INTRODUCTION

Aplastic anemia has been considered to be rare since this disease was first described by Ehrlich. And it is well known that there are many obscure points on the origin of aplastic anemia and no effective treatments of the disease have been found. Now we believe that it is one of the most interesting and difficult disease in clinical hematology and pathology.

Moreover it is noticed that the disease is reported all over the world with a tendency of increase. Although the recent improvement in the hematological and biochemical investigations, there are a few cases in which the differential diagnosis between the two diseases is difficult and it can be only assured by autopsy.

Erythrocytes in both diseases were studied by cytospectrophotometric method. Some interesting results obtained from this investigation are reported in the present paper as the data should be useful to the understanding of the differential diagnosis and the origin of aplastic anemia.

METHODS AND MATERIALS

1. Method

The erythrocytes on the smears of peripheral blood samples of normal healthy people and of various anemia are marked under the oil immersion.

The Leitz’s microspectroscope is installed in the microphotographic apparatus with these slides, the spectra are photographed and the plates obtained are measured by the recording microphotometer.

Some erythrocytes which is almost the same in sizes, forms and colors are microscopically selected.
This apparatus is designed to photograph five spectra by slipping the plate, and the densities on the same wavelength of every spectra on the plate are measured and the differences from control are plotted on every wavelength, so the absorption curve of the searched substance is obtained. Moreover the light intensity, exposure time and width of slit kept the same. The portion with no blood of the objective glass is used as control. The exposing time within one minute is suitable considering the specific curve of the used Fuji’s panchromatic process plates.

2. Material

The unstained peripheral blood smears of 5 normal and 22 various anemic patients as follows are fixed by formalin vapor.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No. of case</th>
<th>No. of autopsy case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplastic anemia</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Leucemia</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Aplastic anemia or leukemia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Morbus Banti</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anemia due to iron-deficiency</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>22</strong></td>
<td><strong>12</strong></td>
</tr>
</tbody>
</table>

All these cases were hospitalized in our hospital and their blood smears are furnished by Dr. Hasegawa, instructor of the internal medicine. The 12 cases among these are observed by the usual pathological studies.

RESULTS

The obtained absorption curves by the above mentioned microspectroscopic studies can be classified into the following 3 groups. Avoiding the complexity, three cases are summed up in every figure.

1. The I group (Fig. 1)

All the absorption curves in Fig. 1 are of normal erythrocytes and their characters are noticed as follows.

a) The most intense absorption peak can be found on 400–420 mμ with the slight difference of density.

b) Mostly the peaks on 510–540 mμ with lower density than (a).

c) It is regarded that the peaks are also seen on 580–620 mμ in spite of considerable variations.
2. The II group (Fig. 2)

The absorption curves belonged to this group have the characteristics which are so different to be distinguished from those of the group I. That is,

a) The most intense peak is shown on 400–420 m\(\mu\) as the group I, but their densities are slightly lower.

b) and c) are the same as the group I.

The absorption curves of this group are mostly derived from the erythrocytes of the patients except aplastic anemia.

3. The III group (Fig. 3)

These curves have the distinctly different characteristics from the I and II
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group. Namely,

a) In comparison with the I and II group, no intense absorption peak can be found on 400–420 m\(\mu\).

b) The other peaks have the same tendencies as the I and II group on 510–540 m\(\mu\) and 580–620 m\(\mu\).

Fig. 3. Absorption spectra of a single erythrocyte (III group)

The erythrocytes with these absorption curves are ones in aplastic anemia and its suspicious cases.

Now the absorption spectra of hemoglobin and its derivatives are well known. Oxyhemoglobin has an alpha band on 579 m\(\mu\), a beta band on 542 m\(\mu\) and a much stronger absorption band, the gamma or Soret band with its peak on 415 m\(\mu\). On reduced hemoglobin the alpha and beta bands are fused with each other and the Soret band is moved to 425 m\(\mu\).

So we are able to suppose that the absorption curves of erythrocytes have the characteristics of hemoglobin and the most intense peak corresponds to the Soret band. Metcalf has also come to the same conclusions on this point by almost the same method.

Comparing the cytospectrophotometric studies on erythrocytes in cases with their clinical and pathological diagnosis, the results obtained are summarized as in Table 1. The erythrocytes in aplastic anemia which were verified by clinical and pathological observations have the absorption curves of the group III as the table shows.

Namely, the absorption curves of the erythrocytes in aplastic anemia have no intense absorption of the Soret band as differing from those in the other patients, while those in leukemia represent the curves of group II. Therefore the erythrocytes in aplastic anemia are cytospectrophotometrically distinct from those in leukemia.
Table 1. Relation between the clinical and pathological diagnosis of the cases and absorption group of their erythrocytes

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>56 M Chr. Myel. Leucemia</td>
<td>Myel. Leucemia</td>
<td>II</td>
</tr>
<tr>
<td>2</td>
<td>47 F Aplas. Anemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>3</td>
<td>25 M Leucemia</td>
<td>Subacut. Myel. Leucemia</td>
<td>II</td>
</tr>
<tr>
<td>4</td>
<td>65 F Heart disease</td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>5</td>
<td>60 F Aplas. A. or Myel. Leucemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>6</td>
<td>48 M Hodgkin’s disease</td>
<td>Lymphogranulomatosis</td>
<td>II</td>
</tr>
<tr>
<td>7</td>
<td>47 M Morbus Banti</td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>8</td>
<td>23 F Aplas. Anemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>9</td>
<td>25 F Aplas. Anemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>10</td>
<td>31 M Aplas. Anemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>11</td>
<td>22 F A. due to iron-deficiency</td>
<td>Aplas. Anemia</td>
<td>II</td>
</tr>
<tr>
<td>12</td>
<td>18 M Chr. Myel. Leucemia</td>
<td>Chr. Myel. Leucemia</td>
<td>II</td>
</tr>
<tr>
<td>13</td>
<td>37 F Aleuc. or Aplas. Anemia</td>
<td>Myel. Aleucemia</td>
<td>II</td>
</tr>
<tr>
<td>14</td>
<td>14 M Myel. Leucemia</td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>15</td>
<td>75 M Morbus Banti</td>
<td></td>
<td>II</td>
</tr>
<tr>
<td>16</td>
<td>44 M A. due to iron-deficiency</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>17</td>
<td>42 F Aplas. Anemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>18</td>
<td>57 M Aplas. Anemia</td>
<td>Aplas. Anemia</td>
<td>III</td>
</tr>
<tr>
<td>19</td>
<td>54 M A. due to iron-deficiency</td>
<td>Aplas. Anemia</td>
<td>II</td>
</tr>
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<td>20</td>
<td>31 M Myel. Leucemia</td>
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</tr>
</tbody>
</table>

DISCUSSION

It is a well known fact that the microspectroscopic studies with ultraviolet ray on the biological field as studied by Caspersson (1936), various results were reported in connection with the metabolism of nucleic acid. On the other hand the microspectroscopic observation in the visible range has been out of the question, but only Jope applied at first this method on the biological materials and Metcalf (1948) has observed the intracellular hemoglobin and the eosinophile leucocytes in camel by the same method.

It is natural that the degree of reliability of the results thus far obtained depended upon the purity of the material and preciseness of the spectroscopic apparatus we used in our experiment. It may be so in chemical studies where purity of the material is paramaunt importance. But in studies related with...
biological materials such as substances in vivo under natural conditions chemical
purenness and homogeneity of a material can not be expected.

The studies on the eosinophilic granulation as a part of cytospectro-
photometric studies of the blood cells were reported by Watanabe in another
paper. In that report he discussed that variations of their absorption curves
were ascribed to the superpositions of the optical conditions, for instance,
number and form of granulation in slit, both absorption and refraction within
granules and reflexion and scattering from their surfaces. In these spectroscopic
studies, it is most noteworthy whether the materials studied are homogenous or
not, and it will be inevitable that the nonhomogenous materials show modified
spectra of their chemical constituents. The spectra of molecules were invested-
gated on this point by Caspersson, his coworkers and Jope.

Erythrocytes are considered to be the most suitable ones for cytospectro-
photometry among biological materials, as they are more homogenous than
eosinophilic granulations and many detailed spectral data of hemoglobin have
been reported which gave us bases for our studies further.

The spectral absorption of hemoglobin has two distinct groups: one, in the
ultraviolet range, is due to the aromatic amino acids of the protein; the other,
a system of bands in the near ultraviolet and visible ranges, is due to the
haem group. Absorption band in the ultraviolet is beyond the sphere of our
discussion at present. The band system due to the haem group is responsible
for the color of hemoglobins and consists of several bands in the visible range,
and the most intense absorption band is called the gamma or Soret band.

However Hoppe-Selye advocated in 1877 that hemoglobin in erythrocytes
is different from that in solution. After that this idea was reinspected by
Adams et al. in 1941 on the basis that the Soret band may be impossible to be
obtained from suspension of horse erythrocytes and the absorption band was
first made clear in dissolving hemoglobin.

Since Keilin, Hartree, Thorell and Jope used better experimental apparatus,
and as a result of their studies they came to a conclusion against Adams's
hypothesis that the Soret band of hemoglobin was noticed even in a suspension
and so that intracellular hemoglobin must be the same as extracellular hemoglobin.
Recently Metcalf has also reported his microspectrographic study that the Soret
band of hemoglobin is demonstrable in individual erythrocytes and is not affected
by fixing and staining and that hemoglobin in cell and solution is identical
spectrographically.

But against Adams's opinion Jope proposed as follows: the bands due to
the haem group also dependent to some extent upon the nature of the protein
carrier, and probably upon the manner of linkage between protein and haem. And the relation between the nature of the protein and the wavelength and intensity of these absorption bands due to the haem group may prove to be related to the nature of the linkage between protein and haem. Its further study may help to clarify such relations. Ponder has also referred in his detailed studies on hemolysis that stromatin is a gel-like matrix occupying the interior of red cell and we agree with a view that the phenomenon of hemolysis consists in breakdown of such a compound rather than the membrane becoming permeable to hemoglobin.

Although this problem is at present being further studied, the results thus far obtained by our microspectrophotometric studies on erythrocytes it is certain that the erythrocytes in aplastic anemia have no absorption waves corresponding to the Soret band (the group III), while erythrocytes in normal, leukemia and the other anemic patients have distinct absorption band corresponding to the Soret band (the group I and II).

Among the erythrocytes in aplastic anemia there are a few which show absorption curves belong to the group II. A large number of erythrocytes in each case must be studied and the percentage of absorption group must be found further. Our optical apparatus however is not perfect. However these results are fairly useful for the differential diagnosis with a single erythrocyte between leukemia and aplastic anemia.

Hasegawa and Honda reported results on the Soret band by Beckman’s Model DU photoelectric quartz spectrophotometer. The used specimens are hemoglobin solution, erythrocytes (fixed in gelatine gel) and protoporphyrin (extracted by Grinstein Wintrobe’s method) in normal, aplastic anemia and the other anemic patients. It has been described on their paper that hemoglobin solution has always showed the maxima of absorption bands on 415 mµ but the erythrocytes in aplastic anemia have their maxima on shorter region and especially on 409 mµ in the cases without blood transfusion.

It is very interesting fact that the erythrocytes in aplastic anemia have the different absorption data from ones in normal, leukemia and the others by the both methods with microspectroscope and Beckman’s spectrophotometer. Though the spectral data of both methods are not identical, it may be due to the differences of treatments of specimens and methods of investigation. Now it could be supposed that the reason of the extraordinary absorption figure in aplastic anemia is ascribed to the difference on the combination between stromatin and hemoglobin. That is, as the erythrocytes in aplastic anemia have the more stable compound of stromatin and hemoglobin than the other, their compound
is difficult to be released by the process of smearing, drying and fixing and so the specific Soret band of hemoglobin will not be found on the erythrocytes in aplastic anemia. This opinion could be supported by the phenomenon that erythrocytes are more resistant in aplastic anemia than in the other anemias.

**SUMMARY**

The erythrocytes of the normal people and various anemic patients are studied cytospectrophotometrically and the following results are obtained.

1. The absorption curve of erythrocytes can be identified with the spectra of hemoglobin as an intense peak which is found to be Soret band.
2. The erythrocytes in aplastic anemia show the different absorption curves from these in the other cases.
3. On the other hand, the erythrocytes in leukemia and the other diseases have slight differences of absorption curves from normal ones. Namely, the most intense absorption tends to be lower.
4. Accordingly the erythrocytes in aplastic anemia are cytospectrophotometrically distinct from those in leukemia.
5. These cytospectrophotometric data are compared with the pathological findings of autopsy cases.
6. The nature of aplastic anemia is discussed from the viewpoint of intracellular structure of hemoglobin.

**REFERENCES**