Numerous reports have been published demonstrating an increase in the activity of white blood cell enzymes among patients with trisomic Down's syndrome. Hook and Engel have suggested that the level of enzyme activity is dependent upon cell age and that a shortened lifespan of the leukocyte in Down's syndrome might result in the presence of younger white blood cells with higher enzyme levels. Two recent studies with conflicting results have attempted to answer this question. Galbraith and Valberg, using the technic of labeling leukocytes in vitro with radioactive diisopropyl fluorophosphate (DFP32) to study the granulocyte half-life, the blood granulocyte mass, and the granulocyte turnover rate were unable to demonstrate any difference between normals and four patients with Down's syndrome. On the other hand, Raab et al., using comparable technics involving DFP32 labeling in trisomic Down's syndrome, were able to demonstrate an increase in granulocyte turnover rate.

In an attempt to demonstrate which white blood cell component might be responsible for the elevation in enzyme activities in Down's syndrome, we have utilized the method of Rabinowitz in the separation of lymphocytes and granulocytes in Garvin's glass bead columns.

Materials and Methods

Twenty-five ml. of heparinized blood was obtained from 10 patients with trisomic Down's syndrome (M) and 10 controls (C), who are residents at the Dixon State School, Dixon, Ill. The two groups were matched for age and sex and had an age range of 6 to 10 years. The blood was sedimented at 37 C. for 30 minutes with 6 per cent dextran in normal saline (Abbott) mixed in a ratio of 1 ml. of dextran per 5 ml. of blood. The supernatant was removed to within 0.2 cm. of the red blood cells and centrifuged at 100 g for 10 minutes. The white cell pellet was then resuspended in 4 ml. of serum. The remainder of the supernatant was centrifuged at 1500 g for 10 minutes and this cell-free serum used for both the preparation procedure and the elution of the column.

The general procedure of preparation of the column and cells and the actual separation
Table 1.—Enzyme Activity in White Blood Cells (W), Lymphocytes (L), and Polymorphonuclear Leukocytes (P) among Ten Controls (C) and 10 Patients with Down's Syndrome (M)*

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>W</th>
<th>M</th>
<th>C</th>
<th>L</th>
<th>M</th>
<th>C</th>
<th>P</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcP (μmoles PNP/hr/10^6)</td>
<td>8.74 ± 2.11</td>
<td>11.39 ± 2.70</td>
<td>5.27 ± 1.1</td>
<td>8.37 ± 1.2</td>
<td>6.11 ± 1.7</td>
<td>9.20 ± 2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AkP (μmoles PNP/hr/10^6)</td>
<td>0.74 ± 0.33</td>
<td>1.37 ± 0.53</td>
<td>0.30 ± 0.12</td>
<td>0.47 ± 0.11</td>
<td>1.07 ± 0.23</td>
<td>1.74 ± 0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-6-PD (μmoles NADPH/hr/10^6)</td>
<td>1.52 ± 0.60</td>
<td>2.80 ± 1.00</td>
<td>0.48 ± 0.16</td>
<td>0.72 ± 0.14</td>
<td>1.78 ± 0.35</td>
<td>2.70 ± 0.64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All value expressed as mean ± standard deviation. All differences between C and D are significant (p<.05).
LYMPHOCYTE AND GRANULOCYTE ENZYME ACTIVITY

A sample of the sedimented white blood cells (W) was placed on the column, the lymphocyte fraction (L) was obtained by elution with 100 per cent cell-free serum, and the polymorphonuclear leukocyte fraction (P) was obtained by elution with EDTA solution (G. I. B. Co.). Aliquots of W, L, and P were used for microscopic analysis, cell count (using a Coulter counter), and enzyme studies. Acid phosphatase (AcP) was assayed at pH 4.90 and alkaline phosphatase* (AkP) at pH 9.3, using p-nitrophenol phosphate as substrate. Glucose-6-phosphate dehydrogenase (G-6-PD) was determined spectroscopically by the reduction of NADP at 340 m, using G-6-P as substrate. Separation of white cells was always performed in pairs with one C and one M being studied simultaneously. The column was considered satisfactory if 50 per cent of P and 50 per cent of L were recovered from W in both columns. Approximately 70 per cent of the columns filled these criteria and were included in the results. The L fraction contained 97 per cent lymphocytes, 3 per cent polymorphonuclear leukocytes, and was contaminated by 2 to 3 red blood cells per lymphocyte. The P fraction contained 95 per cent polymorphonuclear leukocytes and 5 per cent lymphocytes and monocytes. The cell fractions were treated with hypotonic saline to remove any red cells which might interfere with the results of the enzyme assay.

RESULTS

As shown in Table 1, significant differences measured by the Student "T" test could be found between C and D for W, L, and P in all instances.

DISCUSSION

Several explanations have been offered to explain the changes in the peripheral blood of patients with Down's syndrome. A number of investigators have suggested that the extra "G" group chromosome might contain the "structural" genes for certain specific enzymes. Other authors have suggested that the extra "C" group chromosome might contain the "regulator" genes for certain specific enzymes. As of this date, no evidence has been presented localizing a specific genetic locus for an enzyme to the extra chromosome associated with mongolism.

The most recent hypothesis suggesting that the alteration of enzyme activity is related to the age of the white blood cell, specifically the polymorphonuclear leukocyte, has not been convincingly proved. Cartwright et al. has shown that DFP specifically labels the neutrophilic series. Raab et al. were able to demonstrate a shortened lifespan in 4 of 6 patients with Down's syndrome, while Galbraith and Valberg were unable to demonstrate any difference in 4 patients with Down's syndrome.

A number of different enzymes have been reported to be increased in the red blood cells of patients with Down's syndrome. Demars, Cox, and Nadler et al. have failed to demonstrate any difference in enzyme activity of fibroblasts derived from trisomic mongols. The finding of increased activity of AcP, AkP, and G-6-PD in both the W and the L and P fractions in

*0.1 ml. of 0.3M magnesium chloride is required for the P fraction, since this results in a threelfold increase of AkP activity. Neither magnesium chloride nor zinc chloride has any effect upon AkP activity in the W or L fractions.
this study demonstrates that the alteration in white blood cell enzyme activity is not localized to any particular fraction. It would appear that this increase is related to some specific, though generalized, phenomenon of circulatory blood cells which may be characteristic of Down's syndrome.

The distribution of enzyme activities in the specific blood cell fractions is in accord with previous studies. Rabinowitz\(^2\) has demonstrated that the activity of G-6-PD is four times greater in P than L. AkP activity has been shown to reside predominantly in P by numerous histochemical technics.\(^21\)\(^,\)\(^22\) It is of interest to note that after cell separation, the P require magnesium chloride. The requirement for Mg\(^{++}\) may be related to an intracellular loss of Mg\(^{++}\) ion in the active process of adhering to the glass beads or to the elution procedure with EDTA. An alternative possibility is that different isoenzymes are present in the various cell components.

The application of the procedure of white blood cell separation should prove to be a valuable adjunct to the standard methods used in studying genetic, metabolic, and clinical problems.

**SUMMARY**

Patients with trisomic Down’s syndrome were found to have significant increases of acid phosphatase, alkaline phosphatase, and glucose-6-phosphate dehydrogenase in both lymphocytes and polymorphonuclear leukocytes separated from white blood cells by the procedure of Rabinowitz. The alteration in enzyme activities appears not to be directly related to genes located on the chromosome causing Down’s syndrome.

**SUMMARIO IN INTERLINGUA**

Esseva trovate que patientes con le trisomic syndrome de Down ha significativemente elevate nivellos de phosphatase acide, de phosphatase alcalin, e de dehydrogenase de glu-cosa-6-phosphato in le lymphocytos e etiam in le leucocytos polymorphonucleari separate ab le altere leucocytos per le procedimento de Rabinowitz. Il pare que le alteration del activitate enzymatic non es directemente relationate con genes locate super le chromosomas que causa le syndrome de Down.

**ACKNOWLEDGMENTS**

The authors wish to express their appreciation to Drs. Yale Rabinowitz, Fritz Bach, and James Garvin for sharing with us their experience with the columns, to Dr. Paul Tillman for permitting us to study the patients, and to Elvira Kavaliunas, Janet Pi, Rosemary Pribila, and Bonnie Williams for technical assistance.

**REFERENCES**

5. Rabinowitz, Y.: Separation of lymphocytes, polymorphonuclear leukocytes and monocytes on glass columns, in-
Lymphocyte and Granulocyte Enzyme Activity in Patients with Down's Syndrome

HENRY L. NADLER, PATRICIA L. MONTELEONE, TOHRU INOUYE and DAVID YI-YUNG HSIA