Special Article

Studies on the Thyroid Diseases*

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The present report deals with the studies on the epidemiology, etiology, diagnosis, clinical pathology and treatment of thyroid diseases in my clinic.

I. On the epidemiology and etiology of thyroid diseases

1. Type, sex and age distribution and hereditary incidence of thyroid diseases in my clinic

Of 1,960 cases examined at my clinic during the period from April, 1956 to March, 1964, hyperthyroidism, simple goiter, hypothyroidism, thyroid cancer and thyroiditis were 39.9, 50.2, 4.7, 3.2 and 2.0 per cent, respectively, and the ratios of females to males in each disease were 3.1, 6.4, 3.2, 3.0 and 3.8, respectively. The onset of hyperthyroidism was most frequent in ages between 20 and 29, while in simple goiter it was somewhat earlier, and the incidences were also frequent in ages between 10 and 19. Hypothyroidism was seen frequently in ages between 10 and 19, and in ages between 40 and 49. Thyroid cancer and thyroiditis were seen frequently in older people. In some of these diseases, familial occurrences were seen, and hereditary incidences in hyperthyroidism and in simple goiter were 6.7 and 11.6 per cent, respectively.

2. Geographical pathology of thyroid diseases in Gifu Prefecture

I made (1954-1957) a goiter map of Gifu Prefecture which is located in the middle part of Honshu Island and consists of two provinces with different geographical features called Mino and Hida. The former is flat lowland with low incidence of goiter, and the latter is mountainous land of high altitude and with higher incidence. There was seen a mathematically significant difference between incidences of goiter in both regions, as well as a significant correlation of the prevalence rate of goiter to the calcium content of the soil. But, the highest prevalence in young males was only about 2 per cent, while that in young females was several times more than in males. These prevalence rates are not so high as in the European and

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American endemic localities.

3. Defective deiodination of diiodotyrosine in patients with simple goiter

The results of recent studies on familial goitrous subjects have become the basis of a speculation which postulates the role of an inherited metabolic abnormality as the genesis of sporadic goiter.

I have observed 13 patients with simple goiter having a defect in the deiodination of diiodotyrosine. These goitrous individuals could be segregated into three groups: Group 1, consisting of 6 patients, who had a defect both in thyroidal and in peripheral deiodination; Group 2, consisting of 4 patients, who had a defect in peripheral deiodination; and Group 3, consisting of 3 patients, who had a defect in thyroidal deiodination. The familial occurrence of goiter was observed in each of these groups.

Deficiency of deiodinating enzyme in the thyroid or the periphery, or throughout the body may cause the body to waste iodine, and the enlargement of the thyroid would be a compensatory phenomenon for the defect.

From an analysis of pedigrees of familial goitrous subjects who have a defect in deiodination, this defect seems to be transmitted by an autosomal dominant or autosomal recessive gene which shows almost complete penetrance in women. It seems likely that generalized, thyroidal, and peripheral defects may be different manifestations of the same pathological gene. However, there might be some subtle difference between the iodotyrosine deiodinating enzyme in thyroid and that occurring in the periphery, and this possibility could not be eliminated.

II. Thyroid function tests

1. Thyroid $^{131}$I uptake and the estimation of thyroid hormone secretion rate

Japanese foods contain large quantities of iodine, and our inpatients taking ordinary hospital meals excrete 1,510 micrograms of iodine per day in the mean. High iodine intake has an essential influence on iodine metabolism in the thyroid gland.

I noticed two types of thyroid $^{131}$I uptake curves. In the ascending type the uptake increased gradually for 24 hours and decreased gradually thereafter, while in the descending one it increased rapidly for 3-6 hours, then decreased rapidly for 24 hours and gradually thereafter. When urinary iodine amounted to more than 1,000 micrograms per day the $^{131}$I uptake curve showed the descending type, while in cases with daily urinary iodine of less than 500 micrograms, the curve was of the ascending. When the iodine excretion was between 500 and 1,000 micrograms per day, the curves were either ascending or descending.

In order to avoid the remarkable influence of dietary iodine intake on the thyroid $^{131}$I uptake in calculating the thyroid hormone secretion rate from both the thyroid $^{131}$I uptake and the urinary iodine excretion, Rigg's method was used. Normal range of the rate was found to be from 40 to 250 micrograms per day, and hyperthyroidism as well as hypothyroidism differentiated from euthyroidism without overlapping.
2. The calculation of thyroxine degradation rate

A new calculation of the thyroxine degradation rate (TDR) was derived from the thyroxine metabolism studied with $^{131}$I-labeled l-thyroxine in normal subjects and patients with untreated thyroid diseases.

The Daily Volume Turnover (DVT) (1/day), calculated by summing up its thyroidal, urinary and fecal clearances, was found to be roughly proportional to the PBI value ($\mu$g./100 ml.), as shown by the following formula:

$$DVT = 0.41 + 0.17 \times (\text{PBI})$$

Thus, the TDR value could be calculated from the PBI value. The normal range of TDR was from 35 to 125 micrograms per day; hyperthyroidism and hypothyroidism could be differentiated from euthyroidism without overlapping.

High iodine intake from the diet greatly influences the thyroid $^{131}$I uptake, but has little effect on the PBI value. Therefore, it is emphasized that PBI values should prove to be one of the useful indices of thyroid function.

3. Clinical evaluation of thyroid $^{131}$I uptake at 30-minute

After intravenous administration of 30 to 50$\mu$C. of $^{131}$I to normal subjects and patients with simple goiter and hyperthyroidism, who had taken the ordinary hospital meals, the 10-minute, the 30-minute and the 24-hour thyroid $^{131}$I uptake were measured with a scintillation counter. Twenty-four-hour uptakes in some patients with hyperthyroidism became lower, because they had taken much iodine, and much overlapping was observed among the groups. But in the 30-minute uptake, hyperthyroidism could be differentiated from euthyroidism with slight overlapping (Fig. 1). Furthermore, the correlation of the 30-minute uptake to the PBI or BMR was closer than that of

![Fig. 1. Comparison of 10-minute, 30-minute and 24-hour thyroid uptake after intravenous administration of $^{131}$I in various thyroid disorders.](image)
the 10-minute or the 24-hour uptake to the PBI or BMR. Therefore, it can be stated that the 30-minute uptake after intravenous administration of $^{131}$I is a more useful index for evaluating the thyroid function in the state of iodine abundance in Japan.

4. Resin uptake of $^{131}$I-labeled 1-triiodothyronine

By using Triosorb, Abbott's T-3 Diagnostic Kit, which has been employed as routine work in Japan since last autumn, the normal range was from 22 to 35%. Hyperthyroidism was distributed over 40% without overlapping from euthyroidism, and hypothyroidism was distributed around 20%. They had a good correlation to the PBI values for all subjects, and also Triosorb resin uptake (%) was found to be roughly proportional to the thyroxine binding capacity (TBC) minus PBI value ($\mu g./dl.$) (Fig. 2), as shown by the following formula:

$$\text{Triosorb resin uptake} = -1.3 \times (\text{TBC} - \text{PBI}) + 54.2$$

5. The presence of the thyroid autoantibodies in serum of patients with thyroid diseases

The incidences of thyroid autoantibodies in the serum of the patients with thyroid diseases were studied by the precipitation test, tanned red cell hemagglutination test and complement fixation test. The alterations of antibodies titres after treatment of hyperthyroidism and chronic thyroiditis were also investigated.

The precipitation test was positive only in patients with chronic thyroiditis, except in one case of hyperthyroidism. Tanned red cell hemagglutinating antibody and complement fixing antibody in patients with chronic thyroiditis were detected in 61.5% and 46.2%, respectively, and the frequency in patients with at least one of the antibodies was 84.6%.

Hyperthyroidism cases were divided into three groups: untreated cases (group I), cases retaining hyperthyroid state in the course of the treatment with $^{131}$I (group...
II) and cases having clinically attained euthyroid state after the treatment with $^{131}$I (group III). In group I, II and III, red cell hemagglutinating antibody was detected in 52.1%, 45.5% and 58.8%, respectively. Complement fixing antibody in group I, II and III was detected in 41.7%, 54.5% and 70.6%, respectively. And the high titre of complement fixing antibody was found in group III. The hyperthyroidism cases treated with $^{131}$I, who had complement fixing antibody in the serum before treatment, revealed a distinct increase in the titre of antibody after the treatment, and 71.4% in these patients attained clinically euthyroid state after the treatment with $^{131}$I. But in the patients, who had not complement fixing antibody before the treatment, this antibody was not detected until 4 months after the treatment, and only 20 per cent of these patients attained a clinically euthyroid state. These results indicated that $^{131}$I permitted the extravasation of thyroglobulin and thyroid tissue and induced antibody production which, thereby, could result in the thyroid autodestruction. In a case of chronic thyroiditis with a large firm goiter and high titres of thyroid auto-antibodies, the titres of the antibodies progressively fell during the treatment with adrenocortical hormone and the gland decreased in size. However, with cessation of this hormone administration, the gland enlarged and the antibody titres progressively increased. This result indicated that the autoimmunization played a great role in this disease.

III. The biological and biochemical studies on metabolism of thyroid hormone

1. Bioassay of serum TSH activity, serum LATS effect in thyroid diseases

Serum TSH activities were assayed by the beef thyroid weight response method of Bakke et al., and serum LATS effects were assayed by McKenzie's method.

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Fig. 3. TSH activities in various thyroid disorders.
Fig. 3 shows the serum TSH activities in patients with thyroid diseases, and the normal range was from 0.051 to 0.118 μIU/μl. Fig. 4 shows the incidence of serum LATS effect. A particularly high incidence of LATS was found in hyperthyroidism with exophthalmos and malignant exophthalmos.

<table>
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<tr>
<td>Euthyroid</td>
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Fig. 4. LATS effects in various thyroid disorders.

The correlation between 2-hour responses by McKenzie's method and serum TSH activities for subjects with the absence of LATS was closer than the correlation for all subjects. Further, in subjects with the presence of LATS, the correlation between 8-hour responses and serum TSH activities was closer than that between 2-hour responses and serum TSH activities. Therefore, it could be considered that serum TSH activities assayed by Bakke's method contained the LATS activity in serum.

No significant correlation was found between PBI values and serum TSH activities in all subjects, but a reverse correlation between PBI values and serum TSH activities for the subjects with the absence of LATS was significant. Therefore, it could be considered that these facts showed the presence of feed-back relation in subjects with the absence of LATS and the disturbance of feed-back relation in the subjects with the presence of LATS.

2. Studies on the serum butanol insoluble iodoprotein and thyroidal iodoprotein in thyroid diseases

After the administration of 200-500 μc. of 131I to the patients with thyroid diseases,
blood specimens were drawn at 48 hours, and total plasma $^{131}$I (TP$^{131}$I), protein bound $^{131}$I (PB$^{131}$I) and butanol insoluble $^{131}$I (BI$^{131}$I) were measured. The mean BI$^{131}$I concentration in normal subjects was $0.017 \pm 0.008\% / L$, and all the thyroid diseases showed higher values than that in normal subjects. In comparing the ratio of BI$^{131}$I/PB$^{131}$I (Fig. 5), hyperthyroidism distributed in normal range, because of their high PB$^{131}$I. But, those in simple goiter, thyroid cancer, chronic thyroiditis showed higher values compared with that in normal subjects.

![Fig. 5. Comparison of BI$^{131}$I ratio in various thyroid disorders.](image)

Samples of serum after $^{131}$I treatment in hyperthyroidism were subjected to starch-block electrophoresis, and $^{131}$I was found to be associated with inter-alpha globulin and albumin. The iodinated components in the albumin was butanol insoluble. By means of the paperchromatographic analysis of the enzymatic hydrolyte of the extract from the albumin and inter-alpha globulin, it was found that the radioactivity in inter-alpha globulin was thyroxine and triiodothyronine, and about 60 per cent of the radioactivity in albumin was moniodothyrosine.

The thyroid proteins in 12 hyperthyroidism, 12 nodular goiter and 4 thyroid cancer cases were studied by paper electrophoresis, ultracentrifuge analysis and agar precipitation reaction. The investigations disclosed an increase in the relative proportion of a protein with a sedimentation coefficient of approximately 4.2S in the nodular goiter and thyroid cancer. This protein has an electrophoretic mobility in veronal buffer and immunological reactions similar to those observed with serum albumin, and was named thyalbumin by Schuleman.

Fig. 6 shows the distribution of protein components by paper electrophoretic analysis of the thyroid supernatant proteins. The increase of thyalbumin in the thyroid protein of thyroid cancer and nodular part of nodular goiter were remarkable. The relation between serum butanol insoluble iodoprotein and thyalbumin in thyroid protein in these protein diseases can be clarified only when more information is available.
3. Studies on the cellular thyroxine-binding protein

Rats injected with 3 to 5 micrograms of $^{131}$I-labeled 1-thyroxine and 1-triiodothyronine were killed after 60 minutes, and their liver extract was fractionated in a diethylaminoethyl cellulose column; the compounds with radioactivity were identified by paper chromatography. In the in vitro experiments, liver extracts of non-treated rats were chromatographed following the addition of small amounts of radiothyroxine and radiotriiodothyronine. In the distribution of radioactivity of thyroxine, two peaks always appeared in the same positions both in vivo and in vitro experiments (Fig. 7). From a comparison with chromatograms of serum, it could be concluded that the major peak represents cellular thyroxine binding protein (T4BP), and the minor peak probably represents a contamination with serum T4BP.

Triiodothyronine seemed to be bound to cellular triiodothyronine (T3BP), which is different from serum T3BP. The results of studies made on rats injected with a mixed solution of radiothyroxine and radiotriiodothyronine also suggested the presence of specific T3BP, which is different from cellular T3BP in rat liver. These studies provide evidence for the presence of a cellular thyroxine-binding protein and triiodothyronine-binding protein in rat livers.
4. Peroxidase and iodotyrosine deiodinase

1) Peroxidase

Observations were made on iodide-peroxidase tyrosine-iodinase system of the thyroid in patients with various thyroid diseases.

The enzyme activity of the thyroid was higher in hyperthyroidism, and was far lower than normal in nodular goiter and thyroid cancer.

The peroxidase of the normal thyroid was present in the particulate fraction, while the enzyme of hyperthyroid gland was present not only in the particulate fraction but also in the soluble fraction.

2) Iodotyrosine deiodinase

Iodotyrosine deiodinase was present not only in the mitochondria and microsomes but also in the soluble fraction. Ninety per cent or more of the total deiodinase activity was in the mitochondrial and microsomal fractions. When preparation from different organs were compared with each other, it was found that the percentage of total activity in the soluble fraction was somewhat lower in the liver than in the thyroid and was nearly zero in the kidney. It was noteworthy that the activity of the soluble fraction of thyroid was much higher in the hyperthyroid gland than in the normal gland and was almost negative in cancerous tissue.

After electrophoresis on starch block, the iodotyrosine deiodinase of the soluble fraction from the thyroid and the liver migrated toward the anode in one peak which is in a similar
position to the albumin of human serum on a parallel starch block. The iodotyrosine deiodinase of the extract or the mitochondrial and microsomal fractions from thyroid, liver and kidney was in one peak, which migrated toward the cathode in a position between $\beta$- and $\gamma$-globulin of control human serum. This activity peak moved somewhat faster toward the cathode than the peak of protein concentration.

The data of the present studies with thyroid peroxidase and iodotyrosine deiodinase may support the suggestion that the particulate form of these enzymes may be converted into the soluble form, and that these two forms are isomeric. The different percentages of the total deiodinase activity in the soluble fractions from the thyroid, liver, and kidney, and the high peroxidase and deiodinase activity of the soluble fraction from the hyperthyroid gland may be the phenomenon which responds to the metabolic requirement. A mechanism for altering the relative proportion of the enzyme molecules may offer a distinct adaptive advantage to an organism.

IV. Treatment of hyperthyroidism

1. Treatment with antithyroid drug

The prognosis of hyperthyroidism treated with antithyroid drug was studied statistically in 40 patients who had been observed for at least half a year following the cessation of this treatment.

The remission rate was 62.5%.

The age, the duration of disease prior to treatment, the duration of treatment, and the initial BMR were found not to change the ultimate result significantly. But the small sized goiter responded better to the treatment, and the decrease in goiter size during treatment was found to improve the therapeutic result significantly. And also, the remission rate in hyperthyroidism without exophthalmos was more remarkable than that in hyperthyroidism with exophthalmos.

2. Treatment with $^{131}$I

Statistical study on 2,408 cases treated with $^{131}$I from all university hospitals in Japan was made in 1962.

The treatments were continued for 1.5 times in the average of total cases, and total doses amounted to 7.3 mc. in the mean. Excellent effects of this treatment were seen in 60.5 per cent of the cases, but 3.0 per cent of these cases suffered from temporary hypothyroidism. In 4.4 per cent of the cases, the patients complained of various side effects, such as headache, fever, gastrointestinal symptom, circulatory disturbance, skin rash, transient leukopenia, linitus, irritability and depression, depilation and white hair, hemorrhage, abnormality of menses, and dysphagia. The incidence of acute myelogenous leukemia was seen in 2 cases following $^{131}$I treatment. We have been making blood examinations of 20 patients with hyperthyroidism treated with $^{131}$I for one and half year, and until the present time no remarkable changes have been found, except that some influence of alteration of thyroid gland function induced by the treatment on the blood cell count were seen. The problem of the radiation effect on the blood might be solved after further observations.