	Sex	Age in years when			Clinical status	Other clinical features of note	Family history of goitre
				Patient attended hospital	Goitre was noticed	Patient had thyroidectomy			
III	VII	S.S.	M	10	birth	10	Euthyroid	Nil	Nil
IV	VIII	C.K.	F	(i) 6 elsewhere (ii) 13	birth	19	Euthyroid	Nil	Sister of IX, X
	IX	M.K.	F	(i) 9 elsewhere (ii) 18	1	18	Euthyroid, but hypothyroid in childhood	Nil	Sister of VIII, X.
	X	E.K.	F	(i) 11 elsewhere (ii) 21	7	21	Hypothyroid	Nil	Sister of VIII, IX.

Also deceased brother

TABLE X

Sporadic goitre

Uptake of ^{131}I by the thyroid gland.

Group No.	Case No.	Year	I.V. clearance ml/min/1.73m^2	Percentage of dose in thyroid at hours after administration										Remarks
				½	1	2	3	4	6	24	48	96	240	
I	I	1957	637	48	-	-	-	-	-	56	-	-	-	Pre-op.
		1958	188	36	-	-	-	-	-	44	-	-	-	Post-op.
	II	1958	360	47	-	-	-	-	-	91	-	-	-	-
	III	1958 (1)	471	38	-	-	-	-	-	-	-	-	-	-
		(2)	-	-	-	-	-	-	-	43	-	-	-	-
	IV	1958	Nil	Nil	-	-	-	-	-	-	-	-	-	Iodide block
	V	1958	645	67	-	-	-	-	-	-	-	-	-	-

TABLE X Ctd.

Sporadic goitre

Uptake of ^{131}I by the thyroid gland.

Group No.	Case No.	Year	I.V. clearance ml/min/1.73m^2	Percentage of dose in thyroid at hours after administration										Remarks
				½	1	2	3	4	6	24	48	96	240	
II	VI	1957	433	74	-	-	-	-	-	35	-	-	-	-
III	VII	1957(1)	-	-	-	-	-	-	-	67	61	-	-	-
		(2)	676	65	-	-	-	-	-	-	-	-	-	-
IV	VIII	1957	1170	80	-	-	-	86	-	77	66	-	-	-
	IX	1955	-	-	56	71	85	90	88	89	80	74	55	-
	X	1955	-	-	54	65	76	82	88	85	85	73	-	-

TABLE XI

Sporadic Goitre

Urinary Excretion of ^{131}I

Group No.	Case No.	Year	Percentage of dose of ^{131}I excreted in various periods (hrs.)								
			0-6	6-12	12-18	18-24	24-48	0-24	0-48	6-24	T Factor
I	I	1957	18.5	-	-	-	4.2	30.8	35.0	12.3	4.3
III	VII	1957	7.9	-	-	-	7.2	13.4	20.6	5.5	6.9
IV	IX	1955	0.7	1.7	1.1	1.6	3.7	5.1	8.8	4.4	1.8
	X	1955	0.7	3.8	0.8	1.4	4.2	6.7	10.9	6.0	1.1

TABLE XII

Sporadic goitre

Percentage of ^{131}I present in serum at 48 hours as total iodine ($T^{131}I$) and protein-bound iodine ($PB^{131}I$).

Results expressed as per cent of dose per litre.

Group No.	Case No.	Year	$T^{131}I$	$PB^{131}I$	$\frac{PB^{131}I \times 100}{T^{131}I}$
I	I	1957	0.31	0.22	71%
		1958	0.97	0.66	68%
	II	1958	0.20	0.20	100%
	III	1958	0.49	0.42	86%
	IV	1958	-	-	-
	V	1958	-	-	-
II	VI	1957	0.93	0.71	76%
III	VII	1957	1.26	1.01	80%
IV	VIII	1957	0.54	0.55	100%
	IX	1955	0.62	0.49	79%
	X	1955	0.62	0.48	77%

TABLE XIII

Sporadic goitre

Effect of anion block on ^{131}I content of thyroid gland.

Group No.	Case No.	Drug used	Dose given	Time after ^{131}I (mins)	Corrected* thyroid ^{131}I content		Result
					Before	After	
I	I	KCNS	2g.	30	48%	14%	Positive
	II	KClO4	800 mg.	30	47%	21%	Positive
	III	KClO4	600 mg.	38	38%	21%	Positive
	V	KClO4	600 mg.	30	67%	20%	Positive
II	VI	KClO4	600 mg.	30	74%	74%	Negative
III	VII	KCNS	2g.	40	65%	65%	Negative
IV	VIII	KClO4	600 mg.	20	80%	80%	Negative

* Corrected = the uptake observed over the thyroid less the contribution due to general body radioactivity.

TABLE XIV

Sporadic goitre

Urinary excretion of radioactive (^{131}I) monoiodotyrosine after an oral dose of ^{131}I labelled L-monoiodotyrosine.

Group No.	Case No.	MIT + MIT conjugate as per cent of ^{131}I in urine. 0-2 hrs	2-6 hrs	MIT + MIT conjugate as per cent of dose 0-2 hrs	2-6 hrs	0-6 hrs	Remarks
II	VI	97.3	96.1	8.0	46.3	54.3	11 days post-op.on sod. thyroxine.
	Mother of VI	43.4	1.0	14.0	0.3	14.3	non-goitrous euthyroid.
III	VII	14.1	7.7	1.1	2.2	3.3	7/12 post-op. on thyroid.
IV	VIII	16.5	2.0	1.2	0.6	1.8	10/12 post-op. on sod.thyroxine.
	X	18.2	0	3.8	0	3.8	$2\frac{1}{2}$ yrs.post-op on sod.thyroxine.

TABLE XV

Sporadic goitre

Chromatographic analysis of ^{131}I compounds in thyroids removed 24-48 hours after an oral dose of ^{131}I. Butanol acetic acid solvent

Group No.	Case No.	^{131}I concentration as per cent of dose/g.	Zonal distribution of ^{131}I in chromatogram expressed as per cent of ^{131}I present							Remarks
			Origin	I^-	MIT	DIT	Y	T4	MIT/DIT	
I	I	0.26	-	7.7	77.6		-	14.7	?	Alkaline hydrolysate; butanol extract.
	II	0.30 paranodular	10.6	48.8	27.3	13.3	-	-	2.1	Trypsin digest.
		0.71 nodular	13.3	32.5	34.7	19.5	-	-	1.7	
		0.08 cyst	Not examined; radioactivity too low.							
II	VI	0.55 paranodular	8.7	-	52.8	26.7	-	11.8	2.0	Trypsin digest.
		0.12 nodular	7.2	-	86.8	6.0	-	-	14.4	
		0.08 cyst	Not examined; radioactivity too low.							

TABLE XV Ctd.

Sporadic goitre

Chromatographic analysis of ^{131}I compounds in thyroids removed 24-48 hours after an oral dose of ^{131}I, Butanol acetic acid solvent

Group No.	Case No.	^{131}I concentration as per cent of dose/g.	Zonal distribution of ^{131}I in chromatogram expressed as per cent of ^{131}I present							Remarks
			Origin	I^-	MIT	DIT	Y	T4	MIT/DIT	
III	VII	0.42	-	-	46.6	53.4	-	-	0.9	Trypsin digest; butanol extract.
IV	VIII	0.14 paranodular	34.7	16.9*	4.2	36.3	7.9	-	0.1	Trypsin digest. (After alkaline hydrolysis of origin T4 found)
		nodular	24.5	26.7*	5.8	36.6	6.4	-	0.2	
		cyst	53.9	18.6*	6.9	13.5	7.1	-	0.5	

* 'Iodide' peak is double and there is almost certainly an unidentified constituent.

I^- iodide. MIT monoiodotyrosine. DIT diiodotyrosine. T4 thyroxine. Y Unidentified zone, between DIT and T4.

TABLE XVI

Sporadic goitre

Electrophoresis of thyroid proteins

Group No.	Case No.	Q	T	Hb	Thy-g	X	Remarks
I	II	±	±	+	+++ RRR	++ R	
	IV	0	+	+	+++	++	Iodide block due to Lugol's iodine
II	VI	++	0	++	+ RRR	++ R	
IV	VIII	± RR	±	+	? RR	+ R	
Tinker	Case 2	±	±	+	+++ RRR	++	

\+ indicates intensity of protein staining.

R indicates radioactivity.

TABLE XVII

Comparison of protein-bound ^{131}I ($PB^{131}I$) and total butanol-extractable ^{131}I ($TBE^{131}I$) in serum of patients

(a) with dehalogenase defect
(b) with an abnormal serum 'iodinated protein'
(c) with Hashimoto's thyroiditis.

Values expressed as per cent of total serum ^{131}I.

		$PB^{131}I$	$TBE^{131}I$	$TBE^{131}I$ after trypsin	$TBE^{131}I$ after chymotrypsin
Dehalogenase defect					
Case 2 Chapter 8		89	86	-	-
Case VI Chapter 17		76	88	-	-
Abnormal iodinated protein					
Case VIII Chapter 19	(1)	100	43	-	-
	(2)	83	72	68	77
Case IX Chapter 19		79	57	-	-
Case X Chapter 19		77	43	-	-
Case H.McL. Chapter 19		98	37	-	76
Case T.McL. Chapter 19		100	16	-	-
Case A.McD. Chapter 19		84	24	29	58
Hashimoto's thyroiditis					
Case 20 Watson et al. (1959)		99	65	64	81
Case 21 " " " "		91	41	-	-
Case 22 " " " "		100	58	-	-
Case 23 " " " "		74	41	-	-

TABLE XVIII

Non-toxic nodular goitre

Chromatographic analysis of ^{131}I compounds in thyroids removed 24-48 hours after an oral dose of ^{131}I.

Butanol acetic acid solvent.

Case No.	Sex	Age (yrs)	Wt. of thyroid. (g.)	^{131}I concentration as per cent dose/g.	Zonal distribution of ^{131}I in chromatogram expressed as per cent of ^{131}I present.						
					Origin	I^-	MIT	DIT	Y	T4	MIT/DIT
1	F	35	?	1.60	11.8	10.7	40.6	30.7	-	6.2	1.3
2	F	56	25	0.26	14.5	-	55.6	29.9	-	-	1.7
3	F	26	28	1.03	7.9	9.2	40.5	30.6	11.8	-	1.3
4	F	23	35	0.82	2.5	2.5	46.4	18.4	30.2	-	2.5
5	M	52	50	1.13	4.9	8.0	38.9	42.4	5.8	+	0.9
6	F	59	75	1.57	12.6	25.0*	26.5	35.9	-	-	0.7
7	M	60	50	0.35	13.1	29.5*	46.7	10.7	-	-	4.2
8	M	32	100	0.60	15.9	27.5*	40.2	16.4	-	-	2.5
9	F	53	200	0.71	-	-	49.2	43.4	7.4	-	1.1
10	F	57	40	0.97	5.4	+	25.2	63.0	6.3	-	0.4

* Iodide peak is double and there is almost certainly an unidentified constituent (Zone A, Chapter 19).

I^- Iodide. MIT Monoiodotyrosine. Y Unidentified zone between DIT and T4.
DIT Diiodotyrosine.
T4 Thyroxine.

TABLE XIX

Pathogenesis of Goitre