EVIDENCE FOR THE GENETIC BLOCK IN METACHROMATIC LEUCODYSTROPHY (ML)

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The accumulation of cerebroside sulfate and the concomitant reduction of the cerebroside level in nervous tissue from ML patients led to the suggestion that the turnover of cerebroside sulfates to cerebrosides might be blocked as a result of deficient cerebroside sulfatase (Jatzkewitz, 1960). Cerebroside sulfatase activity (Mehl and Jatzkewitz, 1963) was demonstrated in normal mammalian tissues such as kidney and brain, where in ML the cerebroside sulfate accumulation is especially high.

Experiments with a more than 6000 fold purified cerebroside sulfatase from swine kidney revealed that the activity depends upon the presence of at least two higher molecular components, a heat-labile one and a heat-stable one (Mehl and Jatzkewitz, 1964). The heat-labile component of the cerebroside sulfatase and an arylsulfatase type A were found in the same fraction and remained together even after preparative disc electrophoresis, although the isolated protein appeared to be homogenous (Fig. 1). In addition inhibition experiments indicated that the heat-labile component of cerebroside sulfatase and the arylsulfatase-A might be identical (Mehl and Jatzkewitz, 1964).

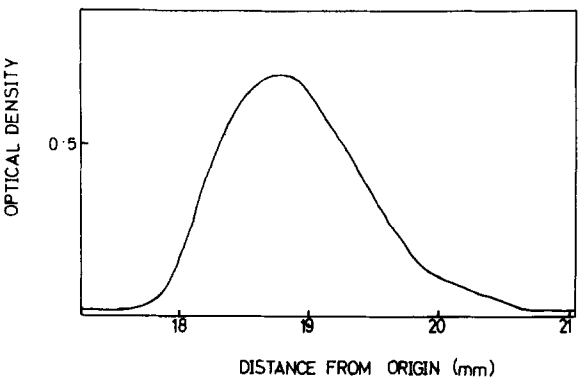

Fig. 1 - Scan of a phoretogram after disc electrophoresis of 100 µg of isolated cerebroside sulfatase component. Electrophoresis on polyacrylamide standard gel (7.5%) revealed only one band which represented both, the heat-labile component of cerebroside sulfatase and the aryl-sulfatase-A activity. On the basis of the density profile the protein appears to be homogenous.

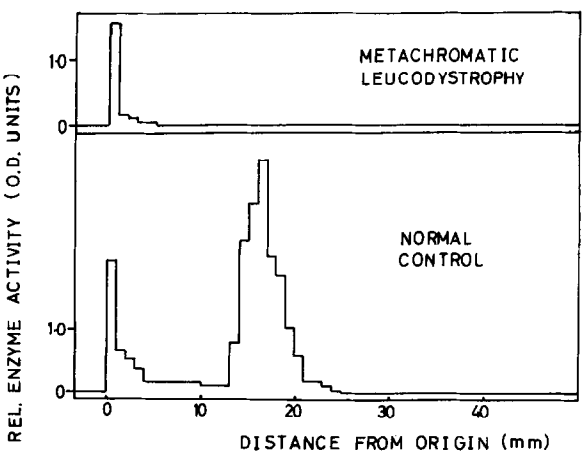

Fig. 2 - Comparison of arylsulfatase activities in normal and ML kidney tissue extracts after disc electrophoresis on polyacrylamide gel (7.5%). For localization of enzyme activity the gel columns were sectioned into 1 mm high discs which were incubated at pH 4.5 (see text). The complete deficiency of the main component (R_F=0.40) in ML tissue extract is obvious.

Since an arylsulfatase type A deficiency has been demonstrated in ML (Austin, et al., 1963, 1964), one might ex-

pect also to find a deficiency of the heat-labile component of cerebroside sulfatase.

In order to prove this, cerebroside sulfatase activity was assayed in tissue extracts of ML and control patients. Using ^{35}S-labeled cerebroside sulfate and extracts of kidney tissue, the control samples gave respectively 3000 c.p.m. and 1550 c.p.m. in the free sulfate fraction, the ML tissue less than 10 c.p.m. after incubation (Table 1). Mixed incubation experiments after combining extracts of ML and control tissues ruled out the presence of reversible inhibitors and interference due to excess amounts of unlabeled cerebroside sulfate in the ML extracts.

Although the cerebroside sulfatase activity was reduced to the limits of detection of our test, arylsulfatase-A activity was found to be 8% of the control value. This discrepancy was settled when samples of kidney extracts containing 700 µg protein were fractionated by standard disc electrophoresis. After a 90 min. run under 2,5 mA the columns were sectioned into 1 mm high discs, which were assayed for arylsulfatase activity at pH 4.5 in the presence of 0.005 M 2-hydroxy-5-nitrophenyl sulfate. Two arylsulfatase activities were present in the control (Fig.2). The minor component (at origin) of the total arylsulfatase activity at pH 4.5 was also present in the extracts of ML kidney with a comparable activity. The main component (R_F= 0.40) gave rise maximally to 3.6 o.d. units per disc after incubation, whereas the activity in ML was within the limits of detection (0.005 o.d. units). This essentially complete deficiency of the arylsulfatase-A component of the cerebroside sulfatase in this patient is the more sur-

TABLE 1

SULFATASE AND ACID PHOSPHATASE ACTIVITIES IN TISSUE EXTRACTS FROM A ML PATIENT AND CONTROLS

Enzyme activity (μmoles/hr/g tissue) in extracts of renal-cortex			
Type of enzyme	Control I	Control II	ML 474
Cerebroside sulfatase* (c.p.m.)	1550	2930±80	<10; <10
(mμmoles/hr/g tissue)	2·47	4·65	<0·02
Arylsulfatase-A	10·4	14·8±1·1	1·07±0·17
(+ 0·0005M sulfite)	<0·10	-	<0·01
Arylsulfatase-B	17·3	52·2	15·8±2·3
(+ 0·0005M sulfite)	<1·0	-	<0·1
Arylsulfatase-C †	23·1±0·4	30·3	51·5±10·1
Acid phosphatase	43·2	28·7±3·2	34·9

Enzyme activity in extracts of liver tissue		
Type of enzyme	Control III	ML
Cerebroside sulfatase (c.p.m.)	840	<10
(mμmoles/hr/g tissue)	1·33	<0·02
Arylsulfatase-A	12·2	1·0
Arylsulfatase-B	10·4	8·4

The tissues were extracted with 0.2M-acetate buffer pH 5·1 in the presence of butanol and 0·4M-$CaCl_2$. After gel filtration (Sephadex G-25, acetate buffer pH 4·5) and concentration by low pressure filtration the extracts were centrifuged at 33,000 g for 30 min. Aliquots of the supernatant were assayed for cerebroside sulfatase (Mehl and Jatzkewitz, 1964), arylsulfatase type A (Austin et al., 1963), arylsulfatase type B and C (Dodgson, Spencer and Wynn, 1956) and acid phosphatase (Gianetto and de Duve, 1955).

* Control II + ML extracts mixed in equal amounts gave 2280±350 c.p.m.

† after incubation of homogenate

prising as he lived to the age of 20.

Since the controls and ML tissues were stored under similar conditions and since other hydrolases (arylsulfatase type B and C and acid phosphatase) exhibited comparable activities in controls and ML extracts, the deficiency of the heat-labile component of the cerebroside sulfatase in ML did not result from improper storage of the ML tissues.

In conclusion, it has been shown that the absence of cerebroside sulfatase activity in ML, a sulfatide lipidosis, is due to a deficiency of the heat-labile component of this enzyme. It is this heat-labile component which exhibits the arylsulfatase type A activity, shown by Austin et al. (1963; 1964) to be greatly diminished in ML tissues. As a consequence of this enzymatic defect the conversion of cerebroside sulfate to cerebroside is blocked.

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