CLINICAL EVALUATION OF THE THYROID STIMULATING HORMONE ACTIVITY IN EXOPHTHALMOS

By

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ABSTRACT

A method for demonstrating the presence of a thyroid stimulating factor in the blood of patients with progressive exophthalmos after thyroidectomy or after treatment with radiiodine is described. The method consists of transfusing freshly drawn blood from the patients to euthyroid recipients and subsequently following the PBI level of the recipients at regular intervals. Six exophthalmic patients tested in this manner were found to have such a factor in their circulating blood. After transfusion of their blood a significant rise in the PBI level of the recipients could be demonstrated. Two other patients, one with exophthalmos of long duration did not show this response nor did it occur after transfusion of blood from two control subjects. In one case the action of this factor was compared with that of animal thyrotrophin and found to be of the same magnitude.

The relationship between exophthalmos and the secretion of the thyrotrophic hormone (TSH) of the hypophysis remains an unsolved problem. Thus, Querido & Lameyer (1956) were unable to find any correlation between TSH-secretion and the degree of exophthalmos. On the other hand Asboe-Hansen et al. (1952) demonstrated an elevated content of TSH in the blood in 9 cases out of 10 with severe exophthalmos. Differences in opinion may be due to technical difficulties. The serum concentration of TSH appears to be very low. All methods hitherto described use animals as test objects for human TSH. The methods have recently been critically surveyed by Brown (1959).

The response to homologous thyrotrophic hormone in man has not been previously studied. This report describes the presence of a factor in the blood from patients with severe exophthalmos which stimulates the thyroid function in euthyroid subjects. This factor is believed to be identical with TSH or some other thyroid stimulating hormone of the hypophysis.
METHODS

The method is based on the transfusion of freshly drawn blood from exophthalmic patients to recipients with normal thyroid function. After transfusion, the level of protein bound iodine of the recipient (PBI) is followed for 72 hours at regular intervals.

Transfusion. – 265 ml of blood was withdrawn from the patient into 45 ml of an anticoagulant, Acid Citrate Dextrose Solution (U.S.P.) Solution A. The blood was then transfused to the recipient within 20–30 minutes. There were no complications.

Protein bound iodine. – Determinations were done according to the method of Barker et al. (1951) with the modification of Skanse & Hedenskog (1955). Mean value: 5.9 ± 0.7 µg. Range 3.8–8.0 µg per 100 ml of serum. The accuracy of the PBI analyses was obtained by analyses performed in duplicate. Each serum sample was analyzed on two different days with the use of two different furnaces. The accuracy of the analysis (σ) was calculated from the differences (d) between the two determinations according to the formula:

σ = ± \sqrt{\frac{\sum d^2}{2n}}

where n is the number of samples. σ was found to be 0.23 µg per 100 ml.

The blood samples for PBI determinations were collected according to a fixed time schedule. Before the transfusion a sample was withdrawn from both the donor and the recipient. Ten minutes after the transfusion was concluded, another sample was drawn from the recipient. Six hours later another sample was drawn. From then on samples were collected twice a day at 12 hours intervals for three days. Thus, the PBI level of the recipient was followed for 3 days after the transfusion.

\(^{131}\)I uptake. – This test was performed by using a tracer dose of 15 µc \(^{131}\)I. The activity over the thyroid gland was measured 24 hours later in the usual way.

Thyrotrophin test. – In one case the PBI response to transfusion was compared with that of a commercial animal thyrotrophin. Actyron® was used (standardized in USP units by Rowlands-Parkes' thyroid weight method; 1 unit corresponding to 4 units of Junkmann-Schoeller).

The dosage used was 20 and 40 USP units respectively.

Clinical material. – Eight patients with severe exophthalmos and 2 normal blood donors were tested, the latter being used as controls. Six of the patients had no signs of active thyroid disease when the test was done. Of the two other cases one had Graves' disease of short duration and the other had had a marked but essentially unchanged exophthalmos for 6 years. The recipients were all patients in the hospital and more or less in need of blood transfusion. The need for transfusion was, however, in no instance acute. None of the recipients had any signs of endocrine disorder.

Brief case histories of donors and recipients are given below.

Case 1 a. – M.S., 55 years old female, donor. In 1957 she had signs of Graves' disease without any eye symptoms. After treatment with iodine a subtotal thyroidectomy was performed. Three weeks later bilateral swelling of the eyelids developed together with progressive exophthalmos. The Hertel values were 16 mm on the left and 17 mm on the right side. The test was performed 6 weeks after the operation. The PBI was then 5.6 µg per 100 ml.

Case 1 b. – E. N., 70 years old female, recipient. She had a carcinoma of the stomach. PBI was 5.0 µg/100 ml. She had bleeding from her tumour but this was not profuse. Electrophoresis showed an albumin of 3.18 g/%.
Case 2 a. — S. C., 44 years old male, donor. Three years before the test he had been subjected to a subtotal thyroidectomy because of thyrotoxicosis. His Hertel values were then 18 mm on both sides. After the operation he was well for two years. He then developed signs of a suspected relapse of thyrotoxicosis with nervousness, sweating and tiredness. The dominating sign, however, was a severe progressive exophthalmos. Hertel values now were 27 mm on the left and 28 mm on the right side. BMR was + 26 %, PBI 8.1 µg/100 ml and 131I-uptake 45 %. His nervous symptoms were relieved during his stay in hospital and it was concluded that no real relapse of thyrotoxicosis had occurred.

Case 2 b. — S. R., 22 years old male, recipient. This patient had a rather benign chronic ulcerative proctitis. His PBI was 6.3 µg/100 ml.

Case 3 a. — M. L., 46 years old female, donor. Six years before the test she had been operated on for mammary cancer. Five years after the operation she was found to have bone metastases. One year later she developed signs of thyrotoxicosis. She had exophthalmos with a Hertel measurement of 22 mm on both sides. The PBI was 10.0 µg/100 ml, BMR + 27 % and the 131I-uptake 65 %. A subtotal thyroidectomy was performed and later also a bilateral oophorectomy. She was also treated with androgens for her metastases. In connection with this treatment her exophthalmos increased and chemosis of both eyes developed. When the dosage of androgens was reduced the chemosis decreased but her exophthalmos seemed to increase. Her PBI was now 4.8 µg/100 ml, BMR — 3 % and the 131I-uptake 45 %. The sella turcica was of normal size.

Case 3 b. — H. S., 76 years old female, recipient. She had a typical myelomatosis with an increase in the β₂-globuline to 4.32 g/%. X-ray and sternal smear showed typical changes. The PBI was 4.0 µg/100 ml.

Case 4 a. — A. N., 33 years old female, donor. Three years before the test she had had a subtotal thyroidectomy done on account of Graves' disease. Later she developed progressive exophthalmos with Hertel values of 20 mm on the left and 21 mm on the right side and also had an anxiety neurosis. She was euthyroid with a BMR + 8 % and a PBI of 6.0 µg/100 ml. The 131I-uptake was 60 %.

Case 4 b. — K. L., 62 years old male, recipient. One year previously he had been operated on for duodenal ulcer. He was admitted because of a moderately severe barbiturate poisoning. He was euthyroid with a PBI of 4.5 µg/100 ml.

Case 5 a. — L. R., 25 years old male, donor. Since the age of six years he had had diabetes, which had been controlled with a rather high dose of insulin. One year before admission he became restless and complained of profuse sweating and palpitations. His thyroid increased in size and he developed progressive exophthalmos. His BMR was + 30 %, and his 131I-uptake was 70 % (48 hours’ uptake). He was diagnosed as having Graves' disease and a subtotal thyroidectomy was performed. Two months later his exophthalmos increased rapidly and chemosis of the eye lids developed. He had ocular pains, his visual acuity diminished to 0.6 and 0.3 and he had choked retinal discs. The BMR was + 4 %, the PBI 3.9 µg/100 ml and his 131I-uptake 47 %. It was now also found that he had a localized myxoedema of both lower legs.

Case 5 b. — G. J., 52 years old male, recipient. This patient was treated in hospital for peptic ulcer. He was clinically euthyroid. BMR ± 0 %, PBI 4.1 µg/100 ml.

Case 6 a. — A. L., 58 years old male, donor. This patient was admitted to the medical department of the hospital of Sundsvall for a typical Graves' disease with a BMR of + 54 %. He was treated with thiouracil for one year. This improved his condition but his nervousness did not abate. His 131I-uptake was still elevated to 67 % and he was now treated with 7 mc of 131I. When after 4 months the uptake was still elevated,
he received a second dose of $^{131}$I of the same size. Three months later he was euthyroid. At this time his eye balls began to protrude and two months later he had an exophthalmos with a Hertel value of 24 mm bilaterally and marked vascular injection of the sclerae. His BMR was $+33\%$, but the PBI was 3.8 $\mu g/100$ ml and the $^{131}$I-uptake $42\%$.

Case 6 b. -- E. L., 53 years old female, recipient.

Clinically euthyroid patient in the same hospital. PBI 4.5 $\mu g/100$ ml.

Case 7 a. -- E. G., 53 years old female, donor. This patient had a typical thyrotoxicosis of short duration with a slight degree of exophthalmos. BMR was $+31\%$, $^{131}$I-uptake 45 $\%$, PBI 10.0 $\mu g/100$ ml.

Case 7 b. -- J. P., 80 years old female, recipient. She was in hospital for a cerebral vascular accident from which she recovered. She had symptoms of advanced arteriosclerosis dementia. PBI was 6.8 $\mu g/100$ ml.

Case 8 a. -- B. F., 58 years old female, donor. For 6 years this patient had had marked exophthalmos but nothing suggesting thyrotoxicosis. Her thyroid gland was, however, enlarged and adenomatous. Two years ago she developed signs of thyrotoxicosis with a BMR of $+18\%$, a PBI 11.7 $\mu g/100$ ml and a $^{131}$I-uptake of 69 $\%$ (48 hours' uptake). She was treated with thiouacil for 1½ years which resulted in clinical improvement. Her PBI was now 5.8 $\mu g/100$ ml but her exophthalmos was essentially unchanged with Hertel values of 28 mm on the left and 27 mm on the right side.

Case 8 b. -- K. B., 53 years old female, recipient. She was treated in hospital for iron-deficiency anaemia. Clinically she was euthyroid with a BMR of $+2\%$ and a PBI of 4.1 $\mu g/100$ ml.

Case 9 a. -- K. A., male, donor. He was an unselected blood donor from the blood bank. He was clinically euthyroid but with a surprisingly high PBI value of 36.5 $\mu g/100$ ml. This value was reported after the transfusion had been given and was interpreted as due to iodine medication.

Case 9 b. -- E. O., 62 years old male, recipient. This patient was treated for a malignant tumour in the abdomen apparently related to a sarcoma for which he had been operated upon two years previously. He was clinically euthyroid. The PBI was 5.2 $\mu g/100$ ml.

Case 10 a. -- Male donor. Unselected blood donor from the blood bank. Clinically euthyroid. PBI 5.6 $\mu g/100$ ml.

Case 10 b. -- Recipient. The same case as case 3 b. This patient was also given thyrotrophic hormone intramuscularly on two different occasions in a dosage of 20 and 40 USP units respectively.

**RESULTS**

The results are given in Table 1. After transfusion with freshly drawn blood from the cases 1a–6a, a rise of the PBI of the recipients occurred. The increase ranged from 0.9 $\mu g/100$ ml (case 5) to 3.8 $\mu g/100$ ml (case 2). The increase of the PBI level after administration of thyrotrophic hormone (20 and 40 units respectively) was 2.4 and 3.9 $\mu g/100$ ml respectively.

After the transfusion, the PBI in cases 1b–6b started to rise within six hours (Fig. 1). The maximal value was reached from 30 to 54 hours. The maximal value of PBI after one single dose of thyrotrophic hormone was reached 30 hours after the injection.
Table 1.
Time-response relationships of PBI in euthyroid subjects after transfusion of blood from exophthalmic patients and controls.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>PBI in μg/100 ml</th>
<th>Time in hours from transfusion to max. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before transfusion</td>
<td>10 min after transfusion</td>
</tr>
<tr>
<td>1</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>6.3</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>4.2</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>4.5</td>
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<td>7</td>
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<td>6.3</td>
</tr>
<tr>
<td>8</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>9 (control)</td>
<td>5.2</td>
<td>5.7</td>
</tr>
<tr>
<td>10 (control)</td>
<td>4.2</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Fig. 1.
Changes in the PBI-level of the recipients after transfusion of blood from exophthalmic patients and a control subject.

In cases 7 and 8 as well as in the controls (cases 9 and 10) no significant rise of the PBI level could be demonstrated. Case 3 b (= 10 b) was transfused twice, first with blood collected from case 3 a, with a subsequent maximal rise of the PBI to 2.4 μg/100 ml, and then from a control (blood donor) without
any increase in the PBI (Fig. 2). Case 3 b was a patient with multiple myeloma whose response to a thyroid stimulating factor could be doubtful. She was therefore also given two separate injections of thyrotrophic hormone with a normal response (Table 2).

![Graph showing changes in PBI level](image)

**Fig. 2.** Changes in the PBI-level in case 3 b after transfusion of blood from an exophthalmic patient, a control subject and after two separate injections of TSH (20 and 40 USP units respectively).

**Table 2.**

Time-response relationships after single intramuscular injections of TSH in case 3 b.

<table>
<thead>
<tr>
<th>Dose given</th>
<th>PBI in µg/100 ml</th>
<th>Time in hours from injection to maximal value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value before injection</td>
<td>Maximal value</td>
</tr>
<tr>
<td>20 U. S. P.</td>
<td>3.6</td>
<td>6.0</td>
</tr>
<tr>
<td>40 U. S. P.</td>
<td>3.9</td>
<td>7.8</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The rise in the PBI level of the recipients after transfusion of blood from the donors with exophthalmos indicates that the transfused blood contained a factor which stimulated the thyroid of the recipients to increased activity. *Adams & Purves (1957)* and *Adams (1958)* have demonstrated a factor in
serum from patients with thyrotoxicosis and exophthalmos with a prolonged action which they believed to be an abnormal fraction of TSH. The amounts of abnormal thyroid stimulator found in sera correlated more closely with the degree of exophthalmos than with the severity of the hyperthyroidism (Purves & Adams 1960). Munro et al. (1960) also found a long acting thyroid stimulator in sera from patients with untreated thyrotoxicosis as well as in sera from patients with persistent eye signs after treatment of thyrotoxicosis. McKenzie (1960) found evidence of the presence of a thyroid activating substance with a delayed response in the blood of patients with Graves' disease, possibly distinct from thyrotrophin. Attempts to isolate a fraction from pituitary extracts different from TSH and with a separate action on the eye balls have been successful in animal experiments (Dobyns & Steelman 1953; Bates & Condliffe 1960). The factor responsible for the PBI elevation in our cases has a delayed action. Even after 67 hours there is still in most cases a significant elevation of PBI as compared with the starting value.

It should be pointed out that all patients were in the euthyroid state. Cases 1 a – 5 a had been thyroidectomized and case 6 a had been treated with thiouracil and radioiodine in a sufficiently high dose.

Case 7 a had had her disease for only a short time and her exophthalmos was not very conspicuous. Case 8 a had had her exophthalmos for six years when the test was performed and her exophthalmos had been stationary for at least 3 years. In the first 6 cases, the test was performed within 1 year after the patients had started to develop signs of progressive exophthalmos. In 5 of the cases this was in time closely related to thyroidectomy and in the sixth, to treatment with radioiodine in high dosage.

It seems likely that there exists a connection between the progressive exophthalmos and the thyroid stimulating factor shown to be present in the blood in these cases. The negative results in cases 7 a and 8 a could be explained by the fact that in case 7 a, the disease was of short duration and the thyrotoxicosis not under effective control and that in case 8 a, the exophthalmos had not showed any progress for at least 3 years.

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REFERENCES

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