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# Changes of Serum Angiotensin-Converting Enzyme Activity During Treatment of Patients with Graves' Disease*

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*Serum angiotensin-converting enzyme activity was measured spectrophotometrically, and serum thyrotropin-binding-inhibitory immunoglobulin (TBII) activity was measured by radioreceptor assay in normal subjects and in patients with Graves' disease serially before and during treatment, and these activities were compared with each other and with thyroid hormone levels in various thyroid functional status. Correlation between serum angiotensin-converting enzyme activity and serum thyroid hormone level was pursued with relation to the changes of thyroid functional status in patients with Graves' disease during treatment.*

*Serum angiotensin-converting enzyme activity was significantly elevated in patients with hyperthyroid Graves' disease before the start of treatment ($35\pm13$ nmol/min/ml, $n=50$), and not in patients with Graves' disease, euthyroid state during treatment with antithyroid drugs or radioactive iodine ($23\pm9$ nmol/min/ml, $n=12$), but decreased significantly in patients with Graves' disease, hypothyroid state transiently during treatment ($15\pm4$ nmol/min/ml, $n=12$), respectively in comparison with normal control subjects. Serum angiotensin-converting enzyme activity was positively correlated with the log value of serum T3 concentration ($r=0.62$, $p<0.001$, $n=95$), and with the log value of free thyroxine index ($r=0.66$, $p<0.001$, $n=91$) but not statistically significantly with serum TBII activity. Serum angiotensin-converting enzyme activity was followed in 11 patients with initially increased activity and the activity decreased in proportion to serum thyroid hormone level during treatment, irrespective of treatment modality. It is suggested that thyroid hormones play a role in the increase and decrease of serum angiotensin-converting enzyme activity directly or indirectly influencing the peripheral tissues (probably reticuloendothelial cells or peripheral endothelial cells) in patients with Graves' disease.*

Key Words: *Graves' disease, Angiotensin-converting enzyme, Thyrotropin binding inhibitory immunoglobulin*

## INTRODUCTION

In thyroid diseases, it is known that the activity of serum angiotensin-converting enzyme(ACE) increases in Graves' hyperthyroidism,[1-4] and that it becomes normal in euthyroid patients after treatment. Even though the mechanism of increased serum ACE activity is still unknown, Yotsumoto et al.[3] and Nakamura et al.[2] reported that the amount of thyroid hormone and the ACE activity in serum had changed proportionately. And other in vitro reports[5-7] suggested that the thyroid hormone has an influence upon the secretion of ACE from tissues to blood and upon the activity of ACE in the cultured

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cells in vitro or in an animal. Therefore, it is now thought that the increase of serum ACE activity in the thyroid disease is generally caused by the increase of the thyroid hormone per se[2-3] rather than by the thyrotropin receptor related antibody, the cause of hyperthyroidism. If serum ACE activity is related to the functional status of the thyroid, serum ACE activity in the patients with hypothyroidism would have been found to be low, but according to Nakamura et al.[2] and Silverstein et al.[4] there was no difference between the activities of hypothyroid patients and those of the normal control group. Mean while, as the autoantibody toward TSH receptor of his own has been regarded as an autoimmune cause of the elevation of the thyroid function, it is necessary to examine the relationship of elevated serum ACE activity in Graves' disease to thyrotropin receptor related antibody, although there have been no reports about these possibilities. We have examined the changes of thyroid functions in the patients with Graves' disease in hyperthyroidism and traced the serum ACE activity and thyrotropin-binding-inhibitory immunoglobulin (TBII) activity and have compared those with each other.

## MATERIALS AND METHODS

### 1. Subjects

The group of normal control subjects consisted of 17 male and 40 female persons, who had no history of thyroid disease. Seventy patients with Graves' disease consisted of 13 male patients and 57 female ones, 28 patients of whom were followed up during treatment. The cases who were in hypothyroid state consisted of 12 patients with Graves' disease who were hypothyroid transiently during treatment and the other 3 patients were one with primary myxedema and 2 patients with Hashimoto's thyroiditis. Graves' disease was diagnosed by clinical examinations (diffuse goiter and hyperthyroidism and/or exophthalmos) and thyroid function tests. The patients were traced during the treatment with only antithyroid drugs or with antithyroid drugs and radioiodine.

### 2. Methods

1) Specimen

The blood of normal persons and the patients were taken and left for appropimately an hour at room temperature. Then, the serum was taken and stored at −70°C and the assay was done within 6 months. The assay of serial samples of a patient was done at the same time.

2) Measurement of serum ACE activity

Serum ACE activity was measured by a modification of the method of Cushman and Cheung. Hippuryl-histidyl-leucin (Sigma®) was used as a substrate, and phosphate was used as a buffer and hippuric acid produced by ACE activity in patient's sera was measured at 228 nm by spectrophotometry. In the normal control serum and the positive standard serum, the intraassay coefficients of variation were both 4.4%, and the interassay coefficients of variation were 11% and 6%(Table 1). To observe serial change of ACE activity in a patient, the specimens having been refrigerated at −70°C, were measured together at the same time. Accordingly, the measurement error of the assay for the evaluation of the change during treatment was 4.4%. It was not found that the enzyme activity decreased or changed significantly during storage for 6 months.

3) Measurement of serum TBII activity

Using the commerical kits, R.S.R.® radioreceptor assay, patient's serum TBII activity was measured by the activity to displace radiolabelled TSH from solubilized membrane TSH receptor.

$$\text{TBII activity(\%)} = 100 \times \frac{\text{specific binding with patient's sera-nonspecific binding}}{\text{binding with normal pooled sera-nonspecific binding}}$$ The normal

Table 1. Data of precision

| | Intrarun assay variance | | Interrun assay variance | | |
|---|---|---|---|---|---|
| | Normal pooled sera | Positive pooled sera | | Normal pooled sera | Positive pooled sera |
| No. of test | 8 | 8 | No. of test | 33 | 24 |
| Mean | 20.3 | 40.9 | Mean | 22.4 | 47.1 |
| S.D. | 0.9 | 1.8 | S.D. | 2.5 | 3.1 |
| C.V.(%) | 4.4 | 4.4 | C.V.(%) | 11.1 | 6.5 |

S.D.: Standard deviation
C.V.: Coefficient of variation

range was less than 15%, the intraassay and interassay coefficients of variation were between 4% and 15%. Triiodothyronine (T3), total Thyroxin (T4), and T3 Bead Uptake in serum were measured by radioimmunoassay (Abbott®). The normal range of T3 was 99-219ng/dl, and that of T4 was 6.6-13.8ug/dl. The normal range of T3 Bead Uptake was 22.8-33.6%. and that of free T4 index was 1.7-4.0.

## RESULTS

### 1. Serum ACE activity in the normal subjects

In the normal control group of 57 persons, the average serum ACE activity was 23.7±6.0 nmoles/min/ml (M±S.D.) There was no significant difference between 17 male and 40 female persons of which the average was 22.2±5.6 nmoles/min/ml and 24.4±6.2 nmoles/min/ml, respectively. No significant difference in serum ACE activity was noted with increasing age.

### 2. Serum ACE activity in the patients with thyroid disease (Table 2)

Serum ACE activity in the 50 patients with Graves' disease in hyperthyroid state before treatment was 34.5±13.3 nmoles/min/ml and it was significantly elevated more than that of the normal control group ($p<0.001$). Of the 12 patients with Graves' disease in euthyroid status during treatment the serum ACE activity was 23.0±7.6 nmoles/min/ml, and there was no significant difference between the normal control group and these patients. In 15 hypothyroid patients including 12 patients with Graves' disease in hypothyroid state during treatment was 15.4×4.0 nmoles/min/ml, it was significantly lower than that of normal control group ($p<0.01$). Figure 1 shows the distribution serum ACE activities in groups.

### 3. The correlation between serum ACE activity and thyroid hormone level

Serum ACE activity was significantly correlated with serum T3 ($\gamma=0.62$, $p<0.01$, Fig. 2), and with free T4 index ($r=0.66$, $p<0.01$, Fig. 3).

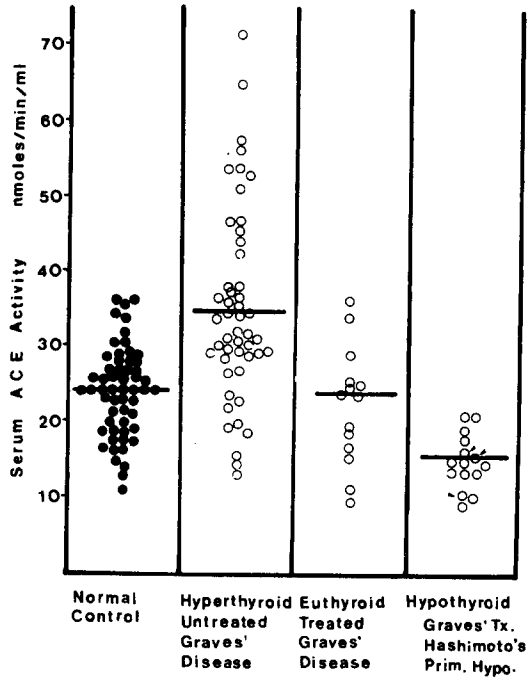


Fig. 1. *Serum ACE activity in healthy control subjects and in patients with Graves' disease in various functional status.*

Table 2. Serum angiotensin-converting enzyme activity in normal and Graves' disease

| | No. of persons | Mean activity* | Standard deviation | Statistical difference** |
|---|---|---|---|---|
| Normal control | 57 | 23.7 | 6.0 | |
| Hyperthyroid-untreated Graves' disease | 50 | 34.5 | 13.3 | P<0.001 |
| Euthyroid-treated Graves' disease | 12 | 23.2 | 7.6 | P>0.1 |
| Hypothyroid-treated Graves' disease | 12 | 15.4 | 4.0 | P<0.01 |

*Activity means nanomoles per milliliter per minute of hippurate produced per serum 1 milliliter.

**Statistical difference means the difference between each group and normal control group. Student's T test was used to calculate statistical significance.

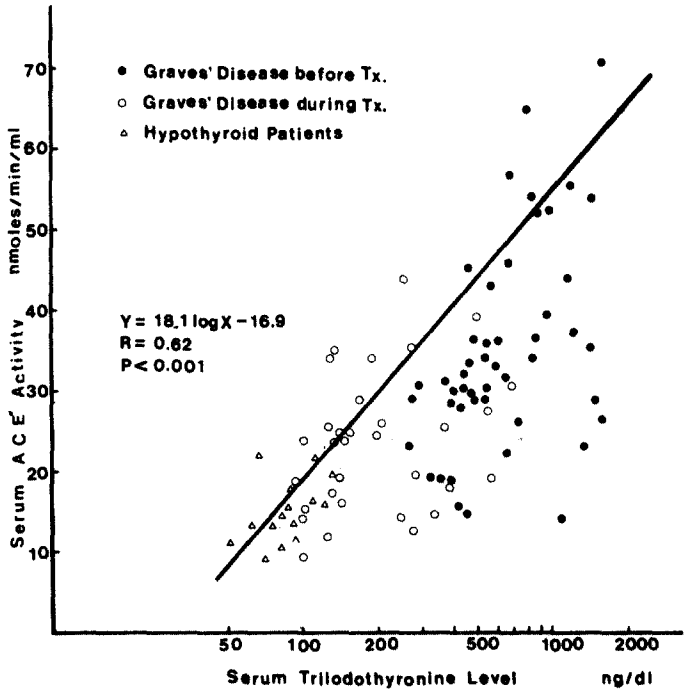


Fig. 2. *Correlation between serum T3 and ACE activity (N=95).*

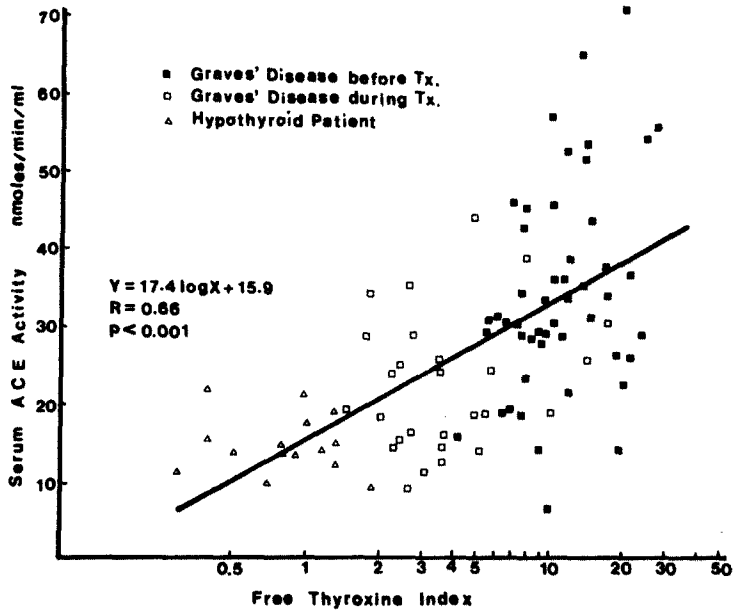


Fig. 3. *Correlation between serum thyroxine and ACE activity (N=91).*

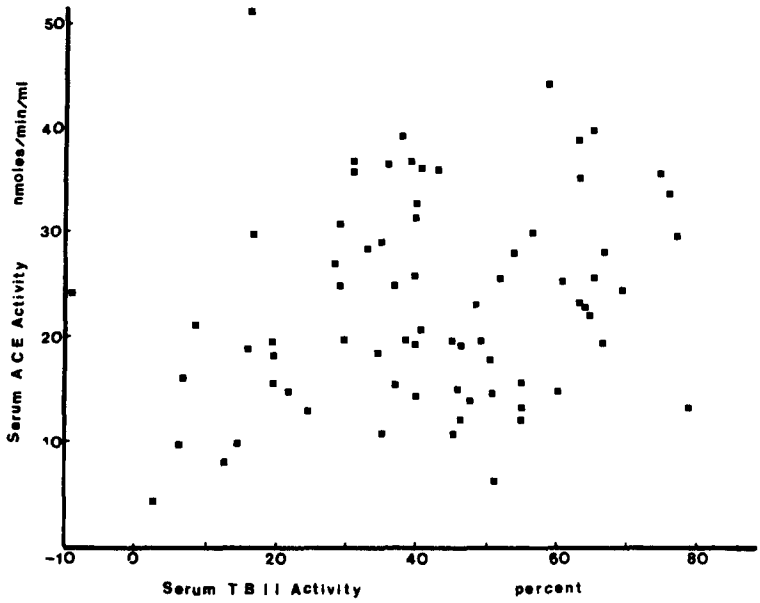


Fig. 4. *Correlation between serum TBII activity and ACE activity.*

4. The correlation between serum ACE activity and serum TBII activity

The distribution of the serum ACE activity and the serum TBII activity is shown in Figure 4 and no significant correlation ($r = 0.2$, $p > 0.1$) was detected.

5. The change of serum ACE activity during treatment in Graves' disease

The serum ACE activities during treatment were continuously decreased according to the recovery of the thyroid functional state as shown in Figure 5. The serum ACE activity decreased into normal range 2 or 3 months after the administration of antithyroid drugs, and these changes did not show any difference in relation to the method of treatment. In Figure 6, in the case to whom only the radioidine was prescribed, we found that the serum ACE activity also changed according to the thyroid functional state irrespective of the transient change in the serum TBII activity after the administration of radioiodine. Four months after treatment, his thyroid began hyperfunctioning again, although serum ACE activity was still in the normal range. Figure 7 showed another case in which the trial of high doses of methimazole had failed and hyperfunction had not declined. After the combined use of radioidine and antithyroid drugs, serum ACE activity began to be lowered as the thyroid function improved. Figure 8 is the results during treatment in another patient to

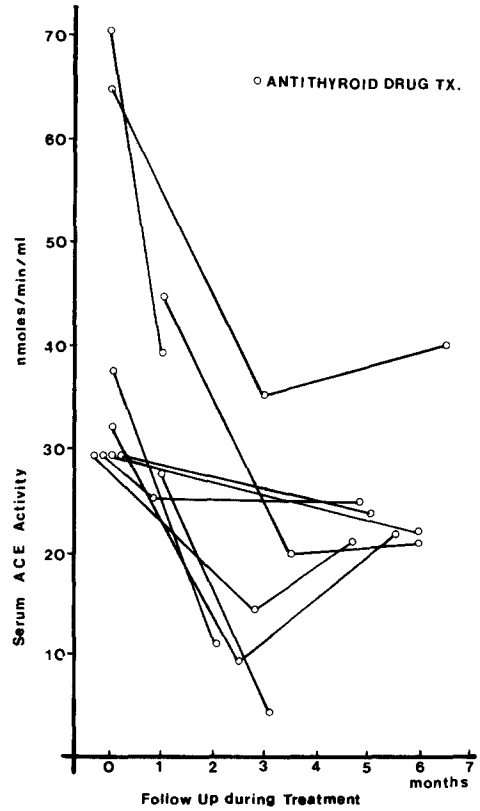


Fig. 5. *Serial changes of serum ACE activity in Graves' disease during treatment.*

whom multiple modalities of treatment were used. The first attempt with radioidine treatment was in vain, and as the second attempt, when the antithyroid drugs was administered for 4 weeks, the TBII activity increased rather than decreased significantly, but serum ACE activity decreased together with an improvement of thyroid function. Only after the treatment with radioiodine and antithyroid drugs did the serum ACE activity and thyroid function begin to normalize regardless of TBII activity. Of the patients with Graves' disease in hyperthyroidism, the ACE activity of 17 patients whose ACE activity before treatment was in the normal range had not showed any different change in the course of treatment (Data, not illustrated).

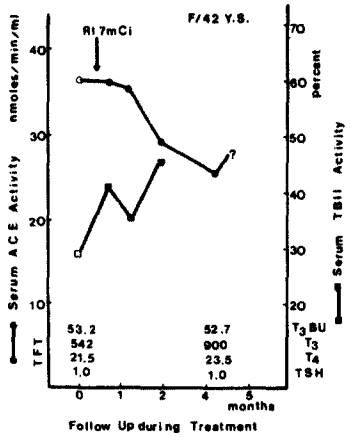


Fig. 6. *Serial changes of serum ACE activity in a patient treated with radioiodine alone.*

## DISCUSSION

Serum ACE activity according to age and sex in normal subjects has been reported variably by several authorities. According to Lieberman,[8] the serum ACE activity was higher in the age group under 20 years old, but Ashutosh et al.[9] stated the contrary. With regard to sexual differences, Lieberman, Beutler et al.[10] and Nakamura et al.[3] said that the activity was higher in men than in women. But Matsuki et al.[11] and Hurst et al.[12] reported that there was no difference in the serum ACE activity between men and women. Our results were similar to these reports in that no differences were detected between older age groups and the young, and between male and female subjects.

Since it has been reported that the serum ACE activities increase in various diseases, it is well known that increased serum ACE activity is not a unique feature of a certain disease. Recently there were a few reports where by the ACE activity increased in proportion to the increase of thyroid function.[2,3,13] Yotsumoto[2] et. al. reported that normal serum ACE activity was 30±9 nmoles/min/ml on an average and that the activity in patients with hyperthyroidism was 65±18 nmoles/min/ml. He also said that 80% of the patients with hyperthyroidism in his series showed the increase of ACE activity

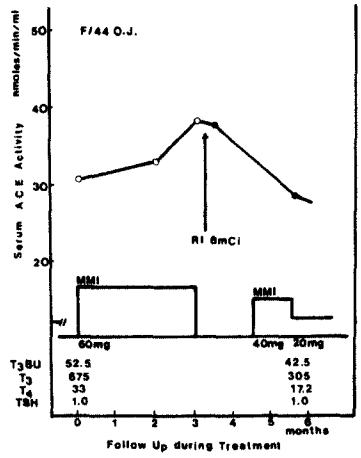


Fig. 7. *Serial changes of serum ACE activity in a patient unresponsive to treatment with antithyroid drug.*

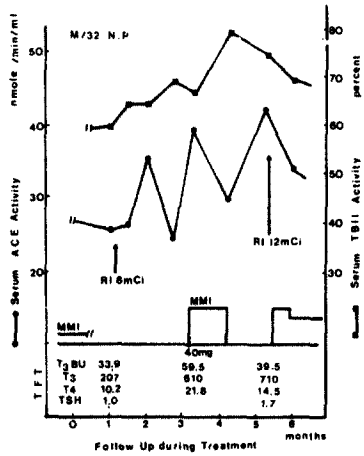


Fig. 8. *Serial changes of serum ACE activity in a patient with fluctuating disease activity despite treatment.*

more than twice the standard deviation range of the normal activity. Nakamura[3] et al. reported that the average was 30 nmoles/min/ml in the normal male and 26 nmoles/min/ml in the normal female subjects and 52 nmoles/min/ml in patients with hyperthyroidism. He also pointed out that serum ACE activity in hyperthyroidism was significantly higher than in the normal subjects, although in hypothyroiidism the activity did not show a statistically significant decrease. Our results showed that the serum ACE activity was $23.7 \pm 6.0$ nmoles/min/ml in the normal control subjects and was $34.5 \pm 13.3$ nmoles/min/ml in untreated patients with hyperthyroid Graves' disease. In addition, only 60% of the patients showed the increase of more than one standard deviation over the mean value of normal controls. These differences in the ranges of serum ACE activities from several reports are now thought to be caused mainly by the difference of buffers chosen in the assay and partly by other factors. According to Cushman et al.[14] the serum ACE was reported to show its maximum activity at pH 8.3. But the buffer capacity of the phosphate used is not perfect at pH8.3. Besides, the phosphate itself inhibits the ACE activity partly, that is, the relative activity was reported to be 0.82 when the phosphate buffer was used as compared with that measured when the borate buffer was used.[12] In addition, Lieberman[8] pointed out that when the absorption of hippuric acid produced was more than 0.6, the linearity of absorbance curve began to decline a little and recommended the assay should be repeated with a diluted sample to get the exact result. But according to our preliminary observation (data, not shown), the linearity of the ratio of absorption to concentration was maintained up to 1.5 of absorption. Our result regarding the serial changes of activity are those obtained within the same assay to avoid the possible large range of error of comparison with interassay variance. This would have contributed to the less sensitivity of the measured activity in our series.

In the 15 patients with hypothyroidism, the serum ACE activity showed a significant decrease than the normal control group. This result was different from those of Nakamura et al.[3] and Silverstein et al.[4] These differences are considered to be caused mainly by the difference of the patients who were included, the assay method and the accuracy of measurement. Because the serum lipid contents increase in the patients with hypothyroidism, the possibility that the interference by the lipid contained in blood had an influence upon the measured activity should also be considered.[15] But above all these limitations and the results of our less sensitive assay showed significant decrease in serum ACE activity in the patients with hypothyroidism, it can be infered that the serum ACE activities decrease in a hypothyroid state in proportion. But before extrapolating the results of this report, we should clarify the interference of the serum lipid especially when the blood sample was drawn from patients for the assay after meals. Nakamura et al.[3] and Kwon et al.[13] reported that there was significant correlation between thyroid hormone levels and the serum ACE activity. Nakamura et al.[3] included patients with Graves' disease before treatment and during treatment, and Kwon et al.[13] excluded the hypothyroid patients with variable ACE activities for the analysis of correlation. We included all the activities measured in concert with thyroid function tests and found a significant correlation between them with the correlation coefficient of about 0.6. Though we intended to ascertain whether the thyrotropin receptor related antibody itself increased the serum ACE activity in the thyroid or the periphery, there was no correlation between serum TBII activities and ACE activities. Because the ACE is not distributed in especially large quantities in the thyroid tissue,[16,17] we could think that the enlarged thyroid did not produce and secrete ACE into the serum according to TBII activities. Though we should take the limitations of TBII in representing abnormal stimulators in Graves' disease into consideration and the least possibility that TBII would have an effect on the peripheral tissues, it might be suggested that the TBII would have no effect on the changes of the serum ACE activity in Graves' hyperthyroidism.

Yotsumoto et al.[2] observed that in the patients with hyperthyroidism, after measuring both the change of the thyroid hormones and the change of the serum ACE activities during treatment, the serum ACE activities decreased together with the decrease of the thyroid hormones. He also reported that there was a trend toward increase in serum ACE activity about two weeks later when the level of thyroid hormone in blood increased again due to the insufficiency of treatment. In this study, we can observe similar features in the cases of which we can follow up both the thyroid functions and the serum ACE activity. In cases who were given antithyroid drugs alone, in accordance with the normalization of the thyroid function, the serum ACE activity decreased as the thyroid function normalized and it showed the same results in cases of combined treatment with radioiodine. In a case, despite the use of high dose of antithyroid drugs the patient was in hyperfunction for four

months and after the additional administration of radioiodine in accordance with the normalization of the thyroid function, the serum ACE activities decreased (Fig. 7). On the other hand, in a case of treatment by radioiodine alone, though the serum TBII activity showed transient fluctuation, the serum ACE activity decreased down to normal range with an improvement of the thyroid functional state (Fig. 6). In addition, the serum ACE activity was yet in the normal range though the function was elevated by the reactivation of the disease in the last month, so there should be a delay in the increase of the serum ACE activity in proportion to the thyroid functional state. We couldn't find any direct correlation between the change of the serum ACE activity and TBII activity, and not in another case, either (Fig. 8). In brief, there was no direct relationship between the abnormal thyroid stimulators, TBII, and the enzyme activities in patients with Graves' disease regardless of the regimen. And the serum ACE activity changed a little later than the increase of the thyroid hormone. These facts can be interpreted to be an indirect evidence of an assumption that the major factor in the changes of serum ACE activity is the peripheral thyroid hormone concentration.

Because the hyperthyroidism itself brings about the hemodynamic excess, the assumption that the hemodynamic excess state in hyperthyroidism should increase the ACE activity by changing the peripheral circulation including the pulmonary circulation is possible as suggested in chronic liver disease.[11] But, since there has been no report that serum ACE activity increased in the other diseases known to cause severe hemodynamic excess, we could pose less possibility that this would be the main mechanism increasing the serum ACE activity in hyperthyroidism. On the other hand, the evidence that thyroid hormones will do much directly and/or indirectly by inducing the ACE secretion from peripheral tissues to the serum has been sought in the animal experiments and in vitro.

Friedland et al.[19] reported that the particulate ACE of endothelial cells has the same activity and characteristics as the serum ACE when the soluble ACE was separated by treating with the proteolytic enzyme of trypsin the particulate ACE of vascular endothelial cells of rabbit. From the fact that the membrane bound ACE and the serum ACE are similar in their physicochemical characteristics and from another observation by immunofluorescence that ACE is bound to the membrane of the endothelial cell of the peripheral blood vessels,[20,23] it can be infered that the membrane bound ACE in the endothelial cells is secreted into serum and shall make the serum ACE activity in certain conditions. Silverstein et al. concluded that the thyroid hormone could not induce the new production of ACE showing that the triiodothyronine was not able to induce ACE in the supernatant of the cultured alveolar macrophage cells,[4] though the enzyme could be induced in the supernatant fluid by the corticosteroid.[5]

These experimental reports cited and our clinical results suggest that, even if the thyroid hormone does not induce the production of ACE in man from peripheral endothelial cells, it will play an important role directly and/or indirectly in the increase or decrease of the serum ACE activities inducing the increased or decreased secretion of ACE into the serum. In conlusion, our results that the serum ACE activities change in proportion to the thyroid hormone levels, from the hyperthyroid state to the normal or even hypothyroid state, was regarded as evidence of an assumption that thyroid hormone will play a role in the secretion and consequent increase in the activity of ACE in the serum.

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