Metachromatic Leukodystrophy: Diagnosis with Samples of Venous Blood

Abstract. *Arylsulfatase A and B have been demonstrated in preparations of human leukocytes. The level of activity of arylsulfatase A is markedly decreased in the preparations from patients with metachromatic leukodystrophy. Acid phosphatase and arylsulfatase B activities were normal. The assay of arylsulfatase A in leukocyte preparations can be useful in the diagnosis of metachromatic leukodystrophy while obviating the difficulties of current methods.*

Late infantile metachromatic leukodystrophy (MLD) is a progressive, degenerative, neurological disease characterized by a defective sphingolipid hydrolase, sulfatide sulfatase (*1*). Prior to 1966, the diagnosis of MLD depended on the detection of sulfatides, the accumulating sphingolipid, in a biopsy of the brain or of a peripheral nerve, or the isolation of metachromatic granules from urine (*2*).

Presently, the assay of urinary arylsulfatase A activity provides a semiquantitative screening test for MLD (*3*). This method relies upon fractionation of the specific enzyme in the urine with ammonium sulfate and is, therefore, subject to such variables as urine osmolality, diurnal variation in enzyme excretion, cellular or microbial contamination, and primary renal disease. Although these difficulties need not be insurmountable, considerable problems may be encountered until the conditions of the assay are satisfied.

The utilization of human white blood cells has facilitated the establishment of specific diagnostic tests in two other disorders of sphingolipid metabolism, namely, Gaucher's disease and Niemann-Pick disease (*4*). Following this precedent, it became of interest to examine the quantity of arylsulfatase A activity in human leukocytes. Our data indicate that leukocytes from patients with MLD are markedly deficient in arylsulfatase A activity when compared with normal controls and with patients with other neurological disorders. Concentrations of acid phosphatase and arylsulfatase B compare favorably with the various controls.

Leukocytes from 10 ml of venous blood were prepared as previously described by means of differential sedimentation in a solution of dextran and heparin (*4*). The leukocytes were washed twice with 1 ml of isotonic saline solution, and after centrifuging at 500*g*, the cells were suspended in 1 ml of distilled water. The suspended cells were frozen (in a mixture of dry ice and acetone) and thawed six times in order to obtain quantitative determination of arylsulfatase activity (*5*). (The leukocytes derived from 10 ml of venous blood provide adequate material for multiple determinations.) These leukocyte preparations have been stored deep-frozen for up to 6 months without detectable loss of enzyme activity.

Arylsulfatase A (*p*H 5.0) and B (*p*H 6.0) were assayed as previously described by utilizing the artificial substrate, *p*-nitrocatechol sulfate (*6*). The methods elaborated by Baum and his co-workers employ appropriate inhibitors to provide a more exact evaluation of each enzyme at its respective *p*H optimum. Acid phosphatase was determined with the use of *p*-nitrophenyl phosphate (*7*).

The diagnosis of MLD among the patients we tested had been previously established. Case 1 was diagnosed by biopsy of a peripheral nerve. A sibling had died following a similar clinical course, and postmortem examination established the diagnosis of MLD. Case 2 was diagnosed by biopsy of the brain and of a peripheral nerve and by the absence of arylsulfatase A in the urine. Cases 3, 5, and 6 were diagnosed by peripheral nerve biopsy. Case 3 also had a sibling in whom the diagnosis of MLD was confirmed at autopsy. Urinary arylsulfatase A was consistently low in case 6. A peripheral nerve biopsy in case 4 did not show metachromasia but metachromatic granules were detected in the urine sediment.

Arylsulfatase A activity in leukocyte suspensions from normal and disease controls was compared with similar determinations in leukocyte preparations from patients with MLD (Table 1). There is a marked diminution of arylsulfatase A activity in the leukocyte preparations from the cases of MLD. This difference was highly significant ($P = .0005$).

Table 1. Arylsulfatase A activity. Incubation mixture includes 0.20 ml of 0.01*M* *p*-nitrocatechol sulfate in 0.5*M* sodium acetate–acetic acid buffer containing $5 \times 10^{-4}M$ $Na_4P_2O_7$ and 10 percent NaCl (weight/volume) (*p*H 5.0) and 5 to 50 μl of white-cell preparation. The reaction was terminated, after incubation for 30 minutes at 37°C in air, by the addition of 0.2 ml of 1*N* NaOH, and the released *p*-nitrocatechol was determined by absorption at 515 nm in a Beckman DU spectrophotometer versus a zero-time enzyme control. MLD, metachromatic leukodystrophy; S.E., standard error.

Age of subjects (yr)	Sex	Type of disorder	*p*-Nitrocatechol released per hour per milligram of protein (nanomoles)
		Normal individuals	
25	F		90
25	F		66
23	F		65
27	F		93
28	M		133
35	F		130
29	M		98
35	M		85
39	M		122
24	M		86
8	F		133
		Neurological disorders other than MLD	
18	M	Duchenne-type muscular dystrophy	104
8	M	Post-infectious leukoencephalopathy	68
6	M	Undefined lipidosis	76
8 mo.	F	Undefined lipidosis	127
5	M	Juvenile-type amaurosis	139
		Mean ± S.E.	100.9 ± 6.5
		MLD	
4½	M	Stage* 2 to 3	8.4
14	M	Stage 2	6.9
10	M	Stage 2	15.9
4	M	Stage 4	14.5
34 mo.	F	Stage 2	16.0
26 mo.	F	Stage 2	8.4
		Mean ± S.E.	11.7 ± 2.4†

* Clinical staging as defined by Hagberg *et al.* (3). † $P = .0005$.

Levels of leukocyte arylsulfatase B (*6*) and acid phosphatase (*8*) were determined as previously described. The mean value (± standard error) of arylsulfatase B activity in 15 controls was 90 ± 13.6 nmole of nitrocatechol released per hour per milligram of protein as compared with 94 ± 14.9 nmole in the six patients. Similarly, the mean value of acid phosphatase activity among the controls was 74 ± 6.9 nmole of *p*-nitrophenol released per minute per milligram of protein, whereas in the preparations from patients the mean was 79 ± 9.9 nmole.

It is appropriate to consider the relation of arylsulfatase A with the accumulated product in MLD, that is, sulfatide. Studies by Mehl and Jatzkewitz (*9*) suggest that arylsulfatase A is the heat-labile, nondialyzable factor in their preparation, capable of hydrolyzing cerebroside sulfate (sulfatide). Sulfatide may then be the natural substrate of arylsulfatase A, as recently reported (*10*), and nitrocatechol sulfate represents the artificial substrate that was used in the earlier characterization of this class of enzymes.

Arylsulfatase activity has been detected principally in the granulocyte series and only to a slight degree in lymphocytes. It is not detectable in red blood cells or platelets (*5*). In suspected cases it is therefore essential to determine the white cell and differential counts in peripheral blood to control for low activity that might vary with the white-cell population. In the patients examined in this study, the number and distribution of white cells were within the normal range. Therefore, the decrease in arylsulfatase A activity in the leukocytes of patients with MLD is indicative of an enzymatic insufficiency in this condition. Leukocyte preparations from the parents of three patients with MLD were also tested. The values obtained for arylsulfatase A and B did not differ significantly from those that were obtained in the control population.

This assay should provide a simple and reliable method for the diagnosis of MLD. From these studies, however, it is not possible to delineate heterozygous carriers of this abnormality.

ALAN K. PERCY
ROSCOE O. BRADY
National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland 20014

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Reversible Cryogenic Blockade of Neural Function in the Brain of Unrestrained Animals

Abstract. *Reversible cryogenic blockade of neural systems in the brain may be accomplished by local cooling. A small cryoprobe is described which may be implanted in the brain of an unrestrained, behaving animal. Cooling may be restricted to the tip of the cryoprobe and the temperature of the tip and the shaft may be regulated and monitored independently by miniature thermocouples and appropriate control and indicator devices. Electrophysiological results are presented which show that the cryogenic blocking effect may be localized to within a few millimeters of the tip of the cryoprobe and that the size of the region blocked is proportional to the tip temperature. The system described has been shown to be effective in both electrophysiological and behavioral studies.*

An important experimental approach to the study of the brain and its role in behavior has been to destroy parts of it and observe deficits, malfunctions, or aberrant behavior. Destruction of brain tissue has been accomplished by ablation, thermocoagulation, high-energy ionizing radiation, focused ultrasonics, and local application of chemical and radioactive substances. However, lack of functional reversibility poses a serious constraint upon behavioral study.

Among methods capable of reversibility are (i) use of local anesthetics, (ii) production of spreading depression by KCl, (iii) polarization by direct current, and (iv) application of local pressure, heating, or cooling. Each of these methods has disadvantages when used to block localized subcortical structures, and especially so in freely moving animals. There may be difficulties in application, control of degree and duration of effectiveness, and reversibility. Restricted local cooling of brain tissue appears to be the most precisely controllable and completely reversible method available (*1*). Functional blockade of electrophysiological and behavioral activities has been demonstrated in cats by cooling to 10°C (*2*); complete recovery of function occurs even after cooling to −10°C and without evident pathologic damage (*3*).

Cryogenic methods previously used for reversibly blocking restricted subcortical structures have presented technological and application problems in chronic, behaving, animal preparations. These, in part, have been resolved by developments reported here: (i) provision to localize cooling at the tip by heating the shaft of the probe, (ii) reduction of probe size, and (iii) use of flexible, light-weight connections to the freely moving animal.

The cryoprobe is constructed of 24 and 18 gauge (0.55 and 1.2 mm), stainless steel, hypodermic-needle tubing soldered together (*4*) to form a concentric, double cannula system (Fig. 1C). A thin, insulated, low-resistance heater-wire (0.025 mm in diameter) is wrapped around the outer cannula except for 2 mm at the tip. Alcohol (95 percent ethanol or methanol), cooled in a dry-ice and alcohol bath, is circulated by pressure through the probe, and direct current is passed through the heater-wire warming the shaft to body temperature while the tip remains cold. The degree of cooling, determined by the flow rate of the coolant through the cryoprobe, is controlled by the pressure in the steel reservoir tank (Fig. 1D). This in turn is determined by a variable pressure regulator (0 to 400 psi) (0 to 27.2 atm) attached to a nitrogen gas pressure source. The cold alcohol is carried to and away from the probe in