Leukocyte Enzymes in Birth Defects—
A Review

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ABSTRACT

The utilization of peripheral leukocytes, mixed preparations or specific populations for the detection of homozygous and heterozygous states of inborn errors of metabolism via enzyme assay or detection of accumulated metabolites are reviewed. Additionally, present knowledge of metabolic activities in leukocyte populations and areas of potentially fruitful investigation are discussed.

Introduction

Peripheral circulating leukocytes often provide a convenient "tissue biopsy" for detection of birth defects resulting in specific enzymatic deficiencies and/or metabolite accumulation. Such approaches are reviewed in this paper. Attention is directed to the articles by Hsia,24 Frie et al,18 and Cone et al11 promoting the utilization of leukocytes for the study of inborn errors of metabolism. Known leukocyte physiology and biochemistry have been compiled by Cline,10 and leukocyte separation techniques up to 1970 have been reviewed by Cutts.12 Inborn errors detectable via leukocytes in the homozygous or heterozygous state are summarized in table I, and discussed in the text.

Carbohydrate Metabolism

Glycolytic enzymes are detectable in polymorphonuclear leukocytes (PMNs) and in lymphocytes,15,21 their activities being very dependent upon cell preparation technique and upon anticoagulant choice.15 The lymphocyte hexose monophosphate pathway is only slightly active22 while that of the PMN is quite active.3 Deficiency of glucose-6-phosphate dehydrogenase has been demonstrated in granulocytes concomitant with deficiency in the erythrocyte.38 Tricarboxylic acid cycle activity is present in granulocytes10 and in lymphocytes.22 Lymphocyte citrate synthesis occurs at a rate comparable to that in skeletal muscle, lungs, and pancreas.36 Blass et al5

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Inborn Error Detection via Leukocyte Assay

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<th>Leukocyte Population</th>
<th>Disease State (form)</th>
<th>Leukocyte Population</th>
<th>Disease State (form)</th>
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<tr>
<td>Lymphocytes</td>
<td>Granulocytes</td>
<td>Mixed</td>
<td>Ref.</td>
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<tr>
<td>Carbohydrate Metabolism</td>
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<tr>
<td>Glucose-6-phosphate dehydrogenase deficiency*</td>
<td>X 38</td>
<td>Lipid Metabolism</td>
<td></td>
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<tr>
<td>Pyruvate dehydrogenase deficiency*</td>
<td>X 5</td>
<td>Hypercholesterolemia*†</td>
<td>X 17</td>
</tr>
<tr>
<td>Ketoglutarate dehydrogenase deficiency*</td>
<td>X 5</td>
<td>Juvenile ceroid lipofuscinosis*</td>
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</tr>
<tr>
<td>Glycogen synthetase deficiency*</td>
<td>X 19</td>
<td>Batten's disease*</td>
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<tr>
<td>Glycogen storage disease II*</td>
<td>X 65, 51</td>
<td>Gaucher's disease*†</td>
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<tr>
<td>Glycogen storage disease III*</td>
<td>X 62, 51</td>
<td>Niemann Pick disease*†</td>
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<tr>
<td>Glycogen storage disease IV*</td>
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<td>Gl-gangliosidosin*†</td>
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<tr>
<td>Glycogen storage disease VI*</td>
<td>X 61, 51</td>
<td>Fabry's disease*</td>
<td>X 29</td>
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| Amino Acid and Urea Cycle Metabolism |                      |                      |                      |
| Maple-syrup-urine disease* | X 7 | Muco-lipid, Muco-poly saccharide Metabolism |                      |
| Isovaleric aciduria* | X 7 | Fucosidosis*† | X 4 |
| o-Ketoadipic aciduria* | X 63 | Mannosidosis*† | X 33, 54 |
| Cystinosis*† | X 34, 60 | Mucolipidosis II* | X 30 |
| Hyperprolinemia, type II* | X 56 | Hunter syndrome*† | X 32 |
| Homocystinuria*† | X 20 | Hunter syndrome* | X 31 |
| Carbaryl phosphate synthetase deficiency* | X 64 | Maroteaux-Lamy syndrome* | X 52 |
| Arginino-succinase deficiency* | X 64 | Sanfilippo, type A*† | X 40 |

<table>
<thead>
<tr>
<th>Lipid Metabolism</th>
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<th>Heterozygous state.</th>
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<tr>
<td>Wyss et al65 discuss the choice of leukocyte preparation in diagnosis of type II. Leukocyte glycogen synthetase activity has been studied in the hepatic deficiency state.19</td>
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**Lipid Metabolism**

Fogelman et al17 utilized unfractionated leukocytes from subjects with heterozygous familial hypercholesterolemia to demonstrate an abnormal induction of 3-hydroxy-3-methylglutaryl coenzyme reductase.

Altered concentration of leukocyte docosahexaenoic acid has been demonstrated in the juvenile form of ceroid lipofuscinosis.37 Leukocyte myeloperoxidase

employed a platelet enriched blood fraction containing 1/3 leukocytes for the diagnosis of pyruvate and ketoglutarate dehydrogenase deficiencies.

Lymphocytes can synthesize glycogen22 but do not store appreciable amounts.42 The glycogen content of the granulocyte is comparable to that of liver and muscle,42 but Esman's16 demonstration that neutrophil glycogen content is increased upon in vitro incubation suggests caution in using neutrophil glycogen to demonstrate tissue storage. The neutrophil and the liver utilize similar mechanisms of glycogen degradation, thus allowing the neutrophil to be utilized for demonstrating the enzyme defects of glycogen storage diseases types II, III, IV, and VI.6,51,61,62 Wyss et al65 discuss the choice of leukocyte preparation in diagnosis of type II. Leukocyte glycogen synthetase activity has been studied in the hepatic deficiency state.19
activity is markedly deficient, while p-phenylenediamine-peroxidase activity is normal in Batten's disease.

Lipidoses owing to deficient lysosomal enzymes are readily demonstrable in leukocytes, several assays employing convenient artificial substrates. Synthetic substrate beta-glucosidase activity in leukocytes can detect Gaucher's disease homozygotes and heterozygotes. Niemann-Pick disease homozygote and heterozygote detection via leukocyte sphingomyelinase still requires radiolabeled sphingomyelin substrate. Leukocyte beta-galactosidase activity is deficient in GM₂-gangliosidosis, possibly secondary to mucolipidosis or mucopolysaccharidosis. Alpha-galactosidase, present in neutrophil granules, is reduced in patients with Fabry's disease. Metachromatic leukodystrophy has been diagnosed via leukocyte arylsulfatase A assay, but low activity has been reported in a family without the disease. The carrier state of Tay-Sach's disease, suggested by serum assay, is reliably confirmed by leukocyte hexosaminidase A activity.

Mucolipid and Mucopolysaccharide Metabolism

Peripheral blood leukocytes have been utilized to detect the carrier state of fucosidosis, with lymphocytes being a more reliable indicator than serum or unfractionated leukocytes. The α-fucosidase(s) of leukocytes appears to differ from that of serum. Mannosidosis and the heterozygote state are demonstrable in mixed leukocytes. Thompson et al have shown that mannosidosis heterozygote detection is more reliable when referenced to leukocyte protein than to other lysosomal hydrolase activities. I-cell disease, or mucolipidosis II, is detected by leukocyte decrease and serum increase of several lysosomal acid hydrolases, including β-galactosidase, β-glucosaminidase, β-glucuronidase, α-galactosidase, and arylsulfatase A. Leukocyte incorporation of radiolabeled substrate into mucopolysaccharides, following phytohemagglutinin stimulation, was utilized to detect patients with Hurler, Hunter, Morquio, or Maroteaux-Lamy forms of mucopolysaccharidosis. α-L-iduronidase of leukocytes has been utilized to detect homozygotes with Hurler and Schie syndromes and heterozygotes with Hurler syndrome. Hunter syndrome has been diagnosed by lymphocytic activity of iduronate sulfatase. Arylsulfatase B and N-acetyl-galactosamine-4 sulfate sulfatase, deficient in Maroteaux-Lamy, are demonstrable in leukocytes. Leukocyte activity in sulfamidase can distinguish heterozygous and homozygous forms of Sanfilippo disease type A. Mucopolysaccharidosis owing to β-glucuronidase deficiency is expressed in leukocytes.

Amino Acids and Urea Cycle Metabolism

Enzymes for the oxidative deamination of branched-chain keto acids of leucine, isoleucine, and valine are active in the neutrophil. These cells have been utilized to demonstrate enzyme deficiency in maple-syrup urine disease and in isovaleric acidemia. Wilson et al has optimized the assay for the pathway converting ω-(1-14C)-aminoadipic acid to 14CO₂ and recommends its use in further elaboration on the nature of α-ketoadipic aciduria. Simell has utilized granulocytes to study transport of diamino acids in lysinuric protein intolerance, showing that one transport system is present for lysine, ornithine and arginine. Cystinosis is manifested by increased cystine in neutrophils, with typical increases of 80-fold in homozygotes and 6-fold in heterozygotes. Unfractionated leukocyte uptake of 35S-cystine has also been utilized to diagnose cystinosis. Cystinuric patients have not demonstrated an accumulation of cystine or a dibasic amino acid transport defect in their neutrophils.
hyperprolinemia has been characterized by deficient pyrroline-5-carboxylic acid dehydrogenase in lymphocytes. Homocystinuria heterozygotes have been detected employing phytohemagglutinin-stimulated lymphocytes. As cell lysis and deproteinizing agents interfere with quantitative extraction of free amino acids from leukocytes, little study of leukocytic amino acid concentrations has been reported.

Moore et al. studied the synthesis of urea from arginine by unfractionated leukocytes, and estimated that approximately one percent of the daily urea production occurs in these cells. Wolfe et al. have demonstrated the presence in leukocytes of the four urea cycle enzymes (ornithine transcarbamylase, carbamyl phosphate synthetase, and argininosuccinase plus its lyase), and point out the utility of this tissue in diagnosis of hereditary hyperammonemic states when a liver biopsy might be quite harmful. In this instance, leukocytes demonstrate urea cycle enzymes not present in cultured fibroblasts, ornithine transcarbamylase and carbamyl phosphate synthetase I. Snodgrass has recently demonstrated that leukocyte ornithine transcarbamylase activity is not deficient concomitant with deficiency of the liver enzyme.

Purine, Pyrimidine and Folate Metabolism

Enzymes for de novo synthesis of pyrimidines and purines are most active in immature granulocytes, with little activity remaining in mature ones. Leukocyte orotidylic decarboxylase is deficient in orotic aciduria. Lymphocyte purine 5'-nucleotidase deficiency occurs in at least some patients with primary hypergammaglobulinemia. In light of the observation that 5'-nucleotidase-positive and -negative sub-populations of peripheral lymphocytes exist, this deficiency may reflect absence of a population. 5'-Nucleotidase does not appear to be present in human polymorphonuclear leukocytes, even though it has classically been utilized as an enzymatic plasma membrane marker. Enzymes of the purine metabolic cycle have been evaluated in normal and leukemic lymphocytes. Lymphoid cells from patients with Lesch-Nyhan syndrome are deficient in hypoxanthine-guanine phosphoribosyl transferase. The 14C-formate to N-formylglycinamide ribonucleotide portion of the purine synthetic pathway has been shown to be enhanced in lymphocytes from patients with primary gout, but values overlap rates measured in secondary hyperuricemia and control states. A "functional folate" assay of lymphocytes, utilizing 14C-formate incorporation into serine has been reported to detect folate deficiency sensitively, and theoretically should provide a means to evaluate simultaneously several of the folate interconversion enzymes.

Studies employing leukocytes for detection of homozygote or heterozygote states of inborn errors of metabolism are increasingly being reported, indicating the vast untapped diagnostic and prognostic potential of the numerous "tissue biopsies" circulating in peripheral blood.

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