THE RELATIONSHIP BETWEEN THE LEVEL OF CIRCULATING BASOPHIL LEUCOCYTES AND THYROID FUNCTION

By

Satoshi Inagaki

Turin in 1910 reported that the count of circulating basophils decreased in thyrotoxicosis. Subsequently, in 1920, Naegeli stated in his textbook on blood that "fast ausnahmslos vermindert fand sie (basophils) Turin bei Basedow (also trotz Lymphocytose!). In this country Nomura (1928) also reported that basophils nearly always decreased or disappeared in thyrotoxicosis, so that the number of basophils was considered to be a valuable means of estimating the therapeutic effect of irradiation of the hypothalamopituitary system in thyrotoxicosis. They counted the basophils indirectly, by examining the stained blood film as performed in the usual differential leucocyte count. The counting of human basophils, however, is tedious and difficult because they are very scarce and their metachromatic granules are extremely water-soluble.

The author has developed a direct counting method for basophils which will be described below, and has also used this method for investigating the correlation between the number of circulating basophils and thyroid function.

METHODS

The composition of staining and diluting fluid used is as follows:

1% Toluidine blue in distilled water 6 ml.
10% Lead acetate solution in distilled water 6 ml.
3% Acetic acid in distilled water 10 ml.
Distilled water ad 100 ml.

The solution is made by mixing and filtering, and will keep for a long time.

Fresh unclotted blood obtained by ear-lobe puncture is diluted with the above mentioned counting fluid in a proportion of one to 10 in a standard leucocyte pipette. The pipette is shaken manually for 2 minutes. The 4 chambers of Fuchs-Rosenthal
hemocytometer are filled by capillary action in the usual manner. The counting
chambers are allowed to stand for 5 minutes so that the leucocytes can settle. A mag-
nification of $15 \times 10$ or $5 \times 40$ is used and all the 4 chambers are counted. The
number of basophils counted in all of the 4 chambers is totaled, averaged and multi-
plied by the dilution factor, 3.125, which gives the absolute count in terms of basophils
per cmm.

The blood was obtained between 8 and 9 a.m. in basal metabolic state.

Observations were made on patients suffering from thyrotoxicosis, myxoedema,
simple goitre and from several other diseases diagnosed from the clinical symptoms
and signs and from several thyroid function tests. They had had no treatment for
thyroid disease until they were seen in this clinic. The estimation of the number
of basophils (BL), serum protein-bound iodine (PBI) and of the basal metabolic rate
(BMR) were made at the same time.

RESULTS

1) Basophil Counts in Normal Adults and in Untreated Patients with
Thyrotoxicosis, Myxoedema and Simple Goitre

The normal level of circulating basophils was obtained from 95 healthy
subjects. These included 53 males and 42 females.

The mean absolute basophil count was $32.8 \pm 18.0$ (S. D.) per cmm. In the
male group, ranging in age from 21 to 62 years, the mean absolute basophil
count was $35.3 \pm 20.3$ (S. D.) per cmm. The counts ranged from 12 to 101 per
cmm. In the female group, ranging in age from 18 to 53 years, the mean
absolute basophil count was $29.7 \pm 13.9$ (S. D.) per cmm. The counts ranged
from 13 to 62 per cmm. The difference between the mean values of these 2
groups was not statistically significant. These data are summarized in Table 1.

Twenty cases of thyrotoxicosis showed a marked decrease in the basophils.
The mean absolute basophil count was 9.7 per cmm. and ranged from 1 to 20
per cmm. Twelve cases were lower than 10 per cmm.

In the seven cases of myxoedema the basophil count was above the normal
range. The mean absolute basophil count was 46 per cmm., ranging from 33
to 69 per cmm. In the cases of simple goitre, the count was in the normal range.

The values obtained in these patients are shown against serum protein-bound
iodine (PBI) and basal metabolic rate (BMR) in Figs. 1 a and b.

<table>
<thead>
<tr>
<th>Normal subjects 95.</th>
<th>32.8 $\pm$ 18.0 per cmm</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Sex</td>
</tr>
<tr>
<td>53</td>
<td>M.</td>
</tr>
<tr>
<td>42</td>
<td>F.</td>
</tr>
</tbody>
</table>
II) The Relationship of the Number of Circulating Basophils to the Serum Protein-Bound Iodine Value and to the Basal Metabolic Rate

As shown in Figs. 1 a and b, there is a correlation between the number of basophils in the peripheral blood and the thyroid function. These figures were obtained exclusively from patients with thyroid diseases before the treatment.
The same relationship, however, is observed in thyroid patients even after treatment. Fig. 2a shows the correlation between the basophil count and the serum protein-bound iodine level in patients both before and after treat-

---

**Fig. 2a and b.**

*Relationship between basophils (BL) and serum protein-bound iodine (PBI) in thyrotoxicosis and myxoedematous patients before and after treatment.*

*b. Relationship between basophils (BL) and basal metabolic rate (BMR) in thyrotoxicosis and myxoedematous patients irrespective of treatment.*
ment. Fig. 2 b shows the correlation between the basophil count and the basal metabolic rate in the same patients as shown in Fig. 2 a.

III) The Relationship of the Number of Basophils to the Course of Treatment of Thyrotoxicosis and Myxoedema

i) Thyrotoxicosis

The number of basophils was determined in 6 cases of thyrotoxicosis during treatment with methylthiouracil. It was observed that when the treatment was successful, the basophil counts increased. There was usually an inverse relationship between the basophils and serum protein-bound iodine or basal metabolic rate. These data are shown in Fig. 3.

Patients 1, 2 and 3 (Figs. 3 a, b and c) were classified as cases of mild thyrotoxicosis. Initial number of the basophils was 6, 12 and 16 per cmm., respectively.

Patient 1. I. T., male, 35. This patient was given 300 mg. of methylthiouracil a day for one month, after which the basophil counts were 22 per cmm. (Fig. 3 a).

Patient 2. S. N., female, 44. The patient was given 375 mg. of methylthiouracil per day for one month and then 300 mg. daily for one month. Three months later, the basophils increased to 57 per cmm. (Fig. 3 b).

Patient 3. N. I., male, 41. The patient was given 300 mg. of methylthiouracil per day for 2 weeks and then 375 mg. per day for 4 weeks, at the end of which period the basophils were 37 per cmm. (Fig. 3 c).

The patients had a good remission of their symptoms, and the serum protein-bound iodine and basal metabolic rate had dropped to the normal range.

Patient 4. H. K., female, 25. This patient had had fairly severe thyrotoxicosis for two years before treatment. Initial basophils were 7 per cmm. She was treated with 375 mg. of methylthiouracil a day for one month and then 300 mg. per day continually. Three months later, the basophils were 19 per cmm. (Fig. 3 d). Her symptoms improved, and the serum protein-bound iodine and basal metabolic rate has fallen to the normal range.

Patient 5. M. S., female, 21. The disease in this patient was classified as active and severe. Initial basophils were 9 per cmm. In spite of the ingestion of 300 mg. of methylthiouracil daily for more than three months, the basophils have not increased (Fig. 3 e). There was no change in any of her symptoms or physical signs at the end of this period.

Patient 6. G. Y., male, 25. This patient was also classified as a case of severe thyrotoxicosis. Initial basophils were 5 per cmm. He was treated with 450 mg. of methylthiouracil daily for two months. At the end of one month, after an increase to 25 per cmm., the basophils rapidly decreased. At that time granulocytopenia was observed in the peripheral blood. He was then treated with a therapeutic dose of radioactive iodine, and by the end of three months had had a fair remission, and the basophil count was 34 per cmm. (Fig. 3 f).

ii) Myxoedema

As to the 5 cases of myxoedema treated with dried thyroid or l-thyroxine sodium, it was found that when treatment was successful, the basophil counts decreased. The relationship between the basophils and serum protein-bound
Fig. 3 a-f.

Showing changes after administration of methylthiouracil in basophils (BL), serum protein-bound iodine (PBI) and basal metabolic rate (BMR) in thyrotoxicosis.
iodine or basal metabolic rate was almost always reciprocal. These data are shown in Fig. 4.

Fig. 4 a-e.
Showing changes after the administration of dried thyroid or l-thyroxine sodium in basophils (BL), serum protein-bound iodine (BPI) and basal metabolic rate (BMR) in myxoedema.
Patients 1 and 2 were classified as fully developed spontaneous adult myxoedema.

Patient 1. H. Y., female, 26. Initial number of basophils was 56 per cmm. She was given 40 mg. of dried thyroid per day for 20 days, after which the basophils were 5 per cmm. About one month later, the basophils were 43 per cmm. She was then treated with 0.2 mg. of l-thyroxine sodium daily for one month, at the end of which period the basophils were 10 per cmm. (Fig. 4 a).

Patient 2. H. I., female, 29. The initial number of the basophils was 69 per cmm. She was given 0.2 mg. of l-thyroxine sodium per day for 17 days and then 0.3 mg. daily for 13 days, at the end of which time the basophils were 35 per cmm. (Fig. 4 b).

Patient 3. K. K., female, 13. The patient was a case of juvenile myxoedema. The initial number of the basophils was 33 per cmm. The patient was given 0.1 mg. of l-thyroxine sodium per day for 10 days and then 0.2 mg. daily for 17 days, after which the basophils were 5 per cmm. (Fig. 4 c).

Patients 4 and 5 were cases of Hashimoto’s disease. The initial number of the basophils were 39 and 38 per cmm., respectively.

Patient 4. Y. Y., female, 60. She was treated with increasing doses of l-thyroxine sodium up to 0.4 mg. per day for about one month, at the end of which time the basophils were 6 per cmm. (Fig. 4 d).

Patient 5. K. Y., female, 47. This patient was given increasing doses of l-thyroxine sodium up to 0.5 mg. daily for about 2 months by which time the basophils were 20 per cmm. (Fig. 4 e).

IV) Relationship Between the Number of Basophils and Thyroid Hormone

The above data show the existence of an intimate correlation between the number of basophils and thyroid hormone. In order to clarify this relationship, the author counted the number of basophils in normal subjects and in cases of nephrosis and thyrotoxicosis before and after the administration of thyroid hormone.

i) Normal Subjects

Three normal subjects who were treated with dried thyroid or l-thyroxine sodium were investigated. They were in a euthyroid state as revealed by the thyroid function tests and serum protein-bound iodine or basal metabolic rate. Initial basophils were all in the normal range. A decrease in the number of basophils always occurred after treatment and a rapid increase at cessation of medication (Fig. 5). Serum protein-bound iodine and basal metabolic rate increased slightly.

ii) Nephrosis

Nephrosis is similar to myxoedema in showing a decrease in the serum protein-bound iodine, basal metabolic rate and an increase in the serum cholesterol. Nine cases of nephrosis showed a marked increase in the basophils. The mean absolute count was 85 per cmm., ranging from 135 to 49 per cmm. The two
Fig. 5

Showing changes in basophils (BL), serum protein-bound iodine (PBI) and basal metabolic rate (BMR) in normal subject after treatment with l-thyroxine sodium.

Fig. 6.

Showing changes in basophils (BL), serum protein-bound iodine (PBI) and basal metabolic rate (BMR) in patient with nephrosis, after treatment with dried thyroid.
cases of nephrosis were treated with dried thyroid. The decrease in the number of basophils was accompanied by an increase in serum protein-bound iodine and basal metabolic rate. One of these cases is shown in Fig. 6.

iii) Thyrotoxicosis

Each of the five cases of thyrotoxicosis was given 300 mg. of dried thyroid per day for two weeks (in one case this was extended to three weeks). The number of basophils decreased in one case of mild thyrotoxicosis, but in other cases it did not change or only slightly increased. These data are shown in Fig. 7.

COMMENTS AND CONCLUSIONS

Asboe-Hansen (1950) states that in myxoedema and in a rare condition called circumscribed thyrotoxic myxoedema, there is an accumulation of hyaluronic acid and large mast cells, and that in thyrotoxicosis there is usually no demonstrable hyaluronic acid in the dermal connective tissue and only a few small mast cells. Chemical and histochemical studies have shown that mast cells contain heparin and that they are the only type of cell in the connective tissue containing this substance. On the basis of the similarity in chemical structures between heparin and hyaluronic acid, Asboe-Hansen now suggests
that the heparin or a heparin-like precursor is secreted by the mast cells into the tissue where it is gradually desulphated and becomes hyaluronic acid; that it appears to be beyond doubt that the thyroid hormone exerts a regulating influence on the hyaluronic acid content of dermal connective tissue, and that it is reasonable to assume that the mast cells play a rôle as the peripheral transmitters of hormonal action, while their localization in the vascular walls may support this interpretation.

The relationship between tissue mast cells and blood basophils has not yet been fully elucidated (Riley, 1954). But as it has already been shown that heparin is contained in both mast cells and basophils, Asboe-Hansen’s suggestion and the present author’s study definitely indicate that not only mast cells but also basophils are closely connected with thyroid hormone.

Andersen et al. (1955) suggest that the histological examination of the dermal connective tissue is valuable as a diagnostic test of hypothyroidism, because there is an accumulation of mucinous substances in the connective tissue ground substance and an accumulation of mast cells which contain similar mucinous substance, and there are also several other signs which are more or less constant.

The results of this study indicate that there is a close correlation between the basophils of the blood and thyroid function.

This suggests that the estimation of the basophil count is of value as a thyroid function test.

ADDENDUM

The author has subsequently improved the staining and diluting fluid for the direct counting method of basophils. By the use of this fluid, the contrast with the other leucocytes is made clearer, and counting becomes easier than with the former fluid. With regard to the counting of basophils in rabbits, however, the former fluid seems to be more satisfactory.

The composition of the improved staining and diluting fluid is as follows:

- 1% Toluidine blue in distilled water 5 ml.
- 1% Lead acetate solution in distilled water 10 ml.
- 1% Acetic acid in distilled water 50 ml.
- Distilled water ad 100 ml.

The solution is made by mixing and filtering, and will keep for a long time.

SUMMARY

The author, using lead acetate solution for fixing the basophil leucocytes has devised a new direct counting method for the basophil leucocytes in the circulating blood.
The present investigation was carried out in order to determine whether there is a correlation between the basophils of the blood and thyroid function, as estimated by the author's direct method for counting the basophils.

1) In almost all cases of thyrotoxicosis, the absolute basophil count is markedly decreased, in myxoedema increased beyond the normal range, while in struma simplex it is within the normal range.

2) After methylthiouracil therapy, the basophil count was markedly increased in thyrotoxicosis. There was a good correlation between the basophil count and the decrease in serum protein-bound iodine or basal metabolic rate, and the improvement of symptoms.

3) In myxoedema, after treatment with dried thyroid or thyroxine the basophil count was markedly decreased. A fair correlation was noticed between the decrease in the basophils and the increase of serum protein-bound iodine or basic metabolic rate, and the improvement of symptoms.

4) Dried thyroid treatment caused the basophil count to decrease in nephrosis and in cases of euthyroid, but when treatment was discontinued, it was rapidly restored.

5) In nephrosis the basophil count increased to above the mean absolute basophil count of normal subjects.

6) In thyrotoxicosis after treatment with dried thyroid, the basophil count showed no change or only increased slightly.

7) The above results demonstrate that there is a good correlation between the basophils of the blood and thyroid function, indicating that the estimation of the basophil count is valuable as a thyroid function test.

ACKNOWLEDGMENT

The author wishes to express his thanks to Professor T. Torikai of Niigata University School of Medicine, for much helpful advice and criticism.

REFERENCES