Prenatal diagnosis of Wolman's disease

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Summary. Amniocentesis was performed in the 15th week of a pregnancy at risk for Wolman's disease. The cultured amniotic fluid cells were found to have a severe deficiency of acid esterase activity consistent with homozygosity of the fetus. The pregnancy was terminated in the 19th week and the prenatal diagnosis confirmed by enzymic and chemical evaluation of the fetal tissues.

The autosomal recessive lipidosis known as Wolman's disease (Abramov *et al.*, 1956; Wolman *et al.*, 1961; Patrick and Lake, 1973) is characterized clinically by the onset in early infancy of recurrent vomiting, persistent diarrhoea, hepatosplenomegaly, abdominal distension, and calcification of enlarged adrenals. The severely wasted infants usually succumb before the age of 6 months. Foamy cells are abundant in the viscera and are found to contain excessive amounts of triglycerides and cholesteryl esters confined to intracellular vacuoles that have been identified as swollen lysosomes (Lake and Patrick, 1970). Storage is the result of a congenital deficiency of a lysosomal acid esterase that normally hydrolyses triglycerides and cholesteryl esters entering the lysosomes for degradation. A profound deficiency of acid esterase has been found in the tissues (Patrick and Lake, 1969; Sloan and Fredrickson, 1972), leucocytes (Young and Patrick, 1970), and cultured skin fibroblasts (Kyriakides *et al.*, 1970) of affected infants. The presence of acid esterase in cultured normal amniotic fluid cells obtained in early pregnancy also provides the opportunity for prenatal diagnosis of Wolman's disease. We report here the prenatal identification of an affected fetus.

Case history

One normal boy and two affected boys had been born to the family. The parents learned of the possibility of prenatal diagnosis after beginning the latest pregnancy and requested the investigation after genetic counselling. Amniocentesis was performed in the 15th post-menstrual week.

Materials and methods

Amniotic fluid cells were cultured in medium 199 containing 15% fetal bovine serum and added glutamine (1 mmol/l). Cultures were incubated at 37°C in pyrex Petri dishes (50 mm diameter) in an atmosphere of 5% CO₂:95% air. Cell growth covering half of a dish was adequate for enzyme assays.

Glyceryl tri(1-¹⁴C) palmitate (30-60 mCi/mmol; Amersham) was adjusted to a specific activity of 0.1 mCi/mmol with tripalmitin (Sigma) and purified according to Kaplan (1970). p-Nitrophenyl palmitate was obtained from Koch-Light Ltd.

Substrate solutions containing 10 µmol labelled tripalmitin and 100 mg Triton X-100/ml, or 12 µmol p-nitrophenyl palmitate and 100 mg Triton X-100/ml were prepared by mixing a solution of the ester in light petroleum (b.p. 40-60°C) with the Triton and removing the light petroleum under reduced pressure at 80°C. Warm water was then added to the required volume with rapid mixing.

Cultured amniotic fluid cells were homogenized in cold water to yield preparations containing 1-5 mg/ml soluble protein. Assay medium employing p-nitrophenyl palmitate as substrate contained, in a final volume of 160 µl: 10 µmol glycine-HCl, pH 4.0; 0.6 µmol p-nitrophenyl palmitate, and 10 µl of whole homogenate. After incubation for 30 min at 37°C, the reaction was stopped with 20% perchloric acid (50 µl) and the mixture was centrifuged after standing 5 min. Supernatant (50 µl) was added to 350 µl mol/l Tris-HCl, pH 9.0, and the optical density at 400 nm measured within 1 min.

The esterase assay using tripalmitin as substrate was essentially that of Kaplan (1970). The medium (final volume 400 µl) contained: 30 µmol glycine-HCl, pH 4.0; 2 µmol tripalmitin (0.2 µCi ¹⁴C-tripalmitin), and 25 µl of whole homogenate. After incubation at 37°C for 2 hr, the liberated fatty acid was extracted and estimated by scintillation counting. Acid esterase activity of fetal tissues was measured as for cultured cells, above, using 20 µl of a 2% whole homogenate, and incubation for 10 min with p-nitrophenyl palmitate, or 25 µl of a 5% homogenate and incubation for 2 h with tripalmitin.

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Thin-layer chromatography of lipids in chloroform:methanol (2:1 by volume) extracts of fetal tissues was run on silica gel G with a solvent system, n-heptane:diethyl ether:acetic acid (80:20:1).

Results and discussion

Amniotic fluid cells were harvested after 3 weeks in culture and assayed for acid esterase activity against both tripalmitin and p-nitrophenyl palmitate as substrates. Normal control cultures (10, of which three were grown at the same time as that from the patient) were assayed simultaneously. From these results and the previously determined acid esterase activities of homozygous, heterozygous and normal cultured skin fibroblasts, given in Table I, it was concluded that the fetus was homozygous for Wolman's disease. With regard to the reliability of differentiation of homozygous and heterozygous levels of activity in amniotic fluid cells, it is of interest to note that the activity ranges of normal

control cultures of skin fibroblasts and amniotic fluid cells were similar, and that the activities of heterozygous fibroblasts fell within the normal range.

At parental request, prostaglandin termination of pregnancy was performed in the 19th post-menstrual week. Fetal tissues were immediately frozen in solid CO₂ for enzymic evaluation, the results of which (Table II) confirmed the prenatal diagnosis. Hydrolysis of tripalmitin was undetectable in all the affected tissues examined, and the hydrolysis of p-nitrophenyl palmitate by liver and spleen was less than 10% of that of normal controls. Other control tissues were not available for comparison but the low levels of p-nitrophenyl esterase activity found for intestine, adrenal, kidney, and lung of the affected fetus are presumed to reflect a similarly severe depression of activity.

Lipid analysis of chloroform-methanol extracts of liver and spleen by thin-layer chromatography (Fig. 1) provided further proof of positive diagnosis and, as in other lysosomal disorders, demonstrated that

TABLE I
ACID ESTERASE ACTIVITY OF CULTURED CELLS

Cultured Cells	Substrate	
	¹⁴ C-Tripalmitin*	p-Nitrophenyl Palmitate†
Amniotic fluid cells Pregnancy at risk	0.04	6.0
Controls (n = 10); range (mean)	1.36–3.65 (2.50)	39.1–104.7 (66.2)
Skin fibroblasts		
Patient with Wolman's disease	0.13	3.1
Father	1.72	31.0
Mother	3.15	42.1
Controls (n = 8); range mean	1.67–3.75 (2.65)	29.6–54.7 (41.4)

* nmol fatty acid released per mg soluble protein per min.
† nmol substrate hydrolysed per mg soluble protein per min.

TABLE II
ACID ESTERASE ACTIVITY OF FETAL TISSUES

Tissue	Substrate	
	¹⁴ C-Tripalmitin*	p-Nitrophenyl Palmitate†
Affected fetus		
Liver	0	1.2
Spleen	0	8.4
Small intestine	0	9.0
Adrenal	0	11.4
Kidney	0	7.0
Lung	0	5.4
Controls		
Liver (n = 5); range (mean)	5.1–15.7 (12.0)	114–219 (145)
Spleen (n = 2)	22.4, 17.8	339, 147

* μmol fatty acid released per g wet weight of tissue per hour.
† μmol substrate hydrolysed per g wet weight of tissue per hour.

FIG. 1. Thin-layer chromatography of chloroform:methanol extracts of fetal tissues. 1: normal liver; 2: liver, affected fetus; 3: spleen, affected fetus; 4: normal spleen CE=cholesteryl esters; TG=triglycerides.

storage was already well advanced in early fetal life. Both tissues showed marked accumulation of triglycerides and cholesteryl esters, the latter being particularly abundant in the liver.

These findings demonstrate the feasibility of intrauterine diagnosis of Wolman's disease and early abortion in affected cases. The results also confirm the equivalence of acid esterase assays employing either triglycerides or nitrophenyl esters as substrates, previously documented in the use of tissues and leucocytes for the diagnosis of Wolman's disease (Patrick and Lake, 1969; Young and Patrick, 1970). The simplicity and higher reaction rate of the nitrophenyl esterase assay commend it as the method of choice in prenatal diagnosis. Its sensitivity could be increased considerably by micro-scale adaptation and longer incubation periods, thereby permitting the earliest possible evaluation of smaller numbers of cultured cells obtained within 2 to 3 weeks after amniocentesis in the 14th to 16th week of pregnancy.

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