The Histochemical Study of Zinc Content in Granulocytes in Normal Adults and in Hematologic Disorders

By Stanislaw Szmigielski and Joanna Litwin

The physiologic and biochemical role of zinc (Zn), as a cofactor of many important enzymes (carboxypeptidase, dehydrogenases, alkaline phosphate, carboanhydrase) is now well established, but our present knowledge of zinc metabolism and transport in the human body is still incomplete. Vallee estimated Zn content in whole blood at 880 gamma/100 ml. He also showed the existence of an exchange of Zn between the plasma proteins (containing 12 per cent of whole blood Zn) and the Zn compounds in erythrocytes and leukocytes (accounting for about 85 per cent and 3 per cent of whole blood Zn, respectively). The individual granulocytes, especially eosinophilic or basophilic, are relatively rich in Zn (14.2 gamma of Zn per 10^6 cells) and probably contains more Zn than does any other cell in the body. A protein containing 0.3 per cent of Zn, accounting for about 80 per cent of the granulocyte's Zn, has been extracted from normal leukocytes by Hoch and Vallee. This Zn containing protein is probably an enzyme, but the nature of its enzymic activity is still unknown. It has no carboanhydrase activity. Gibson and Vallee reported decreased values of Zn in leukemic leukocytes and Dennes and Tupper found most leukemic cells contained approximately only half the amount of Zn found in normal leukocytes separated from blood by the use of dextran technic. The difference was statistically significant. The histochemical demonstration of Zn in blood and bone marrow smears was performed by Mager and McNary. These investigators described a method for histochemical detection of Zn in blood cells and discovered decreased amounts of Zn in leukemic granulocytes. Quantitative estimations, however, were not done.

Zinc in Granulocytes

The present report presents the results of histochemical (dithizone) determinations of Zn in blood and bone marrow smears in normal adults and in patients with various hematologic diseases. The "score" method for quantitative determination was used.

Material and Methods

The dithizone method for histochemical demonstration of Zn as described by McNary was used. Blood was obtained by finger prick and bone marrow by sternal puncture. The samples were smeared on chemically clean slides and rapidly air dried. After 1 hour the smears were dipped quickly three times in distilled water and dried. The hemolysed smears were then stained for 7-10 minutes in dithizone-acetone solution.

Staining solution:
0.02 per cent dithizone in absolute acetone 24 ml.
ZINC CONTENT IN GRANULOCYTES

**Fig. 1.**—“Score” method for Zn in granulocytes.

<table>
<thead>
<tr>
<th>Zn 0</th>
<th>Zn 1</th>
<th>Zn 2</th>
<th>Zn 3</th>
<th>Zn 4</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Image of granulocytes" /></td>
<td><img src="image" alt="Image of granulocytes" /></td>
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<td><img src="image" alt="Image of granulocytes" /></td>
</tr>
</tbody>
</table>

18 ml.

Zinc-free water

pH was adjusted 3.7 with 1 N acetic acid complex-forming buffer

20 per cent sodium potassium tartrate

Complex-forming buffer:

- Sodium thiosulfate penta hydrate
- Sodium acetate tri hydrate
- Potassium cyanide

Dissolve in zinc-free water

Adjust pH 5.5 with glacial acetic acid

Add zinc-free water to make 2000 ml.

Zinc-free water was prepared from redistilled water by use of ion exchange Amberlite-OR-120.

After 7–10 minutes, smears were rinsed three times in distilled water, stained 15 minutes in 0.1 per cent aqueous azur II and mounted in glycerol-gel. The results were expressed as a “score.” The cells with no red granules were classified as Zn 0, and those with increasing amounts of red granulation as Zn 1, 2, 3 and 4 (fig. 1).

One hundred metamyelocytes, 100 juvenile and 100 polymorphonuclear granulocytes were counted in every case. The determination of Zn was performed both in bone marrow and peripheral blood.

The normal values of Zn in granulocytes were obtained from ten healthy adults (blood donors). Histochemical determination of Zn in granulocytes was performed in 62 various hematologic disorders: chronic granulocytic leukemia, 18 cases; acute myeloblastic leukemia, 14; multiple myeloma, 9; chronic lymphocytic leukemia, 4; Hodgkin’s disease, 8; and myelosclerosis, 9 cases. Diagnosis was based on clinical and biochemical investigations (bone marrow biopsy being a standard procedure). Lymph node biopsy was also performed when necessary.

**Results**

**Normal Values**

Red dithizone-Zn granules appeared in metamyelocytes, juvenile and polymorphonuclear granulocytes. Myelocytes occasionally contained dithizone positive material in quantities no more than 7–10 score. Myeloblasts and pro-
All results are presented in figure 2 where average values are also drawn.

Fig. 2.—Zinc in granulocytes of bone marrow and peripherical blood in normal adults and in hematologic disorders.

myelocytes contained no Zn. The values of Zn in normal granulocytes are presented in figure 2. Besides granulocytes, the dithizone positive material was found in mast cells, reticulum cells, and in lymphoid reticulum cells. Monocytes occasionally contained a few red granules. Small lymphocytes, lymphoblasts, plasmocytes and erythroblasts of all types contained no Zn.

The normal values of Zn in granulocytes in human bone marrow are as follows:

<table>
<thead>
<tr>
<th>Average Values</th>
<th>Standard Deviation*</th>
<th>$\frac{1}{\sqrt{n}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metamyelocytes</td>
<td>93.8 score</td>
<td>± 8.21</td>
</tr>
<tr>
<td>Juvenile</td>
<td>111.6 score</td>
<td>± 7.31</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td>138.8 score</td>
<td>± 14.32</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

Mature granulocytes in peripheral blood contained about 30 per cent more Zn than did granulocytes in bone marrow. Average value 178.5 standard deviation 12.42.

Hematologic Disorders

All results are presented in figure 2 where average values are also drawn.
ZINC CONTENT IN GRANULOCYTES

**Average Values** | **Standard Deviation**
--- | ---

### Chronic granulocytic leukemia—18 cases
- **Metamyelocytes**: 62.0 score / 13.75 / 3.2
- **Juvenile**: 78.3 score / 10.31 / 2.2
- **Polymorphonuclear**: 93.1 score / 11.96 / 2.7
- **Blood granulocytes**: 101.1 score / 11.13 / 2.6

### Acute myeloblastic leukemia—14 cases
- **Metamyelocytes**: 43.8 score / 11.77 / 3.2
- **Juvenile**: 49.5 score / 13.05 / 3.5
- **Polymorphonuclear**: 63.6 score / 18.54 / 5.1
- **Blood granulocytes**: 72.4 score / 16.91 / 4.6

### Multiple myeloma—9 cases
- **Metamyelocytes**: 28.8 score / 5.36 / 1.8
- **Juvenile**: 39.1 score / 7.14 / 2.4
- **Polymorphonuclear**: 45.0 score / 7.92 / 2.8
- **Blood granulocytes**: 52.3 score / 8.54 / 2.8

### Hodgkin's disease—9 cases
- **Metamyelocytes**: 66.1 score / 8.41 / 2.8
- **Juvenile**: 78.6 score / 10.15 / 3.4
- **Polymorphonuclear**: 93.4 score / 7.80 / 2.6
- **Blood granulocytes**: 108.0 score / 8.31 / 2.8

### Myelosclerosis—8 cases
- **Metamyelocytes**: 166.4 score / 25.51 / 9.1
- **Juvenile**: 200.6 score / 22.38 / 7.9
- **Polymorphonuclear**: 251.8 score / 27.41 / 9.8
- **Blood granulocytes**: 213.5 score / 21.91 / 7.9

### Chronic lymphocytes leukemia—4 cases
- **Metamyelocytes**: 126.2 score / — / —
- **Juvenile**: 160.4 score / — / —
- **Polymorphonuclear**: 170.2 score / — / —
- **Blood granulocytes**: 199.5 score / — / —

**Discussion**

The results presented in this paper indicate that Zn is incorporated into metamyelocytes and from this moment the content of the metal increases during the maturation of the granulocytes. It is probable that the Zn-protein extracted from normal granulocytes by Hoch and Vallee⁴ is synthesized in metamyelocytes, or Zn is incorporated into the protein in these cells. Since about 80 per cent of the total granulocytes' Zn is found in this Zn-protein and only about 10 per cent in alkaline phosphatase, determination of Zn in granulocytes is independent of the alkaline phosphatase activity.

Erythroblasts of all types, including normoblasts, contain no dithizone-positive material. This indicates that active carboanhydrase appears only in erythrocytes. In this method, mature erythrocytes are hemolysed by distilled
Fig. 3.—Neutrophil leukocytes in chronic myelogenous leukemia. Bone marrow stained with dithizone.

water. The importance of lacking erythrocytes was stressed by McNary. Lymphocytes and lymphoblasts contain no Zn as it was described by McNary. Presence of dithizone-positive material in reticulum cells, both young and mature, make it possible to perform the histochemical differentiation of reticulum cells from lympho- and myeloblasts, the latter being deprived of Zn. Decreased values of Zn in chronic granulocytic leukemia are in conformity with the results obtained by Gibson and Vallee, Dennes and McNary. Statistically significant decreases of Zn in granulocytes were obtained both in bone marrow and peripheral blood. During the remission of leukemia, the Zn content increased, but it was still below the normal values. Our studies confirmed previous observations that the amount of Zn in granulocytes is decreased in multiple myeloma, acute myeloblastic leukemia and Hodgkin's disease.

In myelosclerosis the Zn content of bone marrow granulocytes was considerably elevated, while in peripheral blood granulocytes, the increase of Zn was not so marked. It is of interest that in the cases of myelosclerosis, peripheral blood granulocytes contained Zn 0, 1 and Zn 3 and 4 score, while bone marrow granulocytes were predominantly Zn 3 and 4. It is possible that Zn 0 and 1 granulocytes, morphologically similar to leukemic granulocytes are produced by extramedullary foci of hemopoiesis.

In four cases of chronic lymphocytic leukemia investigated by us, an increased amount of Zn was found in granulocytes, while Dennes and Tupper found that the Zn content in leukocytes separated from blood with use of Dextran is approximately inversely proportional to the number of leukocytes. Because lymphocytes, both normal and leukemic, contain no Zn, the decreased
ZINC CONTENT IN GRANULOCYTES

Fig. 4.—Neutrophil leukocytes in myelosclerosis. Bone marrow stained with dithizone.

amount of Zn obtained by Dennes was probably due to determination of Zn in all leukocytes separated from blood which contained predominantly lymphocytes. Individual granulocytes in these cases contain more Zn than in normal individual.

CONCLUSIONS

1. Zn is incorporated into metamyelocytes and its amount increases in maturation of granulocytes. In granulocytes from peripheral blood Zn content is about 30 per cent higher than in bone marrow granulocytes.

2. Zn is present in mast cells and reticulum cells but not in plasmacytes.


SUMMARY

Zinc in granulocytes was determined using a dithizone histochemical method. Investigations were performed in both bone marrow and in peripheral blood from ten healthy adults and in 62 patients with various hematologic disorders. In bone marrow, zinc appears in metamyelocytes. The amount increases with maturation. In the granulocytes of peripheral blood, zinc values are about 30 per cent higher than in bone marrow.

Zinc is significantly decreased in granulocytes in chronic granulocytic leukemia, acute myeloblastic leukemia, multiple myeloma and Hodgkin’s and increased in chronic lymphocytic leukemia and osteomyelosclerosis.
SuMMA.1uO IN INTERLINGUA

Le contento de zinc de granulocytos esseva determinate per medio de un methodo histochimic a dithizona. Le investigationes esseva executate in medulla ossee e in sanguine peripheric ab dece adultos normal e ab 62 patientes con varie disordines hematologic. In le medulla ossee, zinc appare in metamyelocytos. Le quantitate cresce con le processo maturatori. In le granulocytos del sanguine peripheric, le valores pro zinc es circa 30 pro cento plus alte que in le medulla ossee.

Zinc es reducite significativemente in le granulocytos in chronic leucemia granulocytic, acute leucemia myeloblastic, myeloma multiple, e morbo de Hodgkin; illo es augmentate in chronic leucemia lymphocytic e osteomyelosclerosis.

REFERENCES


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