The Clinical Chemistry and Pathologic Physiology of Thyroid Tissue

J. B. Stanbury and Leslie J. DeGroot

Familial goiter with hypothyroidism can be subdivided into at least 8 groups and each appears to result from an inherited disorder at a specific stage in thyroid hormone synthesis, mobilization, or peripheral effect. At times, the intense hyperplasia which accompanies one of these disorders evolves into malignant disease. Criteria for diagnosis of each are presented.

The rapidly growing list of inborn errors of metabolism has largely defied satisfactory classification (1). Some of these disorders fall into neat biochemical analogues with no unifying clinical theme, whereas others can be grouped into anatomic pigeonholes or into schemes related to organ systems although there is not the slightest biochemical relationship. There are a few instances where order has emerged and an agreeable coherence can be discerned.

For example, there are now at least 7 biochemically distinct disorders in the biosynthesis, structuring, and dismantling of glycogen. These have clinical features in common, and each is the result of a unique biochemical lesion at a specific single step along the route of glycogen metabolism. There are at least 3 separate diseases of the sulfur-containing amino acids—methionine, cystathionine, homocystine, and cysteine—and man himself may be thought of as a methionineless mutant since he is unable to synthesize this amino acid. There are at least 3 biochemically distinct disorders of the urea cycle, all with overlapping clinical manifestations. Perhaps as we learn more of the nature of inherited disease and of biochemical pathways, it will be possible to arrange these diseases into rational groupings; and if this serves no other purpose, it will at least make the learning process easier.

The familial diseases of thyroid function characterized by the paradox of goiter and hypothyroidism are an interesting group in which sepa-

From the Unit of Experimental Medicine, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

542
rate and identifiable biochemical lesions interrupt a metabolic sequence concerned with the synthesis of the thyroid hormones and their effective delivery to the cells of the body. The principal metabolic pathways of iodine metabolism are illustrated in Fig. 1. Missing from this diagram is an indication of the many important aspects of thyroid function not directly related to iodine metabolism. Among these are synthesis of thyroglobulin; regulation of the host of systems involved in the everyday cellular housekeeping of the thyroid cells; and the synthesis and supply of cofactors, substrates, and energy for the continuing synthesis, storage, and release of the thyroid hormones. It may be readily appreciated that in so complex a scheme—in which each step is dependent upon the preceding and upon a supply of enzymes of more or less specificity, appropriate cofactors, usefully transduced energy, and suitable cytologic architecture—the chances for serious error are many.

Inborn errors of the thyroid which have been identified with any degree of clarity are types of familial hypothyroidism with goiter:

1. Iodide transport defect (6)
2. Iodide organification defect (5); Pendred syndrome (4)
3. Dehalogenase deficiency syndrome (5, 6)
4. Iodotyrosyl coupling defect (7, 8)
5. Thyroglobulin deficiency in sheep (9)
6. Abnormal plasma iodopeptide group (10–12)
7. Abnormal thyroglobulin of man and cattle (13, 14)
8. Syndrome of hormone unresponsiveness (15)

While it is possible to assign each to a particular locus in Fig. 1, the ultimate nature of each has eluded detection, except perhaps only in the dehalogenase-deficiency syndrome. Nevertheless, by appropriate laboratory maneuvers, it is possible to identify each with precision. The criteria which must be satisfied to make the separate diagnoses will be given.

**Iodide Transport Defect**

The first patient for whom this diagnosis was established was a 15-year-old male who was clinically hypothyroid and who had a large goiter (2). There had been evidence of hypothyroidism shortly after birth, but treatment with replacement medication restored his growth rate and development to normal. When this medication was withdrawn, there was a rapid enlargement of the thyroid, and signs and symptoms of hypothyroidism supervened. The patient came from a consanguine marriage, and there was a high incidence of goiter on both sides of the family. The initial laboratory test revealed that only a small fraction of an administered dose of radioactive iodine accumulated in the thyroid gland. Analysis of a biopsy specimen from the gland demon-
strated intense hyperplasia (Fig. 2) and only a small amount of organically bound iodine.

These findings suggested the possibility of a failure of iodide transport in the gland. This hypothesis was tested in 2 ways. In the first place, the function of other iodide-transporting tissues was measured. After a dose of radioactive iodine, simultaneous samples of plasma and saliva were obtained. Normally, the concentration of inorganic iodide in saliva is 20 or more times that in the plasma. On numerous occasions, the ratio of iodide between plasma and saliva in the patient was found not to differ from unity. The salivary glands normally concentrate thiocyanate as well. It was found that in the patient there was no concentration of thiocyanate by the salivary glands. The same failure of iodide transport was demonstrated for the gastric mucosa. Thus, several structures with embryologic origin similar to that of the thyroid were shown not to transport iodide, whereas many controls with various kinds of thyroid disease or without thyroid disease were shown to be normal in their ability to transport iodide. Furthermore, it was reasoned that if the sole difficulty accounting for the thyroid disorder in this patient was an inability to transport iodide, then increasing the blood concentration of iodide to a level comparable to that normally existing in the thyroid might enable the gland to resume normal hor-

Fig. 1. Metabolic pathways of iodine. Arrows pointing up indicate stimulation; those pointing down, inhibition.
mone synthesis. To test this hypothesis, the patient was given approximately 20 times the normal daily supply of iodide. On this regimen, his basal metabolic rate rose in the course of 4 weeks from $-36$ to $+8$ and the signs and symptoms of hypothyroidism disappeared.

**Fig. 2.** Hyperplasia of thyroid typical of familial goiter with hypothyroidism.

Transport of iodide across the plasma membranes of epithelial cells is not well understood. Transport in the thyroid is against an electrochemical gradient and requires an expenditure of energy. It is dependent upon the presence of an active sodium-potassium activated ouabain-sensitive ATPase and probably on a specific lipoprotein carrier in the plasma membrane (16). The defect in the patient described here (and in similar patients) has not been further defined. It may possibly be the disordered structure of a carrier protein or of the flow of energy necessary for the transport process, or to other molecular disturbances. Full understanding of this disease awaits disclosure of the precise mechanism of iodide transport. Diagnosis of an individual patient depends upon satisfying the points specified below:

1. Low thyroid uptake of iodide; thyroid hyperplasia
2. Low thyroid content of iodine
3. No iodide transport by salivary glands or stomach
4. Hormone synthesis restored with iodide

**Iodide Organification Defect**

The second principal step in iodide metabolism in the thyroid is oxidation to iodine and displacement of a proton from the 3 position of
peptide-linked tyrosine to form 3-moniodotyrosine. This oxidative step is evidently accomplished by a tissue-specific peroxidase. A number of patients have now been described who are hypothyroid and have hyperplastic thyroid glands because they are unable to perform this step (3). Nevertheless, the avidity of their thyroid glands for iodine is high.

The first patients recognized with this disorder were 4 siblings in a family of 7 children from a consanguineous marriage. Each was severely retarded, had short stature, and a huge hyperplastic goiter. Uptake of radioactive iodine was high in each, but the iodine was not long retained within the gland. Careful measurements disclosed that the release of iodide from the thyroid was governed by the excretion of iodine by the kidney and disappearance from the thyroid at a rate which paralleled the renal clearance of iodide. Thus, the iodide in the thyroid behaved exactly as if it were in free equilibrium with the iodide of the blood. This would not have been so had the iodide been in organic form. The most telling demonstration that the iodide was in organic form was the rapid discharge of thyroid iodide which occurred immediately upon the administration of thiocyanate or perchlorate. Both of these ions displace inorganic iodide from the thyroid but not iodine in organic form. Normally 99% of all the iodine in the thyroid is in organic form. Thus, in these particular patients all the iodine appeared to be in inorganic form. This was confirmed by analysis of thyroid tissue removed surgically from these patients. Diagnosis of this disorder may be made in accordance with the points listed below:

1. High rapid 131I uptake and loss
2. 131I discharge with ClO₄⁻ or SCN⁻
3. Plasma and urine chromatography: only iodide
4. Low thyroid content of organic iodine

These patients are rare.

A more common syndrome which may be related physiologically was originally described by Pendred as quoted by Fraser et al. (4). In this condition there is also discharge of accumulated radioiodide by thiocyanate or perchlorate but the discharge is slower and less complete and the patients with the disease are not nearly so severely affected. Characteristic of this syndrome is nerve deafness. Most of the patients are either mildly hypothyroid or not hypothyroid at all, and typically they have a small goiter. Criteria for the diagnosis of the Pendred syndrome are:

1. Goiter, usually small
2. Euthyroid or mildly hypothyroid
3. Nerve deafness
4. Partial iodide discharge after perchlorate
The relationship of the deafness to the thyroid disturbance is obscure, particularly in view of the fact that the patients who have the much more severe form of hypothyroidism with inability to oxidize iodide are not deaf. It has been suggested that the Pendred syndrome may be a disorder of iodine transferase rather than iodide peroxidase. This suggests that iodization of tyrosine in the thyroid is a 2-step process. There is no unequivocal evidence for this at the present time.

**Dehalogenase Defect**

A large number of patients have now been described (5) who have hypothyroidism and goiter because of an inability to deiodinate mono- and diiodotyrosine (MIT and DIT, respectively). In the normal thyroid, when it becomes necessary to dismantle thyroglobulin in order that triiodothyronine and thyroxine be released as hormones, MIT and DIT are also released within the gland. These hormone precursors are deiodinated by a potent dehalogenase in the gland, and the iodide is reutilized for iodination of other tyrosyl residues in thyroglobulin. Normally, a small part of this iodide may escape in the blood. A similar or identical deiodinase is also present in many other tissues of the body including the liver, so that if one of these substances is administered intravenously, it is rapidly deiodinated at many sites and the iodine appears in the urine as inorganic iodide. When the dehalogenase is missing, MIT and DIT cannot be deiodinated in the thyroid but leak into the blood, are cleared by the kidney, and appear unchanged in the urine. This loss of hormone precursors from the thyroid impairs hormone synthesis. The resulting thyroid hyperplasia is accompanied by a more rapid uptake of iodide, increased synthesis of hormone precursors, breakdown of thyroglobulin, release of hormone precursors, and further loss of these precursors into the blood. Thus a vicious cycle is created which often leads to goiter and hypothyroidism.

Diagnosis can be readily made by demonstrating the presence of large amounts of MIT and DIT or their conjugates in the blood and urine, and also by demonstrating that intravenously administered MIT and DIT are not deiodinated but appear unchanged or conjugated in the urine. It may also be demonstrated that by reversing the cycle through administration of tyrosine or iodine, the gland can come back into equilibrium and maintain some degree of hormone synthesis for a time before the cycle becomes re-established. It can also be shown that administration of large amounts of iodide will enable a gland to compensate and synthesize normal amounts of thyroid hormone (17, 18). Thyroid tissue obtained at biopsy from these patients, in contrast to that from normal glands, is unable to deiodinate MIT and DIT. A variant
of this disease has been described in which the loss of deiodinating activity seemed to be confined to the thyroid; other tissues were able to deiodinate MIT and DIT (17). It cannot be said that this variant has been unequivocally established as a pathophysiologic entity.

The conditions which must be satisfied before a diagnosis of dehalogenase deficiency is established are:

1. High rapid ¹³¹I uptake and fast turnover
2. MIT and DIT in plasma and urine
3. Intravenous MIT and DIT not metabolized

There is some evidence that heterozygotes for this condition may also have impaired ability to deiodinate diiodotyrosine. Unfortunately, the impairment is not sufficient to unequivocally identify heterozygotes.

**Iodotyrosyl Coupling Defect**

The iodothyronine hormones, thyroxine and triiodothyronine, are formed by the coupling of 2 iodotyrosyl precursors to form 1 iodothyronine molecule. An alanine side-chain is lost. This coupling reaction is complex, incompletely understood, and occurs while the precursors are in peptide linkage within the thyroglobulin molecule (8). It requires oxidizing conditions, is presumably dependent upon a more-or-less specific peroxidase in the thyroid, and may or may not require a specific coupling enzyme. No such enzyme has yet been described, and indeed, it is difficult to demonstrate any coupling whatever under in-vitro conditions in thyroid biopsy specimens or in cell preparations. Coupling is a slow process at best and is undoubtedly a bottleneck in thyroid hormone biosynthesis. It probably is an exceedingly vulnerable step.

A number of patients have now been described who appear to have impaired coupling (7). The first patients to be specifically so designated were sisters with mental retardation, huge hyperplastic goiters, and an extremely high radiiodine uptake. Examination of the glands disclosed ample amounts of MIT and DIT, but very little thyroxine or triiodothyronine. Indirect evidence drawn from kinetic studies with radioactive iodine indicated that there was rapid recycling of iodine within the thyroid between the iodide pool and the MIT and DIT pool; thus, the gland appeared to be synthesizing and degrading MIT and DIT, but to be forming only small amounts of finished hormone.

It seems probable that the so-called coupling defect includes a number of distinct biochemical abnormalities. Any defect in the thyroid cell which would impair the coupling step would give the same set of findings which we are able to measure at the present time. A coupling defect could be due to abnormal thyroglobulin structure which would impair coupling, to impaired peroxidase activity, to impairment in
energy supply, or to supply of such cofactors as might be required in execution of this complex chemical event. Nevertheless, it is possible to establish a set of guidelines which, if fulfilled, would permit one to make a diagnosis of a defect in coupling:

1. Hypothyroidism; thyroid hyperplasia
2. Elevated radioiodine uptake and turnover
3. Thyroid iodine normal or not very low
4. High ratio of iodotyrosine to iodothyronine in the gland
5. Other defects ruled out: no iodine deficiency

This diagnosis may be made with the full understanding that it refers only to a step in hormone synthesis but implies no mechanism whereby this step is impaired.

**Iodoprotein Defect**

The thyroid hormones, triiodothyronine and thyroxine, are largely transported in the plasma tightly bound to carrier proteins but not in covalent linkage. A number of patients have been described with goiter and hypothyroidism in whom a large fraction of the circulating iodine is covalently bound to protein. This circulating iodoprotein is not thyroglobulin, but whenever precise methods of identification have been employed, it has proved to be identical with or similar to iodinated albumin.

Iodinated albumin appears in the plasma under a number of circumstances. It is found in thyrotoxicosis, Hashimoto's thyroiditis, and perhaps, in certain patients with nodular goiter. It may be that hyperplasia of any origin permits formation of iodinated albumin—either de novo or in the gland—or the iodination of plasma albumin during passage through the thyroid. Nonetheless, there is a specific group of patients in whom the only identifiable defect seems to be the production of large amounts of this component (19); in at least one instance, the sole iodinated component in the peripheral blood was an iodinated protein of this type. Diagnostic criteria are:

1. High \(^{131}I\) uptake and slow turnover
2. NBIE in plasma (non-butanol extractable iodine), > 20%
3. Iodoalbumin demonstrable in plasma

Whether this condition can be dignified as a separate and distinct entity at the present time is a fair question, but perhaps by categorizing these patients separately some useful heuristic purpose may be served.

**Goiter with Failure to Synthesize Thyroglobulin**

A few patients have been described with hypothyroidism and goiter in whom it was impossible to demonstrate the presence of thyroglobulin
(10). In these patients, large amounts of iodinated albumin appeared in the gland.

Recently, Falconer has described a genetic disorder of sheep in Australia (9). The disease is characterized by the hypothyroid state, poor development, and a large hyperplastic goiter. The most exacting criteria failed to demonstrate the presence of any thyroglobulin in these glands. The hypothesis has been made that the disease is one of failure to synthesize the specific glycoprotein thyroglobulin. Failing this, the animals produce little or no thyroid hormone but grow a compensatory goiter. Since the rigid criteria of Falconer for absence of thyroglobulin have not been applied to analogous situations in man, and since intense hyperplasia of the thyroid may in itself limit the amount of stored thyroglobulin, it cannot be said with certainty that such a disease exists in man as well, but there should be an awareness of the possibility.

Several patients have been described who are reported to have had abnormal iodinated proteins in the thyroid. A patient with an iodoprotein in the thyroid which was unusually insoluble at low salt concentrations was reported by Michel et al. (20). An iodoprotein peculiarly resistant to tryptin digestion was found in the gland of a patient with familial goiter by McGirr et al. (19). Lissitzky et al. (12) have reported the occurrence of an iodinated prealbumin as the predominant protein in the thyroid of a patient with familial goiter. These conditions may prove to be primary errors in protein synthesis by the thyroid.

**Familial Goiter of South African Cattle**

Van Zyl has described a disease of an inbred strain of South African cattle which is of some economic importance. It is characterized by hyperplastic goiter and an elevation of the protein-bound iodine in plasma (13). Together with Robbins, van Zyl has described an abnormal nonthyroglobulin iodinated protein in the thyroid of these animals (14). This disease is presumed to be an error of protein synthesis in the thyroid. It remains to be seen whether there are analogies in clinical thyroid disease.

**Thyroid Hormone Unresponsiveness Syndrome**

A single family has recently been described which contains several members in the sibship which have nerve deafness, markedly stippled epiphyses, small goiters, and a striking elevation in the plasma protein-bound iodine (15) which may be 3–4 times the normal value—careful biochemical analysis has proved that it is thyroxine. Nevertheless, the
clinical evidence is that the patients are either metabolically euthyroid or slightly hypothyroid. They are resistant to relatively large doses of administered thyroid hormone but do develop toxic symptoms when given extremely large doses. The thyroid suppression test to triiodothyronine is negative at ordinary doses, but the thyroid can be suppressed with large doses. In these patients, thyroid function seems to be normal or, indeed, excessive, but the peripheral tissues appear not to

Fig. 3. Scan of ¹³¹I localization in the neck and chest of a 22-year-old female with familial goiter and metastatic adenocarcinoma of the thyroid.
respond to normal amounts of thyroid hormones. The characteristic clinical and laboratory findings of this disease are:

1. Familial disease
2. Characteristic facies
3. Deaf-mutism
4. Euthyroid; goiter with high *¹³¹I* uptake
5. Stippled epiphyses
6. PBI 15–20 µg./100 ml.; all thyroxine
7. Suppression test: resistant

Investigation has failed as yet to give a clue as to the nature of the cellular defect whereby the peripheral cells of the body fail to react to normal concentrations of thyroid hormone. This is not particularly surprising since the exact way in which thyroid hormones affect the cells of the body has not yet been established with certainty.

**Other Groups of Familial Thyroid Disease**

The groups which have been described above by no means exhaust the list of familial thyroid disorders with hypothyroidism and goiter. DeGroot and his colleagues have studied a kindred of more than 70 persons of whom more than half have goiters—many being nodular prior to puberty. Intensive laboratory studies on a number of these subjects have failed to disclose any abnormality of thyroid function. We have studied a pair of identical twins both with mental retardation and large goiters but without evidence of hypothyroidism. Exhaustive investigation has failed to disclose biochemical evidence of any thyroid disorder. We have also studied a 22-year-old patient who had a large hyperplastic goiter removed at the age of 12 with recurrent growth during the intervening years. The mother and 2 siblings of the patient also have large goiters. At surgery, a large follicular carcinoma of the thyroid was removed. Scanning studies with radioactive iodine disclosed localization of radiiodine coincident with nodular masses in the lung fields (Fig. 3). This patient emphasizes the relationship which may exist between hyperplasia caused by familial inborn error and malignant change. The exact category of metabolic defect in the family has not yet been established.

**References**


