Studies of Leukocyte Alkaline Phosphatase in Mongolism: A Possible Chromosome Marker

By Aaron A. Alter, Stanley L. Lee, Mohamed Pourfar and Gerald Dobkin

In 1959 Lejeune, Gauthier and Turpin reported the finding of 47 chromosomes, one more than the normal number, in the cells of children with mongolian idiocy (Down's syndrome). The extra chromosome, one of the small acrocentrics—probably number 21—has since been universally verified.

Nowell and Hungerford, in 1960, showed that chronic granulocytic leukemia is also associated with an abnormality of a small acrocentric chromosome, probably number 21. The abnormality consists of deletion of a portion of the long arm of one of the pair of chromosomes. Subsequent studies have shown that this disease is almost if not always, accompanied by the chromosome abnormality, and that the abnormality is limited to the blood and bone marrow cells.

Neutrophilic leukocytes of patients with chronic granulocytic leukemia are strikingly deficient in alkaline phosphatase. This observation led to the hypothesis that stimulated the present study; namely, that the deleted portion of chromosome 21 carried the gene determinant for leukocyte alkaline phosphatase. Trisomy of this chromosome then might lead to increased levels of the enzyme. Preliminary reports from several sources tend to corroborate this hypothesis.

Materials and Methods

A. Sources

Blood was obtained from 58 subjects with mongolism in whom the diagnosis was established on clinical grounds. Chromosome studies were not done on these patients. Fifty-eight control subjects were chosen to constitute a comparable group. All persons who had any evidence of fever, acute infection or hematologic abnormality were excluded. The distribution of both control and mongolian idiot groups by age, sex and source is given in table 1.

B. Leukocyte Alkaline Phosphatase

Biochemical determinations of alkaline phosphatase were done by modifications of the methods of Valentine and Beck and King and Armstrong. Blood was obtained by venipuncture. From the Departments of Medicine, Maimonides Hospital of Brooklyn, and State University of New York Downstate Medical Center, Brooklyn, N. Y. Aided by a grant (C-5199) from the National Cancer Institute, U. S. P. H. S., Bethesda, Md.

At the time of these studies, Dr. Alter was supported by a Public Health Service Fellowship (CF-14431) from the National Cancer Institute.


The authors are grateful to Drs. George A. Jervis and Henry W. Kaessler, and to Mr. Milton Wilner and Sister Mary Madeleine for the opportunity to study persons under their care.

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### Table 1.—Distribution of Subjects by Age, Sex and Source

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>M.</th>
<th>F.</th>
<th>M. H.*</th>
<th>L. V.†</th>
<th>I. H. B.‡</th>
<th>R. S.¶</th>
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</thead>
<tbody>
<tr>
<td>A. Mongolian Idiots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1-12 mos.</td>
<td></td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>13-24 mos.</td>
<td></td>
<td>7</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>25-48 mos.</td>
<td></td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>49-108 mos.</td>
<td></td>
<td>7</td>
<td>3</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>4</td>
</tr>
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<td>10 years and over</td>
<td></td>
<td>11</td>
<td>6</td>
<td>-</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>37</td>
<td>21</td>
<td>2</td>
<td>25</td>
<td>4</td>
<td>27</td>
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</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>M.</th>
<th>F.</th>
<th>M. H.*</th>
<th>L. V.†</th>
<th>A. G. H.†</th>
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<tr>
<td>B. Control Group</td>
<td></td>
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<td></td>
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<tr>
<td>1-12 mos.</td>
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<td>10</td>
<td>7</td>
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<td>5</td>
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<tr>
<td>13-24 mos.</td>
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<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>25-48 mos.</td>
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<td>3</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>6</td>
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<tr>
<td>49-108 mos.</td>
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<td>5</td>
<td>2</td>
<td>-</td>
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<td>2</td>
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<td>10 years and over</td>
<td></td>
<td>8</td>
<td>9</td>
<td>-</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
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<td>31</td>
<td>27</td>
<td>12</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>

*Maimonides Hospital, Brooklyn, N. Y.
†Letchworth Village, Haverstraw, N. Y.
¶Infants' Home of Brooklyn, Brooklyn, N. Y.
§Richmond Sanitarium, Mount Vernon, N. Y.
¶¶Angel Guardian Home, Brooklyn, N. Y.

Puncture; 5 to 8 ml. were put into a tube containing 3 ml. of 6 per cent dextran and 1 ml. of 4 per cent sodium citrate. The tube was inverted several times, placed in a rack and red blood cells were allowed to sediment. The plasma layer was removed after 30 to 45 minutes and centrifuged at 500 rpm for 3 minutes. The sediment which contained red blood cells, white blood cells and platelets was then washed three times with 0.85 per cent sodium chloride and resuspended in 1 ml. of 0.85 per cent sodium chloride. A leukocyte count was done in duplicate on this suspension, 0.2 ml. of which were promptly added to 4 ml. di-sodium phenylphosphate buffered at pH 9.3. The length of time from the venipuncture until the start of the incubation with substrate was kept under 1 hour. Phosphatase determination then proceeded according to the method of King and Armstrong. Each determination was done in duplicate; pairs which did not agree within 10 per cent of each other were rejected.

Blood smears obtained from each patient at the time of venipuncture were stained by the Wright-Giemsa method. Differential white blood cell counts were done on these smears.

### C. Serum Alkaline Phosphatase

At the time of venipuncture, 5 ml. of whole blood were placed in a separate test tube and allowed to clot. The resulting serum was assayed for alkaline phosphatase by the method of King and Armstrong. Determinations were done in duplicate and results which did not agree within 10 per cent of each other were rejected.

### D. Statistics

Preliminary analysis of results disclosed that normality of distribution and equality of variance could be achieved by logarithmic treatment of leukocyte phosphatase values. Since valid comparisons can be made only when these criteria are met, logarithmic plots and
LEUKOCYTE ALKALINE PHOSPHATASE IN MONGOLISM

Table 2.—Alkaline Phosphatase per 10^10 Leukocytes (APA/C)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean APA/C (log units)</th>
<th>S.D. (log units)</th>
<th>Regression Coefficient (log phosphatase units/month)</th>
<th>Correlation Coefficient</th>
<th>Significance of Regression (co-variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongolian idiots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>41</td>
<td>32 mos.</td>
<td>39 ± .24</td>
<td>-.00112</td>
<td>-.075</td>
<td>p &gt; .60</td>
<td></td>
</tr>
<tr>
<td>10+</td>
<td>17</td>
<td>21 yrs.</td>
<td>20 ± .23</td>
<td>-.28</td>
<td>p &gt; .20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>58</td>
<td>7.9 yrs.</td>
<td>34* ± .31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>41</td>
<td>25 mos.</td>
<td>23 ± .18</td>
<td>-.0018</td>
<td>-.18</td>
<td>p &gt; .30</td>
<td></td>
</tr>
<tr>
<td>10+</td>
<td>17</td>
<td>25 yrs.</td>
<td>20 ± .19</td>
<td>-.00049</td>
<td>-.50</td>
<td>0.05 &gt; p &gt; .02</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>58</td>
<td>8.8 yrs.</td>
<td>22* ± .18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Difference between these means: (Fisher's t) .005 > p > .001.

calculations have been used throughout the present studies. Conversion to anti-logarithmic values has been made in order to facilitate comparisons with values obtained by other workers. Snedecor's Statistical Methods was used as a reference in this regard.

RESULTS

A. Leukocyte Alkaline Phosphatase per 10^10 Leukocytes (Table 2)

When alkaline phosphatase activity was calculated per 10^10 leukocytes, the mean value for the group of 58 mongolian idiots was 34 mg. of phosphorus liberated in 1 hour. For children under 10 years of age, the mean was 39 mg. of phosphorus per hour; for subjects 10 years and older, 20 mg. of phosphorus per hour. The difference between these means was statistically significant (Fisher's t: p < .001) (table 2). However, no significant relationship could be demonstrated by an analysis of co-variance between age and leukocyte alkaline phosphatase calculated in this way (table 2, fig. 1) (p > .60 for under 10 years group; p < .20 for the older group).

For the control population, the mean alkaline phosphatase activity per 10^10 leukocytes was 22 mg. of phosphorus per hour. The difference between this mean and that of the Down's syndrome patients (calculated by Fisher's t) was significant (.005 > p > .001) (table 2).

B. Leukocyte Alkaline Phosphatase per 10^10 Neutrophilic Leukocytes (Table 3)

In both the mongolism and control groups, a definite relationship was noted between age and leukocyte alkaline phosphatase activity per 10^10 neutrophil leukocytes. This relationship was more significant and clearer in the control group than in the subjects with Down's syndrome because of a wider scatter of phosphatase results in the latter group. Thus, the mean leukocyte alkaline phosphatase per 10^10 neutrophils for the 41 control children under 10 years of age was 71 mg. of phosphorus per hour; while for the 17 subjects aged 10 and over, the corresponding value was 29 mg. per hour. This difference is significant at the 0.1 per cent level of confidence (Fisher's t test). Similarly, within each of these age groups there was highly significant negative regression on age (figs. 2 and 3), although the regression coefficients were markedly (and significantly) different in the young children (under 10) as compared with older subjects.
Fig. 1.—A plot of leukocyte alkaline phosphatase activity (mg. of phosphorus liberated by $10^{10}$ leukocytes per hour) (APA/Leuk) plotted against age in months for the mongolian idiot group under 10 years of age. There is no significant regression of enzyme activity on age.

The 41 Down's syndrome subjects under 10 years had mean leukocyte alkaline phosphatase activity per $10^{10}$ neutrophils of 89 mg. of phosphorus per hour, while the mean value for the 17 older mongols was 32. The difference between these means is significant at the 1 per cent level of confidence (Fisher's t test). Within the under-10 mongol group, regression on age was significant at the 5 per cent level, but the relationship in the older Down's syndrome subjects was not statistically significant. Regression coefficients calculated for the younger and older age groups were quite similar to those obtained for the control subjects.

For all subjects tested, the mean phosphatase activity per $10^{10}$ neutrophil leukocytes was 71 mg. of phosphorus per hour for the mongolian idiots and 56 for the control group. This difference is significant at the 5 per cent level of confidence (Fisher's t). If only subjects under 10 years of age are considered, the difference between mongols and controls (89 versus 71) is still significant ($0.05 > p > 0.025$—Fisher's t). However, the mean ages of these groups
Table 3.—Alkaline Phosphatase per 10¹⁰ Neutrophils (ADA/P)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean APA/P</th>
<th>S.D. (log units)</th>
<th>Regression Coefficient (log phosphatase units/month)</th>
<th>Correlation Coefficient</th>
<th>Significance of Regression Co-variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongolian idiots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 0-9</td>
<td>41</td>
<td>32 mon.</td>
<td>89*†</td>
<td>±.27</td>
<td>-.0025</td>
<td>-.315</td>
<td>.05 &gt; p &gt; .02</td>
</tr>
<tr>
<td>2. 10+</td>
<td>17</td>
<td>21 yrs.</td>
<td>32‡</td>
<td>±.24</td>
<td>-.00074</td>
<td>-.38</td>
<td>.20 &gt; p &gt; .10</td>
</tr>
<tr>
<td>3. All</td>
<td>58</td>
<td>7.9 yrs.</td>
<td>71‡</td>
<td>±.31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Control group |
| 4. 0-9 | 41 | 25 mos. | 71† | ±.21 | -.0047 | -.60 | p < .001 |
| 5. 10+ | 17 | 25 yrs. | 29† | ±.21 | -.00050 | -.47 | p < .001 |
| 6. All | 58 | 8.8 yrs. | 56† | ±.25 | | | |
| 7. 0-9 | 41 | corrected to 32 mos. | 66* | | | | |

*Difference between these means is significant at the 0.5 per cent level of confidence.
†Difference between these means is significant at the 5 per cent level of confidence.
‡Difference between these means is not significant.

were different (Down’s syndrome, 32 months; control, 25 months), and leukocyte alkaline phosphatase activity in these children was clearly a function of age. The value for the controls, corrected to the activity expected at 32 months of age by use of the previously determined regression equation, was 66 mg. of phosphorus per hour. When this was compared with the value of 89 mg. of phosphorus per hour for the mongolian idiots, the difference was significant at the 0.5 per cent level (Fisher’s t).

No such difference between mongols and controls was apparent in the older age group. Mean leukocyte phosphatase activity per 10¹⁰ neutrophils was 32 mg. of phosphorus per hour for mongolian idiots, 29 mg. of phosphorus per hour for controls (p > .90).

C. Age and Differential Leukocyte Count (Table 4, Fig. 5)

Mongolian idiots have less than the normal childhood lymphocytosis. The 41 subjects under 10 years of age had a mean value of 50 per cent neutrophil leukocytes, while the corresponding value for the control subjects was 34 per cent. The proportion of neutrophils showed a significant negative correlation with age (covariance: p < .005 for mongols, p < .001 for controls). Older children and adults had a mean neutrophil value of 63 per cent with no significant variation with age within the group (covariance: p > .50) and with no difference between control and Down’s syndrome subjects.

D. Relationship of Leukocyte Alkaline Phosphatase to Sex (Table 5)

No significant difference of leukocyte alkaline phosphatase was present between males and females in either group or for any segment of either group.

E. Leukocyte Alkaline Phosphatase Compared with Serum Alkaline Phosphatase

Serum alkaline phosphatase was determined for 42 mongolian idiot subjects. The mean value was 9.5 King-Armstrong units. As expected, younger subjects had higher values. For 29 patients under 10 years of age (mean age 32
Fig. 2.—Leukocyte alkaline phosphatase activity (mg. of phosphorus per $10^{10}$ neutrophils per hour) (APA/Polys) is plotted against age of Down's syndrome subjects demonstrating a significant regression.

months) the mean serum alkaline phosphatase was 11.9 King-Armstrong units; for the 13 older patients the mean value was 5.7 units (fig. 5). These values are well within the normal ranges for the appropriate ages. Serum alkaline phosphatase shows significant covariance with leukocyte alkaline phosphatase for the total of 42 subjects ($p < .01$); this relationship cannot be demonstrated in the young or older groups analyzed separately.

**DISCUSSION**

The present study was begun as an effort to detect a difference in leukocyte alkaline phosphatase activity between cases of mongolism and a control population. Because this enzyme is thought to occur only in mature neutrophilic granulocytes, it seemed reasonable to express its observed activity as a function of these cells rather than of all leukocytes.

Early results showed that, in the control group, young children had much higher values than did older ones. Analysis of these results led to the conclusion that leukocyte alkaline phosphatase activity is in large part a function of the age of the individual up to the age of 10 years. Beyond this age, although the
Fig. 3.—Plot of leukocyte alkaline phosphatase activity (mg. of phosphorus per hour) per $10^{10}$ neutrophils (APA/Polys) and age showing a significant regression on age for control subjects.

The regression of enzyme activity on age was highly significant, the slope of the curve was much less.

The great majority of subjects with Down's syndrome who were available for study were under 10 years of age.

These subjects (under the age of 10) showed significantly higher leukocyte alkaline phosphatase activity than did control children. The difference is apparent in a crude comparison of the mongolian idiots with the control subjects (table 3). However, the variation of phosphatase with age (figs. 2 and 3) was similar in the mongolian idiots to that seen in the controls, while the mean ages of the two groups were different. Legitimate comparison of the two groups therefore demanded elimination of the age differential. A comparison of lines 1 and 7 of table 3 shows that, after correction for age, the difference between the means is significant at the 0.5 per cent level of confidence.

Beyond the age of 10 years the number of mongolian idiots studied was small. No difference between these subjects and the control subjects was apparent.

The study thus appears to show that up to the age of 10 years the neutrophilic leukocytes of mongolian idiots differ from those of controls as regards alkaline phosphatase activity, while after 10 years of age this difference could not be established in the small group studied. Available information does not explain this possible effect of aging. It is clearly related to the observation...
that leukocyte alkaline phosphatase activity declines progressively with age up to 10 years, both in normals and in mongolian idiots. A rational explanation for the one observation might well clarify the other.

Valentine and Beck\(^1\) have shown that activity of leukocyte alkaline phosphatase is increased in infections, the post-operative state and following adrenal cortical stimulation. Mongolian idiots are more susceptible to infections than are normal children;\(^1\) a high frequency of infections in the study patients could account for the elevated enzyme level. All subjects in the study, however, were screened for infection and all subjects with fever or other

![Bar chart showing percentage of polymorphonuclear leukocytes for mongolians and controls at various age increments.](chart)

**Fig. 4.—**Percentage of polymorphonuclear leukocytes is graphed for both mongolian idiots and controls at various age increments. The mongols have a higher percentage until over 120 months, when both groups reach equal levels. Numbers at the top of each column indicate the number of subjects in each group.

Table 4.—Relationship between Per Cent Neutrophils and Age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No.</th>
<th>Mean Age</th>
<th>Mean Per cent Neutrophils</th>
<th>Regression Coefficient (per cent neutrophils/month)</th>
<th>Correlation Coefficient</th>
<th>Significance of Regression (co-variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongolians idiots</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0–9</td>
<td>41</td>
<td>32 mos.</td>
<td>50*</td>
<td>+.192</td>
<td>+.48</td>
<td>(p &lt; .005)</td>
</tr>
<tr>
<td>10+</td>
<td>17</td>
<td>21 yrs.</td>
<td>63</td>
<td>+.0003</td>
<td>+.037</td>
<td>(p &gt; .50)</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–9</td>
<td>41</td>
<td>25 mos.</td>
<td>34*</td>
<td>+.257</td>
<td>+.625</td>
<td>(p &lt; .001)</td>
</tr>
<tr>
<td>10+</td>
<td>17</td>
<td>25 yrs.</td>
<td>63</td>
<td>+.006</td>
<td>+.075</td>
<td>(p &gt; .50)</td>
</tr>
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</table>

\(\ast\) Difference between these means is significant (Fisher's \(t\): \(p < .001\)).
Fig. 5.—Leukocyte alkaline phosphatase per $10^{10}$ neutrophils (APA/P) is plotted against serum alkaline phosphatase in King-Armstrong units for 42 mongolian idiots. There is significant positive correlation between the enzyme activities of the serum and the neutrophilic leukocytes.

Clinical findings of acute disease were eliminated. Dutton was unable to demonstrate significant differences in adrenal cortical function (as measured by excretion of 17-keto and 17-ketogenic steroids) between mongolian idiots and a group of non-mongolian mentally defective children.

Interpreting the observed increased leukocyte alkaline phosphatase activity as increased content of enzyme in leukocytes involves an assumption which

Table 5.—Sex and Alkaline Phosphatase Activity (per $10^{10}$ neutrophils) (APA/P)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. Females</th>
<th>No. Males</th>
<th>Mean APA/P for Females</th>
<th>Mean APA/P for Males</th>
<th>Significance of Difference (Fisher’s t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongolian idiots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>15</td>
<td>25</td>
<td>96</td>
<td>78</td>
<td>p &gt; .90</td>
</tr>
<tr>
<td>10+</td>
<td>7</td>
<td>10</td>
<td>41</td>
<td>27</td>
<td>p &gt; .50</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-9</td>
<td>18</td>
<td>23</td>
<td>74</td>
<td>68</td>
<td>p &gt; .50</td>
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<td>10+</td>
<td>9</td>
<td>8</td>
<td>35</td>
<td>32</td>
<td>p &gt; .60</td>
</tr>
</tbody>
</table>
has not yet been proved. Cohn and Hirsch\textsuperscript{15,16} have shown that phosphomonoesterases of leukocytes are contained within the specific cytoplasmic granules and are released into the cytoplasm after phagocytosis. Increased lability of the granules could lead to increases of phosphatase activity without any change in the actual enzyme content of cells.

Whether it be due to differences in enzyme content or enzyme release, the high level of alkaline phosphatase in leukocytes of mongolian idiot children belongs in a context of leukocyte abnormalities. Benda\textsuperscript{17} observed that these children did not show the lymphocytosis characteristic of infancy. Ridler and Shapiro\textsuperscript{15} found marked diminution in the number of large lymphocytes. Neutrophilic leukocytes of mongolian idiots have a lower than normal Arneth index.\textsuperscript{15,19} Finally, the increased incidence of acute leukemia in these children has been clearly demonstrated.\textsuperscript{20} Multiple abnormalities of white blood cells thus characterize this syndrome.

Leukocytes of mongolian idiot children show a high level of alkaline phosphatase activity: this is in accord with the hypothesis which stimulated this study. If the hypothesis is correct, it may have considerable potential importance in the mapping of human chromosomes. Markers for the X chromosome in man are known because of sex-linked inheritance patterns: the genes responsible for color blindness, hemophilia A and B, glucose-6-phosphate dehydrogenase deficiency,\textsuperscript{21} Duchenne type of muscular dystrophy and the blood group Xg\textsuperscript{22} all help to map this chromosome. No autosomal marker has yet been reported. Once a given autosome is identified with a genetic trait, linkage studies can be undertaken to see which of the many inherited characteristics can be localized to the same chromosome. If this procedure is to be possible with regard to leukocyte alkaline phosphatase and chromosome 21, genetically determined individual differences in phosphatases will have to be detected. Whether such differences exist is a matter of speculation; however, increasing numbers of cellular and plasma protein constituents (blood group substances, gamma globulin, haptoglobins, transferrins, glucose-6-phosphate dehydrogenases) have been shown to be polymorphic in recent years.

Acceptance of neutrophil leukocyte alkaline phosphatase as a marker for chromosome 21 demands inquiry into conditions associated with reduced activity of the enzyme. Two diseases (in addition to chronic granulocytic leukemia) have been reported to show this abnormality: paroxysmal nocturnal hemoglobinuria\textsuperscript{23} and hypophosphatasia.\textsuperscript{24,25} During the period of this study no patient with either disease has been available for examination. A normal karyotype has been reported in a single case of paroxysmal nocturnal hemoglobinuria;\textsuperscript{26} no cytogenetic information is yet available on hypophosphatasia.

Hypophosphatasia is a syndrome which is thought to be based on defective formation of alkaline phosphatase in all tissues; reduction or absence of this enzyme has been noted in bone, intestinal mucosa and kidney;\textsuperscript{27} cartilage, liver, teeth and serum,\textsuperscript{28} as well as leukocytes. The disorder may be inherited through the action of an autosomal recessive gene.\textsuperscript{29} In hypophosphatasia, then, alkaline phosphatases of all tissues behave in the same way. However, there is some evidence that phosphatases of differing tissue origin can be separated.
LEUKOCYTE ALKALINE PHOSPHATASE IN MONGOLISM

Fig. 6.—Hypothetical schema for genetic control of synthesis of specific alkaline phosphatases.

These apparently antithetical ideas can be reconciled by assuming a “basic” alkaline phosphatase polypeptide (Fig. 6) whose formation (in all tissue) is controlled by one gene, and a second “modifying” gene which is specific for each tissue normally synthesizing the enzyme. The lesion in hypophosphatasia would be in the “basic” gene, while in mongolism and chronic granulocytic leukemia the defect in specific leukocyte phosphatase is mediated through the “modifying” gene specific for leukocytes.

This hypothesis takes into account the fact that serum alkaline phosphatase, in the present series of mongolian idiots, was well within the normal range. It is still quite possible, however, that the abnormalities of alkaline phosphatase observed in leukocytes are also present in other tissues. Measurements in other tissues have not yet been done.

SUMMARY

Leukocyte alkaline phosphatase was measured by a biochemical method in a group of 58 mongolian idiots. The mean activity found in the 41 children less than 10 years of age was equivalent to 89 mg. of phosphorus per 10^10 neutrophilic leukocytes per hour. This was compared to the value of 66 mg. of phosphorus per 10^10 neutrophilic leukocytes per hour obtained in a group of 41 control children in the same age group (after correction of the control mean to the same mean age). The difference is significant at the 0.5 per cent level of confidence.

This difference was interpreted as confirming (but not proving) the hypothesis that leukocyte alkaline phosphatase formation is controlled by a gene on chromosome number 21—the chromosome for which mongolian idiots are trisomic. The hypothesis arose because of the known deficiency of this enzyme in the leukocytes of patients with chronic granulocytic leukemia, and the known partial deletion of chromosome 21 in this disease.

This finding should provide a stimulus to further investigations into the content of alkaline phosphatase in leukocytes, possible polymorphism of leukocyte alkaline phosphatases, linkages with other inherited traits, and relationships between leukocyte and other tissue phosphatases.
SUMMARIO IN INTERLINGUA

Le phosphatase alcalin del leucocytos esseva mesurate per un metodo biochimic in un gruppo de 58 idiotas mongolian. Le activitate medie trovate in le 41 juveneS del gruppo qui habeva minus que 10 annos de etate esseva 89 mg de phosphoro per 10^10 leucocytos neutrophile per hora. Isto se com-
para con le valor de 66 mg de phosphoro per 10^10 leucocytos neutrophile per hora obtenite in un gruppo de controlo de 41 juvenes del mesme gruppo de etate (post correction del valor de controlo al mesme etate medie). Le differen-
tia es significative al nivello de confidentia de 0,5 pro cento.

Le differen
tia esseva interpretate como supporto (sed non como prova) del hypothes
e que le formation de phosphatase alcalin del leucocytos es regulate per un gen in chromosoma numero 21 (i.e. le chromosoma pro le qual idiotas mongolian es trisomic). Le hypothes
e se suggereva a causa del cognoscite carentia del mentionate enzyma in le leucocytos de patientes con chronic leucemia granulocytic e le cognoscite deletion partial de chromosoma 21 in iste morbo.

Le constatation provide un stimulo a investigationes additional del contento de phosphatase alcalin in le leucocytos, del possible polymorphismo del phosphatases alcalin del leucocytos, del ligation con altere characteres heredi-
tari, e del relation inter le phosphatases de leucocytos e de altere tissus.

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Aaron A. Alter, M.D., Assistant Physician and Research Associate in Hematology, Maimonides Hospital of Brooklyn, Brooklyn, N. Y.

Stanley L. Lee, M.D., Director of Hematology Division, Maimonides Hospital of Brooklyn, and Associate Professor of Medicine, State University of New York Downstate Medical Center, Brooklyn, N. Y.

Mohamed Pourfar, M.D., Research Fellow in Pediatric Hematology, Kings County Hospital, Brooklyn, N. Y.

Gerald Dobkin, M.S., Chief Chemist, Maimonides Hospital of Brooklyn, Brooklyn, N. Y.
Studies of Leukocyte Alkaline Phosphatase in Mongolism: A Possible Chromosome Marker

AARON A. ALTER, STANLEY L. LEE, MOHAMED POURFAR and GERALD DOBkin