METACHROMATIC LEUKODYSTROPHY: ARYLSULFATASE-A DEFICIENCY IN SKIN FIBROBLAST CULTURES*

BY MYNA T. PORTER, ARVAN L. FLUHARTY, AND HAYATO KIHARA

RESEARCH DEPARTMENT, PACIFIC STATE HOSPITAL, POMONA, CALIFORNIA

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Abstract.—Fibroblasts cultured from the skin of a patient with metachromatic leukodystrophy have been found to manifest the biochemical defect of this inborn error of metabolism, a deficiency of arylsulfatase A. Diseased cells had less than five per cent of normal arylsulfatase-A activity, while activities of other lysosomal enzymes—including arylsulfatase B, $\beta$-galactosidase, $\beta$-glucuronidase, and $\beta$-N-acetylglucosaminidase—were comparable to those in control cells. The presence of dissociable inhibitors in extracts of the diseased cells was excluded by combination experiments. The deficiency of the enzyme in leukocytes was also confirmed and is comparable to that found in cultured fibroblasts. The finding that readily cultured fibroblasts from easily obtained skin biopsy specimens exhibit the enzymatic defect should prove valuable in the biochemical study of this disease.

Late infantile metachromatic leukodystrophy (MLD) is an hereditary defect in lipid metabolism thought to be transmitted as an autosomal recessive trait. The first clinical symptoms, which usually appear before the second birthday, include hypotonia, muscle weakness, and an unsteady gait. In advanced stages of the disease, the myelin in the nervous system becomes devastated; abnormal metachromatic granules accumulate in the central and peripheral nervous systems$^1$ and in the kidneys, and are excreted in the urine.$^2$ Excessive levels of cerebroside sulfates (sulfatides) have been demonstrated in the metachromatic tissues.$^{1,3}$

The first indication of an enzymatic defect was presented by Austin, who showed that the lysosomal enzyme arylsulfatase A was deficient in brain and kidney tissue obtained at autopsy from leukodystrophic children.$^4$ He subsequently demonstrated the enzyme deficiency in urine$^4$ and proposed the use of this criterion for diagnostic purposes.$^6$ However, the urinary arylsulfatase-A activity has proved useful only as a semiquantitative screening test, since it is affected by many variables. The possibility of a more reliable diagnostic test has been provided by the recent discovery of Percy and Brady$^7$ that the enzyme deficiency is also manifested in leukocytes.

Cravioto et al.$^8$ obtained outgrowths from cultured cerebral white matter (cultured nerve cells) and found that such cells from a patient with MLD exhibited the characteristic brown metachromasia when stained with cresyl violet. However, mitotic figures were not observed in these cultures and the cells began to deteriorate at about 70 days. Thus the usefulness of such cultures as a model system for the study of the disease is limited.

The present communication reports the presence of arylsulfatase-A activity in normal fibroblast tissue culture cells and its deficiency in cells from a patient...
with metachromatic leukodystrophy. Thus, the biochemical abnormality that is characteristic of this disease can be investigated with conveniently established and propagated skin fibroblast cultures. The deficiency in arylsulfatase-A activity of leukocytes in MLD and its usefulness as a diagnostic procedure are also confirmed.

**Materials and Methods.**—**Cell strains:** The MLD cell strain was derived from a skin biopsy of S. P., an eight-year-old male Caucasian who is the fourth member of the sibship to be affected with metachromatic leukodystrophy (Fig. 1). The diagnosis was based on the clinical course, analysis of rectal biopsy, sural nerve biopsy, liver and kidney biopsy, altered nerve conduction time, deficient urinary arylsulfatase-A activity, and analysis of autopsy tissue of his deceased sibs. Cultured nerve cells from this child were shown by Cravioto et al. to be metachromatic. Control cells were derived from foreskin material of three normal infants. Cells from a patient with another storage disease, Sanfilippo syndrome, were derived from a skin biopsy.

**Culture of fibroblasts:** Primary cultures were initiated by the explant technique and outgrowths were cultured under a standardized procedure in Medium 199 supplemented with 10% calf serum and incubated under an atmosphere of 95% air and 5% CO₂. Cultures were fed twice a week and grown to confluence in Blake bottles.

![Pedigree of the P family](image)

**Preparation of cell-free extracts:** The cells were harvested by being scraped, washed twice with 0.85% NaCl, and suspended in an equal volume of glass-distilled water. The suspension was subjected to sonic oscillation by the Branson sonifier with the microtip for a total of 25 sec and centrifuged at 5000 g for 10 min. An aliquot of the supernatant fluid, which contains the lysosomal enzymes, was used for determination of protein.

**Preparation of leukocyte extracts:** Leukocytes were prepared according to the method of Kampine et al. and suspended in distilled water. The suspension was frozen and thawed six times to lyse the cells and then centrifuged at 800 g for 10 min. Protein was determined on an aliquot of the supernatant fluid.

**Enzyme assays:** Arylsulfatase-A activity in leukocyte extracts was assessed according to the method of Perey and Brady. For arylsulfatase-A activity in tissue culture cells, the incubation mixtures contained 0.2 ml of 0.01 M p-nitro catechol sulfate in 0.5 M sodium acetate buffer, pH 5.0, which contained 5 × 10⁻⁴ M Na₄P₂O₇ and 10% w/v NaCl, and 10 to 50 μg of protein in a total volume of 0.24 ml. The 30-min incubation at 38°C was terminated by the addition of 0.2 ml of 1 N NaOH. The released p-nitrocatechol was measured against a zero-time control by absorption at 515 mμ in a Gilford spectrophotometer.

Arylsulfatase B was determined by a modification of the method of Baum et al. Incubation mixtures contained 0.5 ml of 0.05 M p-nitrocatechol sulfate in 0.5 M sodium acetate buffer, pH 6.0, which contained 10⁻⁴ M Ba(OH)₂ and 40–120 μg of protein. The incubation temperature was 38°C. An 0.2 ml-aliquot was removed from each sample
at 30 to 90 min and mixed with 0.2 ml of 1 N NaOH. Arylsulfatase-B activity was then calculated as described by Baum et al.\textsuperscript{13}

$\beta$-Galactosidase was determined by a modification of the method of Gatt and Rapport\textsuperscript{14} in which the protein was not removed prior to color development. $\beta$-Glucuronidase was assayed according to the method of Ockerman.\textsuperscript{15} $\beta$-N-acetylglucosaminidase was measured as described by Levy and Conchie.\textsuperscript{16}

Results.—Arylsulfatase $A$ in cultured fibroblasts: In limited trials, metachromasia with cresyl violet, as observed in cultured nerve cells, could not be induced in MLD fibroblasts,\textsuperscript{17} but a defect of arylsulfatase-$A$ activity was clearly established. These fibroblasts had less than five per cent of the arylsulfatase-$A$ activity of control cells, while activities of other lysosomal enzymes examined—including arylsulfatase $B$, $\beta$-galactosidase, $\beta$-glucuronidase, and $\beta$-N-acetylglucosaminidase—did not differ significantly from those of control fibroblasts (Table 1). Cells from a patient with another storage disease, Sanfilippo syndrome, showed normal levels of all enzymes tested.

Combination experiment: A cell-free extract prepared from MLD cells had no effect on the arylsulfatase-$A$ activity of control cell extracts (Table 2), which indicated the absence of dissociable inhibitors.

Arylsulfatase $A$ in leukocytes: Arylsulfatase-$A$ activity was determined in leukocyte preparations from S. P. and from three age-matched patients with diagnoses other than metachromatic leukodystrophy. The enzyme activity of these three patients was within the previously observed normal range,\textsuperscript{7} whereas the MLD patient showed a marked deficiency of the enzyme (Table 3). The number of subjects in the present study is not as great as in the original leukocyte study,\textsuperscript{7} but the findings are similar and provide confirming evidence in an independent laboratory for a potentially valuable diagnostic procedure.

Discussion.—The decreased level of arylsulfatase $A$ noted by Austin\textsuperscript{4} in postmortem tissue obtained from children with metachromatic leukodystrophy can also be demonstrated in cultured fibroblasts obtained by skin biopsy from

**Table 1.** Activities of arylsulfatase $A$ and other lysosomal enzymes in cultured fibroblasts.

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Arylsulfatase $A$</th>
<th>Arylsulfatase $B$</th>
<th>$\beta$-Galactosidase</th>
<th>$\beta$-Glucuronidase</th>
<th>$\beta$-N-Acetylglucosaminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>940</td>
<td>377</td>
<td>555</td>
<td>—</td>
<td>16,900</td>
</tr>
<tr>
<td>Control 2</td>
<td>750</td>
<td>370</td>
<td>729</td>
<td>40.8</td>
<td>12,300</td>
</tr>
<tr>
<td>Control 3</td>
<td>1250</td>
<td>415</td>
<td>918</td>
<td>95.0</td>
<td>14,400</td>
</tr>
<tr>
<td>Sanfilippo syndrome</td>
<td>870</td>
<td>441</td>
<td>630</td>
<td>80.5</td>
<td>14,900</td>
</tr>
<tr>
<td>MLD (S. P.)</td>
<td>44</td>
<td>—</td>
<td>627</td>
<td>—</td>
<td>12,600</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>428</td>
<td>693</td>
<td>81.1</td>
<td>11,800</td>
</tr>
</tbody>
</table>

* Nanomoles product released per hour per milligram protein.

**Table 2.** The effect of MLD fibroblast extract on arylsulfatase-$A$ activity of control extract.

<table>
<thead>
<tr>
<th>Arylsulfatase-$A$ activity*</th>
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</thead>
<tbody>
<tr>
<td>10 $\mu$l Control extract</td>
</tr>
<tr>
<td>10 $\mu$l MLD extract</td>
</tr>
<tr>
<td>10 $\mu$l Control extract + 10 $\mu$l MLD extract</td>
</tr>
</tbody>
</table>

* The arylsulfatase-$A$ activity was determined as described in Materials and Methods and is expressed as nanomoles $p$-nitrocatechol formed per 30 min.
TABLE 3. Arylsulfatase-A activity in leukocytes.

<table>
<thead>
<tr>
<th></th>
<th>Specific activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. C.</td>
<td>126</td>
</tr>
<tr>
<td>L. R.</td>
<td>106</td>
</tr>
<tr>
<td>B. C.</td>
<td>125</td>
</tr>
<tr>
<td>MLD</td>
<td>4.9</td>
</tr>
</tbody>
</table>

* Nanomoles p-nitrocatechol released per hour per milligram protein.

a living child afflicted with the disease. The enzyme deficiency of these fibroblasts is of the same magnitude as that seen in leukocytes. In both types of cells, the enzyme activity in the patient is less than five per cent that of controls. Since arylsulfatase B is not totally inhibited under the conditions of assay of arylsulfatase A,13 it is not possible to say whether arylsulfatase A is completely lacking in MLD fibroblasts and leukocytes or is present to a very low degree. The finding that there is no loss of activity in normal cell-free extracts when mixed with extract from diseased cells indicates that the low activity seen in cells from MLD patients is probably due to a defect in the enzyme itself rather than to the presence of a reversible inhibitor. It is also clear that in metachromatic leukodystrophy the deficiency in arylsulfatase A is selective and not the result of secondary damage to the lysosymes, since all the other lysosomal enzymes tested fell within the normal range.

At present the relationship between the arylsulfatase-A deficiency and the deposition of cerebroside sulfates has not been established, although there is reason to believe that these compounds are normally catabolized by this enzyme. Mehl and Jatzkewitz18, 19 have presented evidence that arylsulfatase A isolated from pig kidney has weak cerebroside sulfatase activity. The activity is also present in normal human kidney but is absent in MLD kidney. The cleavage of cerebroside sulfates by the enzyme is enhanced by a heat-stable, high-molecular-weight factor, although the activity is still less than that toward nitrocatechol sulfate and tyrosine-O-sulfate.20 Whether or not the principal function of arylsulfatase A is the cleavage of cerebroside sulfate, the fact that its activity is low in all reported cases of metachromatic leukodystrophy4, 5, 7, 21 would indicate that its absence plays a central role in the disease. By comparing the organic sulfate metabolism of normal and MLD fibroblasts, it may be possible to gain a better understanding of the role of arylsulfatase A in normal cells and of the possible consequences of its deficiency in diseased cells.

The arylsulfatase-A deficiency of cultured fibroblasts could be used to confirm the diagnosis of metachromatic leukodystrophy, or even to detect the disease in high-risk infants before the onset of clinical symptoms. However, in this respect it offers no advantage to the much simpler and more rapid assay for the enzyme in leukocytes, the efficacy of which was confirmed in this study. There is at present no method for identifying heterozygous carriers of the disease. Austin was unable to detect metachromatic granules in the urines of parents and sibs of affected children2 nor did their urinary arylsulfatase-A activities differ from those of controls.6 In keeping with these findings, Percy and Brady7 have reported that leukocyte preparations obtained from the parents
of MLD patients have normal arylsulfatase activities. In light of the experience with the mucopolysaccharidoses, where otherwise normal carriers of the abnormal genes can be recognized by the behavior of their cultured fibroblasts,\(^2\) it will be of interest to investigate cells obtained from the parents and other relatives of MLD patients. Skin biopsies, obtained from the parents of the patient reported upon here, are now being cultured.

We are indebted to Dr. Robert E. Carrel for performing the skin biopsies and to Joe L. Trammell and Mrs. Sylvia D. de la Flor for their technical assistance.

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2 Ibid., 7, 415 (1957).
17 Harris, S. E., and H. Kihara, unpublished results.