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Prenatal Diagnosis of Lysosomal Storage Diseases

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The prenatal diagnosis of lysosomal storage disorders can be achieved, once the diagnosis is confirmed in the index case, by a variety of techniques including analysis of amniotic fluid, asay of enzymic activity in cultured amniotic fluid cells, cultured chorionic villus cells and by direct assay of activity in chorionic villus samples. These studies can be accompanied by ultrastructural observations which give an independent means of diagnosis. In some instances molecular genetic studies for mutation detection or linkage analysis are appropriate for prenatal diagnosis. Pseudodeficiencies of some of the lysosomal enzymes, which cause no clinical problems, can complicate the initial diagnosis particularly in metachromatic leucodystrophy where the pseudodeficiency is more common than the disease itself. Mutation analysis as well as enzyme assay is necessary not only in the index case but also in the parents before the same techniques are applied to a sample for prenatal diagnosis. A large number of lysosomal storage disorders may present as fetal hydrops and the diagnosis can be established at this late stage by fetal blood sampling and examination by microscopy as well as by biochemical assay of the appropriate enzyme or metabolite in amniotic fluid. All prenatal diagnoses in which an affected fetus is indicated should have confirmation of the diagnosis as soon as possible to reassure anxious parents, and to act as audit of the laboratory's competence to undertake prenatal diagnosis. A combined approach to prenatal diagnosis involving biochemical, molecular genetic and morphological studies is recommended.

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Introduction

Prenatal diagnosis of the lysosomal disorders has evolved from the recognition of the enzyme defects in the late 1960's and early 1970's and the discovery that the enzyme defects are expressed in cultured fibroblasts. It was a short step to apply the techniques to cultured amniotic fluid cells which shared many properties with fibroblasts although their origin is probably mostly epithelial. Amniocentesis was a relatively new technique at that time and was practiced in only a few centres. Samples of amniotic fluid containing amniotic fluid cells were taken comparatively late (16-18 weeks) in the second trimester and generally the cells required culturing, which meant that a diagnosis could not be achieved until approximately 20 weeks gestation. Analysis of amniotic fluid supernatant was diagnostically helpful in certain circumstances. Tests to measure enzyme activity or to detect the specific metabolites in the amniotic fluid were used as a basis for the diagnosis of those disorders in which the lysosomal enzyme activity is grossly raised (e.g. I-cell disease) or a specific metabolite is increased (e.g. the mucopolysaccharidoses).

Although amniocentesis was an advance on no prenatal diagnostic tests being available for the lysosomal disorders, the late termination of an affected fetus caused much distress. However tissues obtained from these terminations enabled confirmation of the diagnosis and provided valuable morphological evidence that the biochemical tests were accurate and could be relied upon (1, 46). In addition morphological observations on the placenta showed that in some of the disorders there were diagnostically important features to be found at the light microscope level (46), although in some disorders the light microscopy of the fetal organs appeared normal and evidence of the disease was only apparent by electron microscopy (1). Electron microscopical examination of the uncultured cells from the amniotic fluid has been applied to the diagnosis of Pompe's disease (30) and late infantile Batten's disease (12).

The placenta is essentially a fetal organ, reflecting the enzyme activity, cellular morphology and chromo-

Disease	Defect	Material for diagnosis	DNA Analysis Possible #
1. Sphingolipidoses			
A. Gangliosidoses			
G_{M1}-gangliosidosis	β-galactosidase	CV, CCV, CAC	Yes
G_{M2}-gangliosidoses			
Tay-Sachs	hexosaminidase A	CV, CCV, CAC	Yes
Sandhoff	total β-hexosaminidase	CV, CCV, AF, CAC	Yes
B1 variant	hexosaminidase A	CV, CCV, CAC	Yes
AB variant	activator protein deficiency	CCV, CAC	Yes
Galactosialidosis	cathepsin A	CV,CCV,CAC	Yes
Saposins A,B,C,D deficiency	prosaposin	CCV*,CAC	Yes
Farber disease	ceramidase	CCV, CAC	Yes
B. Leucodystrophies			
Krabbe	galactocerebrosidase	CV, CCV, CAC	Yes
Metachromatic	arylsulphatase A	CV, CCV, CAC	Yes
	saposin B	CCV*, CAC*	Yes
C. Visceral storage disease			
Gaucher	β-glucocerebrosidase	CV, CCV, CAC	Yes
	saposin C	CCV*, CAC*	Yes
Niemann-Pick types A&B	sphingomyelinase	CV, CCV, CAC	Yes
Fabry	α-galactosidase	CV, CCV, AF, CAC	Yes
2. Glycoprotein Storage Diseases			
Fucosidosis	α-fucosidase	CV, CCV, CAC	Yes
α-Mannosidosis	α-mannosidase	CV, CCV, CAC	Yes
β-Mannosidosis	β-mannosidase	CV, CCV, CAC	Yes
N-acetyl α-galactosaminidase deficiency (Schindler)	N-acetyl α-galactosaminidase	CV*,CCV*,CAC*	Yes
Aspartylglucosaminuria	N-aspartyl β-glucosaminidase	CV, CCV, CAC	Yes
3. Mucopolysaccharidoses			
All types	Glycosaminoglycans	AF	-
I - Hurler	α-iduronidase	CV, CCV, CAC	Yes
II - Hunter	iduronate-sulphatase	CV, CCV, AF, CAC	Yes
IIIA - Sanfilippo A	heparin sulphamidase	CV, CCV, CAC	Yes
IIIB - Sanfilippo B	N-acetyl α-glucosaminidase	CV, CCV, CAC	Yes
IIIC - Sanfilippo C	Acetyl-CoA:α-glucosamide N-acetyl transferase	CV, CCV,CAC	No
IIID - Sanfilippo D	N -acetyl glucosamine-6-sulphate sulphatase	CV*, CCV, CAC	Yes
IVA - Morquio A	N-acetyl galactosamine-6-sulphate sulphatase	CV, CCV, CAC	Yes
IVB - Morquio B	β-galactosidase	CV, CCV, CAC	Yes
VI - Maroteaux–Lamy	arylsulphatase B	CV, CCV, CAC	Yes
VII - Sly	β-glucuronidase	CV, CCV, AF, CAC	Yes
4. Mucolipidoses			
Mucolipidosis I (Sialidosis)	α-neuraminidase	CV, CCV, CAC	Yes
Mucolipidosis II (I-celldisease)	N-acetyl glucosamine phosphoryl transferase	CCV, AF, CAC	No
Mucolipidosis III (pseudo Hurler polydystrophy)	N-acetyl glucosamine phosphoryl transferase	CCV, AF, CAC	No
Mucolipidosis IV	unknown (abnormal phospholipid composition)	CCV*, CAC (EM)	No
5. Other types			
Wolman and Cholesteryl Ester Storage Disease	acid esterase (acid lipase)	CV, CCV, CAC	Yes
Mucosulphatidosis	multiple sulphatase deficiencies	CV, CCV, CAC, FB	No
Pompe (GSD II)	α-1:4-glucosidase	CV, CCV, CAC	Yes
Infantile Batten (CLN1)	palmitoyl protein thioesterase	CV(EM)	Yes, also**
Late infantile Batten (CLN2)	pepstatin-insensitive protease	AF(EM)	Yes*, also**
Juvenile Batten (CLN3)	unknown (mutated protein of unknown function)	CV(EM)	Yes, also**
6. Transport defects			
Sialic acid storage disease	free sialic acid	CV,CCV,AF,CAC	Yes**
Salla disease	free sialic acid	CV, AF*	Yes**
Cystinosis	cystine	CV,CCV,CAC	Yes**
Niemann-Pick disease type C	cholesterol	CCV, CAC	Yes
Pycnodysostosis	cathepsin K	*	

Abbreviations:
CV: uncultured chorionic villi CCV: cultured chorionic villi
CAC: cultured amniotic fluid cells AF: amniotic fluid
FB: fetal blood morphology and assay EM: by electron microscopy
*:no reports in the literature ** :by linkage analysis
possible if mutations known in index case

Table 1. Prenatal diagnosis of lysosomal storage diseases.

somal make up of the fetus. Therefore it was a logical step to attempt to detect genetic defects in fetuses at risk by analysis of a placental biopsy (chorionic villi). Although chorionic villus sampling for the prenatal diagnosis of genetic disease had been reported in 1968 (25), it was not introduced for the prenatal diagnosis of metabolic disorders by enzyme assay until the early 1980's (35). Chorionic villus sampling (10) is possible at a much earlier stage of pregnancy than amniocentesis and means that a diagnosis can be achieved by 10-11 weeks gestation if direct assay of the villi is possible, and before 13-14 weeks if cultured cells are necessary.

Over the last few years most of the genes encoding proteins that are defective in lysosomal storage diseases

have been cloned, permitting identification of mutations in individual patients. It is now possible to use DNA analysis to confirm or make the diagnosis in cases where there is informative linkage data or the mutations in the family are known. It is particularly valuable in diseases where the defective enzyme is not yet assayed routinely (31, 55).

A potential but real pitfall in the diagnosis of several lysosomal diseases has been the discovery in a number of individuals of an apparent enzyme deficiency which is later proven to be unrelated to their clinical disease. These 'pseudodeficiencies' (76) in which the enzyme activity appears to be deficient are usually uncovered, without their significance being recognized, during screening for causes of metabolic disease. It is therefore essential to carry out further biochemical, histochemical or DNA analysis to confirm the diagnosis, especially in cases with an atypical presentation. Failure to recognize the presence of a pseudodeficiency allele in a family could lead to an incorrect prenatal diagnosis.

Most definitive diagnoses are now made by enzymic analysis (2) but it has been our practice since 1970 to make and confirm prenatal diagnoses wherever possible both by biochemical analysis and by morphology (40). To date approximately 1000 pregnancies at risk for a lysosomal storage disorder have been successfully monitored in this way in our laboratories. However DNA analysis is now playing an increasing role, and in the future we would advocate a combined biochemical, morphological and molecular genetic approach to all who are involved in prenatal diagnosis of the lysosomal disorders.

Requirements for prenatal diagnosis

The prerequisite of accurate prenatal diagnosis of a lysosomal storage disease by biochemical analysis, be it by enzyme assay, storage product detection or DNA analysis, is confirmation of the biochemical or genetic defect in the index case. A clinical diagnosis alone is insufficient. In a few rare situations the initial diagnosis may have been made, or may only be possible by histopathological examination (light or electron microscopy) of a tissue sample. This is the case in some of the forms of Batten's disease. The diagnosis of a lysosomal storage disorder has also been made following histological examination of the placenta to establish the causes of fetal intrauterine growth retardation (64) or fetal ascites/hydrops (23, 52, 56). Routine placental examination in cases of unexplained fetal or neonatal death may also uncover evidence of a storage disease (61). In such cases the parents can be tested for heterozygosity for a range of lysosomal enzyme deficiencies, before prenatal diagnosis is considered. However, in the majority of disorders a large overlap of activity between heterozygotes and normal controls often leads to equivocal results.

G_{M1}-gangliosidosis (β-galactosidase deficiency)
I-cell disease
Infantile sialic acid storage disease
Sialidosis (α-neuraminidase deficiency)
Galactosialidosis (cathepsin A deficiency)*
* expected but no data available

Table 2. Disorders with striking trophoblast vacuolation.

The laboratory undertaking the test must be experienced in the diagnosis of lysosomal disorders, and should have experience of handling chorionic villus samples. In practice this means that there will be regional specialist centres which can undertake the whole range of tests necessary and are highly experienced in the study of lysosomal disorders. In addition where morphological studies are to be made in parallel, the investigator should have a thorough knowledge of the morphological changes not only in the disorder under test but also the range of normal appearances of the chorionic villus sample (CVS), amniotic fluid cells or fetal blood cells. In the cases where the initial diagnosis in an affected child has been made on morphological evidence alone, it is imperative that the investigator assessing the prenatal sample has seen, at first hand, the evidence on which the diagnosis was made.

Appropriate counselling of the parents by a geneticist, and an explanation of the possible limitations of the test are also necessary prerequisites. In some countries it may also be appropriate for a waiver of possible legal action to be signed should the result be inaccurate and fail to detect an affected fetus.

Prenatal diagnosis based on chorionic villus samples (CVS)

The lysosomal storage disorders (69) are probably the largest group of diseases that can be diagnosed prenatally on chorionic villus material. The diagnosis can be made by direct enzyme assay on the CVS, without cell culture, for the majority of these disorders and has generally evolved from analysis of amniotic fluid cell cultures (CAC). DNA analysis, morphological examina-

Disease	Trophoblast changes	Fibroblast changes	Endothelial cell changes	Comments
G_{M1} gangliosidosis	Striking syncytiotrophoblast vacuolation	Vacuolation	Vacuolation	
G_{M2} gangliosidosis (all types)	No changes	No changes	No changes	
Mucopolysaccharidosis types I and II	Some vacuolation of cytotrophoblast	Vacuolation	Vacuolation	
Mucopolysaccharidosis type IA, Morquio A	No changes	Vacuolation expected	Vacuolation expected	
β-glucuronidase deficiency	Patchy syncytiotrophoblast vacuolation	Vacuolation	Vacuolation	
Infantile sialic acid storage disease	Striking syncytiotrophoblast vacuolation	Vacuolation	Vacuolation	
I-cell disease (mucolipidosis II)	Striking syncytiotrophoblast vacuolation	Vacuolation	Vacuolation with lipofuscin deposition	Stromal macrophages may contain ingested glycogen and collagen fibres
Sialidosis (α-neuraminidase deficiency)	Striking syncytiotrophoblast vacuolation	Vacuolation	Vacuolation	
Glycogen storage disease type II (Pompe)	Some vacuoles containing glycogen in cytotrophoblast	Vacuoles containing glycogen	Vacuoles containing glycogen	
Niemann-Pick disease type A	Vacuolation with lamellar bodies in syncytiotrophoblast and cytotrophoblast	Vacuolation with lamellar bodies	Vacuolation with lamellar bodies	
Niemann-Pick disease type C	No changes	No changes	No changes	
Aspartylglucosaminuria	Rare and patchy cytotrophoblast vacuolation	Vacuolation	Vacuolation	
Wolman's disease	No changes	Membrane-bound lipid droplets	Membrane-bound lipid droplets	
Mucolipidosis IV	No changes	No changes	Lamellar bodies found in placenta	
Farber's disease	No information available	No information available	No information available	
Fabry's disease	No changes	No changes	No changes	
Gaucher's disease	No changes	No changes	No changes	
Krabbe's leucodystrophy	No changes	No changes	No changes	
Infantile Batten's disease	No changes	No changes	Granular osmiophilic deposits (GROD)	
Late infantile Batten's disease	No information available	No information available	No information available	Rare curvilin ear bodies in placenta at 20 weeks
Juvenile Batten's disease	No changes	No changes	Atypical fingerprint bodies	
Early juvenile (variant late infantile) Batten's disease	Not known	Not known	Not known	
Cystinosis	No changes	No changes	No changes	

Table 3. Electron microscopy of CVS in a variety of lysosomal storage disorders.

tion and detection of specific metabolites in CVS are also useful. Table 1 shows the disorders and the material that can be used for diagnosis.

Sample preparation. Samples of chorionic villi are taken by standard procedures (10) at around 11 weeks gestation. The villi should be dissected free of contaminating maternal material, transferred to culture medium containing 10-20 U heparin / ml medium and transported to the laboratory undertaking the prenatal diagnostic test by post or courier to arrive within 48 hours. Sent under these conditions CVS travel well and will be perfectly viable. Upon receipt in the laboratory the villi are again rigorously examined for maternal decidua, and, depending on the size of the sample and the disorder being tested for, the material is divided. If possible some material is taken to establish a culture of cells and, if it is likely to be informative a small frond of villus is taken for morphological assessment (see Tables 2 and 3). In pregnancies at risk for the X-linked Hunter and Fabry diseases, some material should be also be sent for chromosomal analysis. The remaining villi are washed thoroughly with cold isotonic saline and divided into approximately 5 mg portions to which are added 50 μl water, except for the diagnosis of cystinosis the washed villi are added directly to medium containing ^{35}S-cystine (58). If the pregnancy is at risk for sialidosis, neuraminidase must be assayed immediately, as this is a very labile enzyme and freezing inactivates the enzyme, but villi for other disorders can be stored frozen at -20°C until assay, or may be assayed immediately.

Enzyme analysis. The methods used to determine enzyme activities in chorionic villi directly are similar to

those used in cultured amniotic cells and fibroblasts (83). However interfering isoenzymes have necessitated modification of the arylsulphatase A assay for the diagnosis of metachromatic leucodystrophy (66). The synthetic substrate (4-methylumbelliferyl β-N-acetyl glucosamine-6-sulphate) (44) , which is specific for hexosamindase A, is used for the diagnosis of Tay-Sachs disease and the B1 variant of G_{M2}-gangliosidosis rather than assay of the enzyme by heat inactivation.

The specific activities of the lysosomal enzymes in uncultured chorionic villi are, with a few exceptions, of much the same order as those found in cultured amniotic cells and fibroblasts (22). Alpha-Iduronidase activity is considerably lower in chorionic villi than in cultured cells and doubts had been expressed (18, 21, 22), as to whether assay of this enzyme in chorionic villi is a reliable test for the prenatal diagnosis of Hurler disease. However Young (84) reported the results of 24 pregnancies tested by direct assay and concluded that it was a highly accurate test.

Prenatal diagnosis of the X-linked Hunter and Fabry diseases can pose a problem because of mosaicism in heterozygous female cells expressing either the normal or mutant gene. Due to non-random X-inactivation it is possible for material from a heterozygous female fetus to have almost as low enzyme activity as an affected male. This has been reported in chorionic villi (8, 13) and in cultured amniotic fluid cells (36). Thus, in the prenatal diagnosis of these disorders it is very important to determine the sex of the fetus.

The lability of α-neuraminidase can present a problem in the prenatal diagnosis of sialidosis and in this disorder the combined approach of enzyme and morphological analysis is particularly useful.

Morphological examination. For the morphological assessment of the various cell types in the CVS any fixative containing glutaraldehyde is suitable and fixation at room temperature for periods of up to 1 month or longer gives results indistinguishable from the usually prescribed cold fixation for two hours. The sample is relatively unaffected by storage in culture medium for periods of up to 24-48 hours, provided the temperature is not excessive, and in effect any sample which is adequate for biochemical assay will be adequate morphologically. Thus samples can be taken for morphological examination from the CVS received by the laboratory undertaking the biochemical test. The sample is processed whole into resin and then divided to give two or three blocks of three or four 'branches' which are then embedded end-on with the thickest ends to be cut first.

Gaucher's disease
Fabry's disease
Krabbe leucodystrophy
Metachromatic leucodystrophy
Mucolipidosis IV (theoretically changes should occur)
Niemann-Pick disease type C
Tay-Sachs disease (and all G_{M2}-gangliosidoses)
Cystinosis

Table 4. Disorders with no morphological evidence of disease found in CVS.

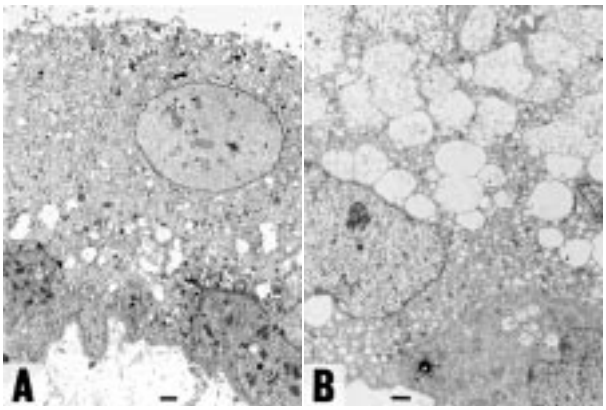

Figure 1. Electron micrographs of chorionic villus samples taken at 10 weeks from a twin pregnancy at risk for **infantile sialic acid storage disease**. Twin 1 (A) shows no vacuolation in the syncytiotrophoblast, while twin 2 (B) has marked vacuolar changes in the syncytiotrophoblast with a few vacuoles in the cytotrophoblast, indicating that twin 2 is affected. These findings agreed with the sialic acid levels on direct CV assay and were confirmed in amniotic fluid and in cultured CVS. Selective fetocide was effected and the pregnancy went to term with a normal outcome. Scale mark 1μm.

This way the greatest area containing older cells, rather than the more rapidly dividing tips of the villi with young cells, can be examined. The examination of the older cells has the distinct advantage that if storage is to be found, the older cells will have had the greatest chance to develop the vacuolar change or for the storage substance to acquire the ultrastructural characteristics of the storage disorder under test. Several villi must be examined (62), in addition to as many different cell types and structures as possible, especially if there is no evidence of storage, because the changes may not be widespread at the age of sampling.

Some of the storage disorders have quite dramatic changes in the trophoblast (Figure 1) with the striking

Disease	Pseudodeficiency
G_{M2}-gangliosidoses	
Tay Sachs	Gene encoding α-subunit of hexosamindase A
Sandhoff	Gene encoding β-subunit of hexosaminidase A & B
Krabbe leucodystrophy	galactocerebrosidase
Metachromatic leucodystrophy	arylsulphatase A
Fabry	α-galactosidase
Fucosidosis	α-fucosidase
MPS I - Hurler	α-iduronidase
MPSVII - Sly	β-glucuronidase
Pompe	α-1, 4-glucosidase

Table 5. Pseudodeficiencies of lysosomal hydrolases.

Disorder	References
Gaucher's disease	(23, 73)
Infantile sialic acid storage disease	(23)
Salla disease	(23)
β-glucuronidase deficiency	(33, 52, 80, 82)
G_{M1}-gangliosidosis	(23, 46)
Galactosialidosis	(72)
Sialidosis	(5, 23)
Morquio syndrome (MPS IVA)	(3, 6)
Wolman's disease	(77)
Niemann-Pick disease type A	(51)
Niemann-Pick disease type C	(50)
Farber's disease	(34)
Hurler (MPS I)	quoted in (34)

Table 6. Lysosomal storage disorders associated wuth fetal ascites (hydrops).

Abnormality	Occurs in
Alder granulation of neutrophils	β-glucuronidase deficiency (MPS VII)
	Multiple sulphatase deficiency
	Maroteaux-Lamy syndrome (MPS VI)
Vacuolated lymphocytes	
a) numerous large bold vacuoles	
	G_{M1}-gangliosidosis type I (but not type II)
	Sialic acid storage disease
	Salla disease
	Mannosidosis
	I-cell disease
	Juvenile Batten's disease (CLN3)
	Sialidosis
	Galactosialidosis
b) fewer smaller discrete vacuoles	
	Niemann-Pick disease type A
	Pompe's disease
	Wolman's disease

Table 7. White blood cell abnormalities in lysosomal storage disorders.

vacuolation readily visible at the light microscopy level (42) (Table 2). In some disorders the fibroblasts and endothelial cells have small vacuoles also visible by light microscopic examination of the semi-thin resin sections. Other disorders require ultrastructural examination to detect the characteristic changes (Table 3). The

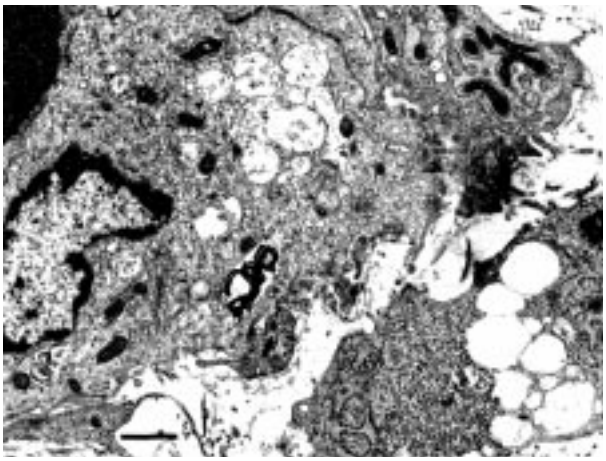

Figure 2. Electron micrograph of chorionic villus sample taken at 11 weeks from a pregnancy at risk for **Hurler's disease (MPS I)**. Membrane bound vacuoles are seen in endothelial cells and in fibroblasts, indicating an affected fetus. Biochemical assay showed deficient α-iduronidase activity. Scale mark 1μm.

macrophages, normally present in large numbers in the CVS at this age, appear very similar to the storage cells of many disorders and have membrane-bound vacuoles. Their presence can be very alarming but because they are normally present in chorionic villi these Hofbauer cells should be ignored in prenatal diagnosis, and all other cells types examined for evidence of storage. However in the term placenta the presence of many large and prominent Hofbauer cells should alert the observer to the probability of a storage disorder, and in particular the mucopolysaccharidoses should be considered.

Many of the storage disorders do not have a specific characteristic ultrastructural morphology but do have membrane-bound vacuoles from which the storage products have been removed during processing for electron microscopy. The vacuoles may be totally empty or contain a few remnants of amorphous granular material with a few wispy membranous fragments (Figure 2). This indicates that a storage disorder is present but does not define the type. The purpose of microscopic (light or electron) examination in most cases is not to make a definitive diagnosis but to confirm or exclude a lysosomal storage disorder. The conditions in which the non-specific appearance occurs include the mucopolysaccharidoses and the glycoproteinoses.

Specific changes include glycogen deposition within membrane-bound vacuoles (Figure 3) in Pompe's disease (29), but similar features may also be found in I-cell disease (7). In the placenta at around 20 weeks in I-

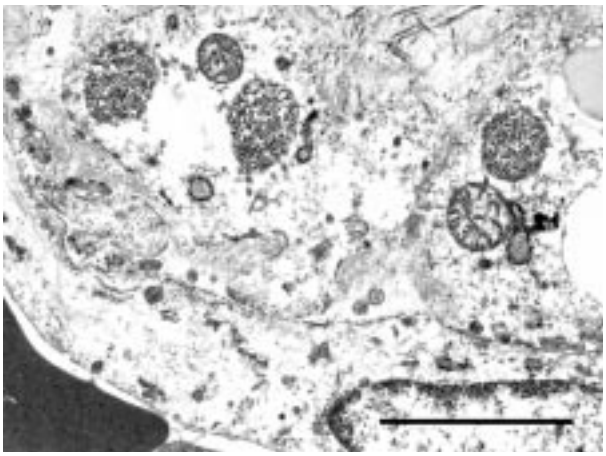

Figure 3. Pompe's disease (GSD II). Electron micrograph of placenta obtained after termination at 20 weeks following a positive prenatal diagnosis on cultured AF cells. Membrane-bound deposits of glycogen are present in a smooth muscle cell, and are also seen in endothelial cells and fibroblasts, confirming the diagnosis. Scale mark 1μm.

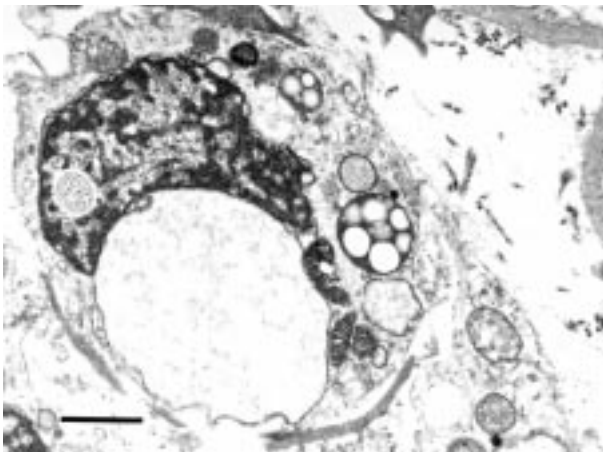

Figure 4. Electron micrograph of a chorionic villus sample at 12 weeks, from a pregnancy at risk for **Wolman's disease**. Membrane-bound lipid inclusions were present in endothelial cells. None were found in the trophoblast cells or fibroblasts. Biochemical assay showed deficient acid esterase activity. Scale mark 1μm.

cell disease there may be collagen fibre ingestion into lysosomes as evidence of defective remodelling in that disorder (Lake, unpublished observation), but this has not been recorded in CVS taken at 10-11 weeks. Clusters of characteristic membrane-bound lipid inclusions are found in the endothelial cells of CVS in Wolman's disease (Figure 4), but although the syncytiotrophoblast is active in lipid metabolism there are no lysosomal inclusions observed in this site. However there is one report that in the milder cholesteryl ester storage disease lysosomal lipid inclusions were found in the syncytiotrophoblast (16). In contrast, the syncytiotrophoblast in Niemann-Pick disease type A accumulates sphingomyelin which could be demonstrated by histochemical analysis of placenta from affected fetuses at 18.5-21.5 weeks (37, 68), but no ultrastructural observations were made in those cases. In our experience, lamellar bodies characteristic of Niemann-Pick disease type A are present in endothelial cells and fibroblasts in CVS at 11 weeks (Figure 5A) and membranous cytoplasmic bodies, similar to those in the gangliosidoses, can be found in the syncytiotrophoblast (Figure 5B). Lamellar bodies were reported in the endothelial cells of placenta at 19 and 23 weeks from fetuses affected with mucolipidosis IV (70), and would be expected to be found in CVS. The preferred route for the diagnosis of mucolipidosis IV is by phospholipid analysis of cultured cells (85) until the enzymology or mutation analysis becomes available. Electron microscopy of cultured amniotic fluid cells has been used to detect mucolipidosis IV (38), but there are problems of discriminating between the diagnostic inclusions and those possibly induced by added aminoglycoside antibiotics (14). Specific ultrastructural changes are present in the CVS in infantile Batten's disease in which granular osmiophilic deposits are found in the endothelial cells in the capillaries of the villus core (24, 63) (Figure 6). In the classic juvenile type of Batten's disease the fingerprint/curvilinear bodies which characterize the disease in the affected child, are not seen. However, inclusions which are very suspicious of early fingerprint/curvilin-

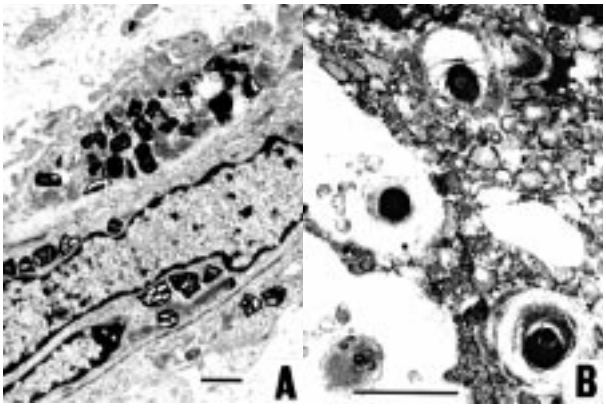

Figure 5. Electron micrograph of chorionic villus at 12 weeks, from a pregnancy at risk for **Niemann-Pick disease type A**. Membrane-bound lamellar bodies are present in endothelial cells (A). They are also seen in fibroblasts. Looser membranous cytoplasmic bodies with a dense core are found in the syncytiotrophoblast (B). Biochemical assay showed deficient sphingomyelinase activity. Scale mark 1μm.

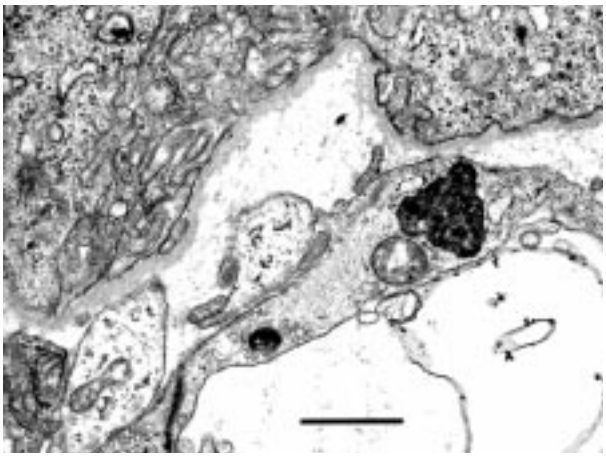

Figure 6. Electron micrograph of a chorionic villus sample at 12 weeks, from a pregnancy at risk for **infantile Batten's disease**. The presence of granular osmiophilic bodies in the endothelial cells indicates the fetus is affected, and this was confirmed by DNA analysis showing that the fetus had an identical genotype to the index case using haplotype analysis with the marker HY-TM1. Scale mark 1μm.

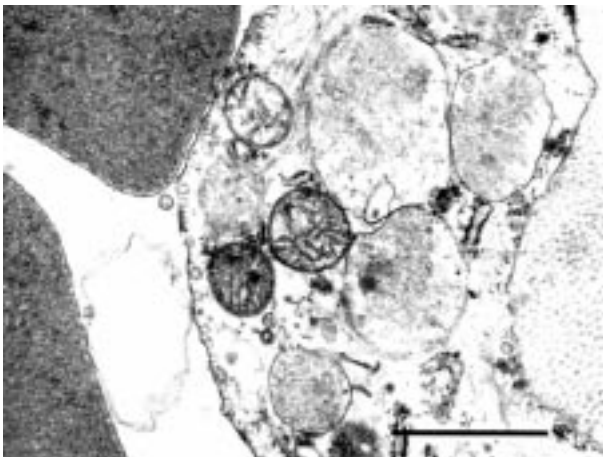

Figure 7. Electron micrograph of chorionic villus sample taken at 12 weeks from a pregnancy at risk for classic **juvenile Batten's disease**. No typical fingerprint or curvilinear/fingerprint bodies were present but unusual loosely membranous inclusions were found within endothelial cells, suggesting that the fetus might be affected. DNA linkage analysis with D16S298 showed that the fetus had an identical genotype to that of the index case and the parents opted for termination of pregnancy. The diagnosis was confirmed by examination of the abortus (see Figure 17). Scale mark 1μm.

ear bodies can be found in endothelial cells in the capillaries of the CVS (Figure 7) in affected pregnancies.

Some of the lysosomal storage disorders do not have a morphological change detectable in CVS (Table 4). The absence of morphological evidence suggests that the cells present in CVS are not metabolically active with regard to the storage substances.

In a few instances (infantile, late infantile and juvenile Batten's disease) electron microscopy either alone or with linkage or mutation analysis is the main diagnostic test, because the enzyme responsible for the disease has yet to be found or is not yet routinely available (74, 81). In mucolipidosis IV, electron microscopy may be used in conjunction with phospholipid analysis of cultured cells.

In rare instances when very low activities of control reference enzymes are obtained as well as the enzyme under test, microscopical examination of the tissue may show evidence of autolysis thus explaining the anomalous results and indicating that the CVS has not been treated correctly (Besley and Lake, unpublished findings).

Prenatal diagnosis of Niemann-Pick disease type C currently takes advantage of the lysosomal accumulation of endocytosed LDL-derived cholesterol, and the accompanying anomalies in intracellular sterol trafficking which are the hallmark phenotypic features of the disease (59). The tests require cultured CVS to demonstrate the intralysosomal accumulation of unesterified cholesterol by fluorescence microscopy after staining with filipin. Filipin is a fluorescent probe which reacts specifically with unesterified free cholesterol (39). LDL-induced cholesterol esterification should also be studied (15, 79). In the filipin-stained cells a characteristic pattern of intense perinuclear cytoplasmic fluorescence is seen in affected pregnancies, while little or no fluorescence is found in normal cells. The studies on cholesterol esterification in contrast show a severely impaired level in affected cells. To date more than 100 pregnancies at risk have been monitored (Vanier, personal communication). The above approach of combining the demonstration of cholesterol accumulation and the impairment of cholesterol esterification can be used not knowing which of the two complementation groups (75, 78) the family belongs to, but will not be reliable in the so-called variant families (about 15% of all Niemann-Pick C families) who have only mild cellular abnormalities (59, 79). It is therefore essential that the biochemical phenotype of the index case is established before genetic counselling and attempting prenatal diagnosis. The recent cloning of the NPC1 gene (11) which shows mutations in 95% of the families opens up new possibilities for the prenatal diagnosis of Niemann-Pick disease type C in the future.

Prenatal diagnosis based on amniotic fluid and amniotic fluid cells

Biochemical examination. Prenatal diagnosis based on biochemical analysis of cultured amniotic fluid cells has now been largely superseded by analysis either in chorionic villi directly or in cultured chorionic villus cells. However, in our experience, amniocentesis is still preferred to chorionic villus sampling (due to the slightly lower miscarriage rate) in pregnancies at low risk for a lysosomal disorder, e.g. where carrier detection is not possible and there is maternal anxiety due to diagnosis of the disorder in a close relative. It is also appropriate where maternal bleeding in the first trimester has made chorionic villus sampling inadvisable, or when a late diagnosis of the proband has been made. In the latter case a placental biopsy in the second trimester would also allow direct analysis of the placental material in the same way as for chorionic villus material taken in the first trimester.

The enzyme and chemical composition of amniotic fluid is a reflection of the urinary output of the fetus and varies with gestational age. An example of increased enzymic activity in amniotic fluid is I-cell disease. At 15-16 weeks gestation amniotic fluid supernatant from a fetus affected with I-cell disease generally shows such a marked increase of arylsulphatase A, β-hexosaminidase and β-glucuronidase activities when compared with gestationally aged-matched controls. A positive diagnosis can be made within hours of receipt of the sample. Besley et al. (9) diagnosed I-cell disease in a fetus as early as 10 weeks gestation in this way but in another pregnancy from a different family the amniotic fluid enzyme activities were not raised although the activities in cultured amniotic cells were very low, consistent with an affected fetus (Besley, personal communication). Amniotic fluid supernatant after 14.5 weeks gestation would be expected to show abnormally high enzyme activities in an affected I-cell pregnancy but a normal result in the supernatant fluid should always be confirmed on cultured amniotic cells. There is considerable residual enzyme activity in uncultured chorionic villi from an affected I-cell pregnancy so prenatal diagnosis can only be made reliably on cultured chorionic villi cells. Cells cultured from a CV biopsy taken at 11.5 weeks gestation may not be available for assay for 3-4 weeks which brings it to the same time that an amniocentesis can be reliably offered for this disorder. A diagnosis will not be available until the second trimester whichever method of sampling is preferred. For both of the X-linked lysosomal storage disorders, Hunter disease (45) and Fabry disease (Young, unpublished observation), the relevant enzymes can be assayed in supernatant amniotic fluid as can total hexosaminidase (deficient in Sandhoff disease) and β-glucuronidase (deficient in MPS VII). However, the finding of normal amniotic fluid activity should always be confirmed in cultured amniotic cells.

Increased storage products in amniotic fluid were shown by Mossman and Patrick (54) who found abnormal glycosaminoglycan (GAG) patterns in amniotic fluid from pregnancies when the fetus was affected with a mucopolysaccharidosis. Although all the different types of mucopolysaccharidosis can be diagnosed by enzyme analysis on CV directly GAG analysis in amniotic fluid is still important in pregnancies where the definitive enzyme analysis has not been made in the index case. We have received requests for prenatal testing from pregnant women whose deceased brothers were diagnosed on clinical grounds as Hurler / Hunter, 20-30 years ago, from anxious sisters of Sanfilippo A cases whose carrier status and that of their partners cannot be established and from centres in the Middle East, India and Turkey where chorionic villus sampling is not widely available or there is no material from the index case for biochemical diagnosis. Quantitative amniotic fluid GAG analysis is not a reliable indicator of an affected pregnancy but an affected fetus can be diagnosed if, after electrophoresis of the component GAGs, either dermatan-, heparan- or keratan sulphates are detected in the fluid in addition to the normal components, chondroitin sulphate and hyaluronic acid. This has proven to be a very reliable test with over 170 pregnancies monitored in this way in our laboratory. Enzymes can be assayed in the cultured amniotic cells to confirm or define the diagnosis. This can have far reaching importance if a deficiency of iduronate sulphatase is found which would therefore be consistent with a diagnosis of the X-linked mucopolysaccharidosis, Hunter disease. If no other material from an affected male in the family is available then DNA can be extracted from the cultured amniotic cells or from the abortus for mutational analysis. Once the mutation is found accurate detection of carrier females within the family is possible. In cases of sialic acid storage disease the increased amount of sialic acid in amniotic fluid is derived not only from the fetal urine but probably also from the trophoblast.

Morphological assessment. Amniotic fluid cells may be used uncultured in a few situations, and is the only sample that can be currently used for the prenatal diag-

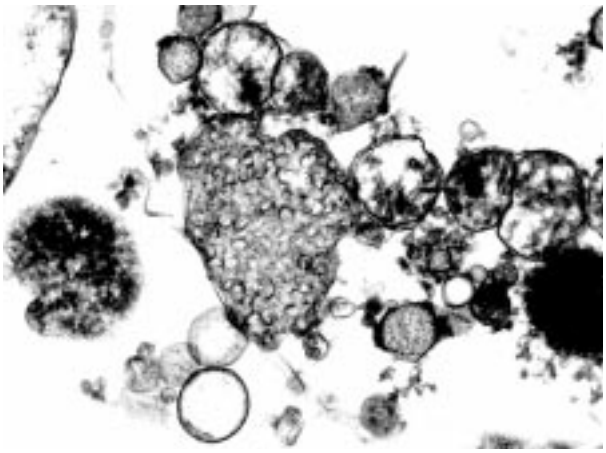

Figure 8. Electron micrograph of uncultured AF cells taken at 17 weeks from a pregnancy at risk for **late-infantile Batten's disease**. Curvilinear bodies are present in some of the cells indicating an affected fetus, which was confirmed by electron microscopic examination of the abortus. It is important that only typical curvilinear inclusions are interpreted as positive findings since a variety of strange membranous inclusions are found in uncultured AF cells. Scale mark 1μm.

nosis of late infantile Batten's disease (*CLN2*) (12, 47, 49). Amniotic fluid, about 10ml, is centrifuged to produce a loose pellet, the supernatant tipped off and the pellet of cells is resuspended in 10% bovine serum albumin (BSA) in PBS. The suspended cells are then recentrifuged and the pellet fixed in a glutaraldehyde fixative. The purpose of the BSA is to provide a proteinaceous medium around the cells which is crosslinked by the glutaraldehyde to give a coherent pellet which will not fragment during processing for electron microscopy.

Membrane-bound inclusions containing glycogen characteristic of glycogenosis II (acid maltase deficiency) were present at 36 weeks gestation in one of three pregnancies at risk for Pompe's disease (30). The diagnosis was confirmed in the newborn infant. Berndon and Hug (7) examined placentas from proven cases of glycogenosis II and commented that the membrane-bound inclusions of glycogen in endothelial cells and villus stromal cells observed in the placenta from these cases should also be present in first trimester CVS, a prediction which was later confirmed (29). Amniotic fluid cells normally contain large quantities of glycogen which could mask any lysosomal deposits so this route for diagnosis has not been widely used for prenatal diagnosis of acid maltase deficiency.

MacLeod and colleagues (47-49) reported that curvilinear bodies, the hallmark of classic late infantile Batten's disease, were present at 16 weeks in a case later (after birth) confirmed as having the disease. A few, mostly unreported, attempts at the prenatal diagnosis of late infantile Batten's disease have been made, but there was no confirmation that any positive diagnosis was correct until Chow and his colleagues (12) showed that a fetus predicted to have the disease (Figure 8) did indeed have curvilinear bodies in the brain, liver, skin and lymphocytes. The amniotic fluid cells with curvilinear inclusions are infrequent, and the problem of how many cells to examine arises if no inclusions are seen. Mutation detection, linkage analysis, and possibly enzyme assays (74) will in the future make this route for diagnosis of late infantile Batten's disease redundant.

Cultured cells, prepared as a pellet, can be used for the prenatal diagnosis of mucolipidosis IV. The appearance of the inclusions, similar to that seen in the cultured fibroblasts of affected individuals, is diagnostic (70), but beware of the effect of added aminoglycoside antibiotics which can mimic the inclusions (14). Analysis of the phospholipid composition of the cultured cells may potentially be used as an alternative means of prenatal diagnosis in mucolipidosis IV (85).

Mutation analysis

The cloning of most lysosomal enzyme proteins permits mutation analysis in affected individuals. Once the mutation(s) in the index case is established and proven to be the disease-causing mutation, in theory, there is no reason why prenatal diagnosis cannot be approached in this way (see Table 1). In practice, however, it is often more cost effective and a diagnosis obtained in a shorter time if prenatal diagnosis can be made by measurement of the relevant enzyme activity in uncultured chorionic villi rather than by mutational analysis. Nevertheless mutational analysis has proved a very useful adjunct to the biochemical tests for prenatal diagnosis of the lysosomal storage disorders, especially in those families with a pseudodeficiency (e.g. metachromatic leucodystrophy), and in families with high residual enzyme activity in the index case where morphological studies on CVS are not known to be informative (e.g. juvenile type II glycogen storage disease). It is also useful where the mutation in the gene causing the disorder is known, but the enzyme defect has not yet been found or is not assayed routinely (55, 67). Often this approach is in conjunction with a morphological assessment as in juvenile Batten's disease (*CLN3*) or infantile Batten's disease (*CLN1*) respectively (see Figures 6 and 7). In Niemann-Pick disease type C, which at present relies on the demonstration of cholesterol accumulation with filipin (79), mutational analysis (11) will be especially useful in those families where it is impossible to

distinguish between homozygotes and heterozygotes by filipin staining on cultured cells.

Mutational analysis may be helpful in the diagnosis of X-linked disorders and for predicting the likely phenotype of an infant born to parents whose carrier status has been detected as part of a screening programme (86).

Pseudodeficiencies

Pseudodeficiencies of many lysosomal hydrolases have been reported - Table 5 (76) so it is essential not only to confirm the enzyme deficiency in the index case in the family but also to assay the enzyme activity in both parents before prenatal diagnosis is offered. The lysosomal enzymes are often assayed using artificial substrates and these, while being very useful in increasing sensitivity of the assay and thus allowing easier recognition of the various disorders, will also detect a pseudodeficiency. With a pseudodeficiency there is no manifestation of the disease and the apparent enzyme deficiency will have been uncovered during investigations for suspected metabolic disease in the index case. Since there is a great diversity of clinical manifestations in any particular disorder the possibility that the low enzyme activity is not related to the clinical symptoms may not have been considered. Therefore it is essential to carry out further biochemical, histochemical or DNA analysis especially in patients with an atypical clinical presentation and their parents must also be investigated. If the results show that a parent has the same low levels of activity, the possibility of an incorrect diagnosis based on the enzyme assays alone should be considered and the pseudodeficiency status investigated. The parent may be a compound heterozygote for the disease-causing mutation and the pseudodeficiency mutation.

Most pseudodeficiencies are uncommon but in screening for metachromatic leucodystrophy (MLD), the pseudodeficiency (PD) will be more frequently detected than the disease itself. Between 7-15% of individuals have been shown to carry the mutation encoding a pseudodeficiency of arylsulphatase A, (28, 57), and it is essential that the presence of this mutation is recognized in a family with metachromatic leuodystrophy (43). It may not be possible on enzyme analysis alone to distinguish between a fetus who is homozygous for metachromatic leucodystrophy and would be clinically affected with the disease, and one who is a compound heterozygote for the *MLD* and *PD* alleles and would be clinically normal. An additional problem can arise if the index case is homozygous for both the *MLD* and *PD* alleles and one parent is also homozygous for the *PD* allele. In such families every effort should be made to determine the mutation(s) in the *MLD* gene, and prenatal diagnosis approached in this way otherwise sulphatide loading studies on cultured cells would be needed to distinguish between a fetus homozygous for *MLD*, and one homozygous for the *PD* and heterozygous for the *MLD* alleles (43). Other assays, including those with natural substrates and metabolite loading tests on cultured fibroblasts may be necessary in situations where the clinical presentation does not match that expected for the disorder. Failure to recognize the presence of a pseudodeficiency allele in a parent could lead to an incorrect diagnosis in the fetus.

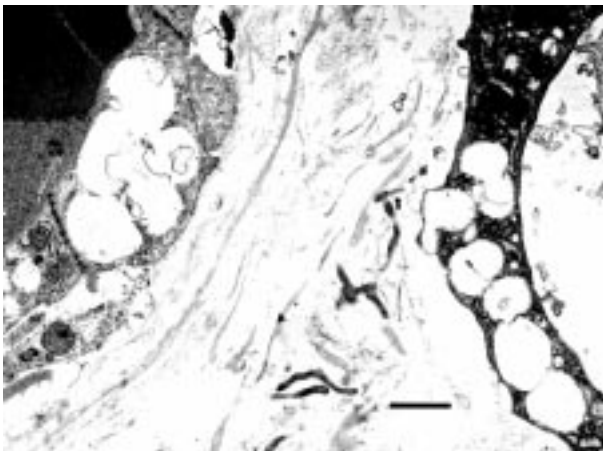

Figure 9. Placental sample from a fetus with severe hydrops at 21 weeks. There was a family history of 3 other pregnancies in which fetal hydrops of unascertained cause resulted in fetal death at around 22 weeks. This electron micrograph shows large membrane-bound vacuoles in endothelial cells indicating that a lysosomal storage disease is present. Fibroblasts also had vacuoles, but there was only rare and patchy vacuolation in the cytotrophoblast, and none in the syncytiotrophoblast. This suggested a diagnosis of β-**glucuronidase deficiency** which was confirmed on biochemical assay and on mutation analysis. Scale mark 1μm.

Fetal hydrops as a presenting symptom of a lysosomal storage disorder

A significant number of the lysosomal storage disorders can present with fetal hydrops or fetal ascites (see Table 6) first detected at the time of the routine scan during the second trimester at around 20 weeks (Figure 9). Amniotic fluid and a fetal blood sample (32) can be taken at this time for investigation of the hydrops for which there are many causes (20, 65), but it should be noted that lysosomal storage disorders are likely to contribute less than 1-2% of the total. Nevertheless fetal white blood cells can be used for assay for some of the conditions listed in Table 7, and this can be done in con-

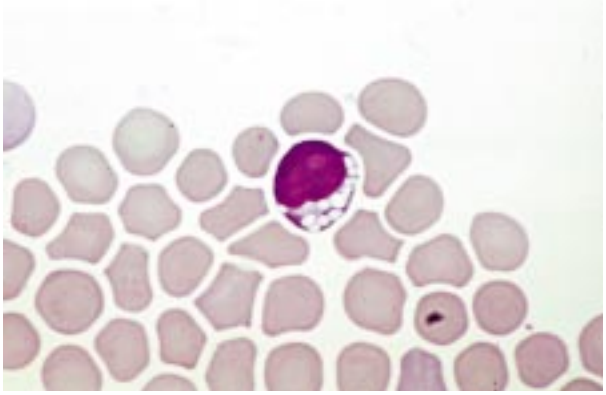

Figure 10. Fetal blood sample taken at 20 weeks gestation for investigation of fetal hydrops showing a vacuolated lymphocyte. Many lymphocytes with bold vacuoles were present, indicating a storage disorder which was confirmed **as infantile sialic acid storage disease** by assay of the amniotic fluid.

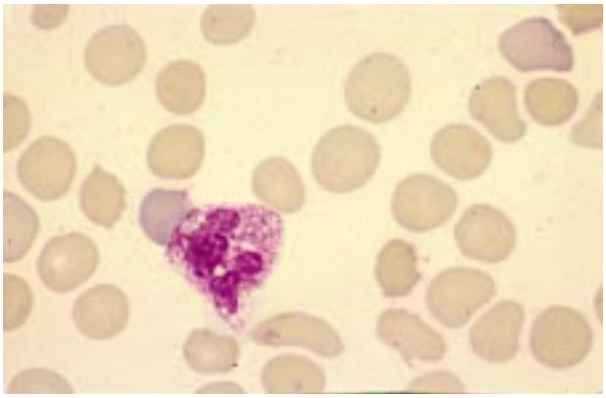

Figure 11. Fetal blood sample from a fetus of 20 weeks affected with **mucosulphatidosis** showing Alder granulation in a neutrophil indicating an affected fetus.. The sample was taken because there was a marked depletion of sulphatase activity in WBC in the index case while considerable residual sulphatase activity was found in cultured fibroblasts.

junction with the examination of white cell morphology. The presence of vacuolated lymphocytes (19) or Alder granulation of neutrophils (Figures 10 and 11) is well established at this age in the fetus affected by the disorders in which these abnormalities occur (see Table 7, and (27, 41)). The light microscopical assessment of white blood cell morphology will assist in the choice of the appropriate biochemical assay on what are necessarily limited amounts of cells or plasma. Amniotic fluid analysis may be appropriate in a few instances e.g. for the detection of oligosaccharides, sialic acid or glycosaminoglycans (60). Fetal hydrops is also recorded in the carbohydrate-deficient glycoprotein syndrome (17) and in a cardiac glycogen storage disease with normal acid maltase activity (4).

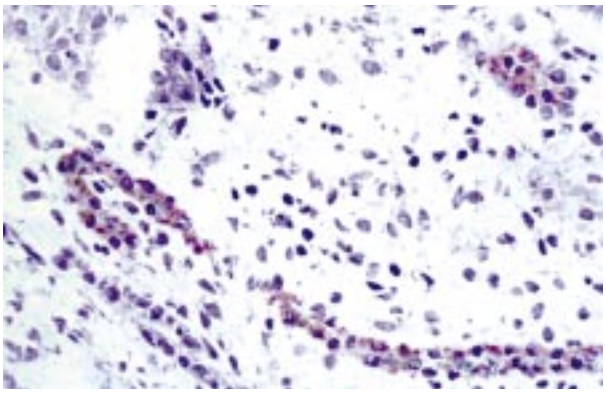

Figure 12. Cryostat section of a snap-frozen sample of kidney from a fetus of 24 weeks gestation, affected with **mucosulphatidosis**. The prenatal diagnosis was made on a fetal blood sample where deficiencies of several sulphatases were found. The section, stained with toluidine blue, shows accumulation of sulphatide (stained brown/yellow) in the renal tubules which confirms the diagnosis of mucosulphatidosis.

Fetal blood analysis

As mentioned in the previous section, fetal blood can be very useful in the diagnosis of a lysosomal storage disorder which may only be suspected late in pregnancy. The relevant enzymes can be assayed immediately in the white blood cells or in plasma. We have also used fetal blood to diagnose a fetus affected with mucosulphatidosis (Young and Lake, unpublished). The index case in this family had been extensively investigated and had shown a marked deficiency of several sulphatase enzymes in peripheral blood, an abnormal urinary glycosaminoglycan pattern and Alder granulation was found in neutrophils. However, there were considerable residual activities of the sulphatase enzymes in cultured fibroblasts which suggested that prenatal diagnosis using cultured cells might not give a conclusive result. Two pregnancies monitored by fetal blood sampling showed normal sulphatase activities and no Alder granulation. Both resulted in the birth of normal infants. In the third pregnancy, fetal blood taken at 20 weeks gestation showed Alder granulation of neutrophils (Figure 11) and a deficiency of plasma iduronate sulphatase, leucocyte arylsulphatase A and heparin sulphamidase whilst other non-sulphatase enzyme activities were normal. The iduronate sulphatase activity in the amniotic fluid was normal but the amniotic fluid glycosaminoglycan electrophoresis pattern was abnormal. In cultured amniotic fluid cells the arylsulphatase A activity was 10% of the lowest control, and the heparin sulphamidase and iduronate sulphatase activities were both 20% of the lowest normal control. Similar results were obtained in fibroblasts cultured from the aborted fetus,

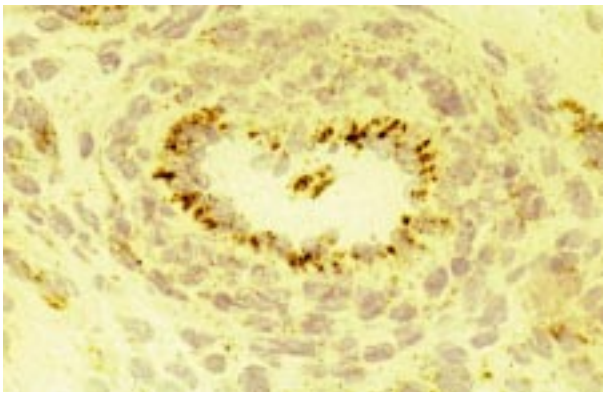

Figure 13. Cryostat section of a sample of snap-frozen limb from a fetus affected with **infantile Batten's disease** diagnosed by EM of CVS and DNA studies. The tissue has been stained to demonstrate acid phosphatase activity (brown) in endothelial cells to indicate that these cells have lysosomal storage. Normal endothelial cells have no demonstrable acid phosphatase activity.

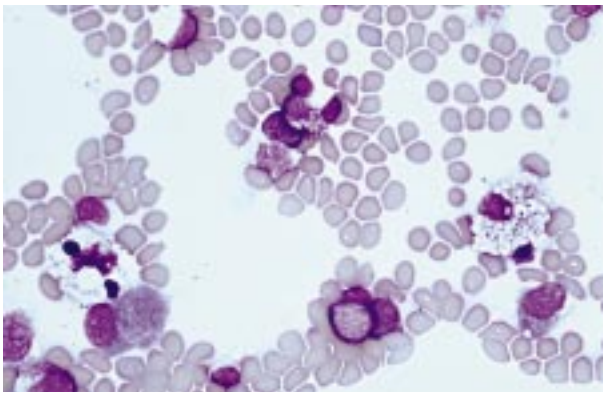

Figure 14. Bone marrow film prepared from the femur of a fetus of 18 weeks gestation, affected with **G_{M1}-gangliosidosis** diagnosed by enzyme assay of cultured AF cells. Storage cells (large foamy cell on right and Buhot cell on left) are present and readily visible in a May-Grünwald-Giemsa stain.

in contrast to the high residual activity in the index case. Sulphatide accumulation was demonstrated in the fetal kidney tubules (Figure 12) and in peripheral nerve from the brachial plexus

Why both enzyme assay and morphology?

Enzyme assay will produce a definitive result in most disorders. However there can occasionally be high residual activity in an affected index case and the degree of overlap of affected and carrier states will cause some concern. It is in these cases in particular that morphological evidence and DNA analysis can be useful to help decide whether the fetus is affected or only a carrier. The big advantage of having a range of tests for prenatal diagnosis of lysosomal storage disease is that these are independent means of making a diagnosis. In our experience spanning nearly 20 years there has not been any disagreement. The time scale usually considered for electron microscopy is in the range of days to a week, but the small size of the CVS means that if the occasion demands a morphological result can be achieved within 24 hours, and DNA analysis within a few days.

Confirmation of the diagnosis

In all instances where the tests indicate an affected fetus, it is important to confirm the diagnosis. This is for two main reasons. Firstly to reassure the parents who will be anxious that the right decision has been made, and secondly to reassure the geneticists and laboratory workers that the tests were correct and reliable. It also acts as audit to ensure that the laboratory is competent to carry out prenatal diagnosis.

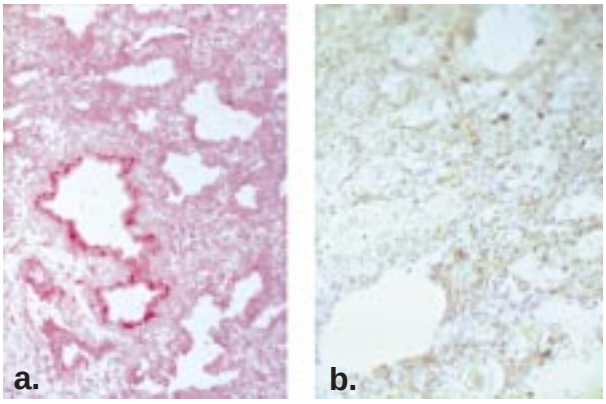

Figure 15. Cryostat sections of samples of snap-frozen fetal lung stained for β-glucuronidase activity (red). **a.** from a fetus with GM1-gangliosidosis with normal β-glucuronidase activity; **b.** from a fetus presenting with fetal hydrops with no detectable β-glucuronidase activity. The **β-glucuronidase deficiency** was confirmed on biochemical assay of fetal lung and by finding heterozygote levels of activity in the parents' blood.

It is often difficult to identify individual tissues in the products of conception from terminations at under 14 weeks, but samples of placenta and occasionally limbs may be found. From these a culture can be established for enzyme assay and/or DNA analysis, and other samples can be used for microscopy (Figures 13 and 14). When a termination is effected slightly later in pregnancy due to a later diagnosis, the abortus may provide more suitable tissue samples (Figure 15). However, even under these circumstances many of the organs may not be identifiable. For culture, samples of skin or lung tissue or placenta can be used. For the morphological confirmation of a neuronal storage disorder the best site is

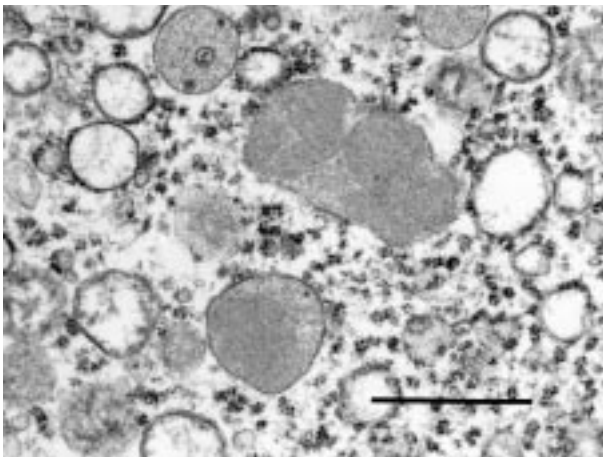

Figure 16. Infantile Batten's disease. Electron micrograph showing granular osmiophilic bodies present in the cytoplasm of a neuron from the spinal cord obtained after termination of pregnancy at 14 weeks following a positive prenatal diagnosis. Scale mark 1μm.

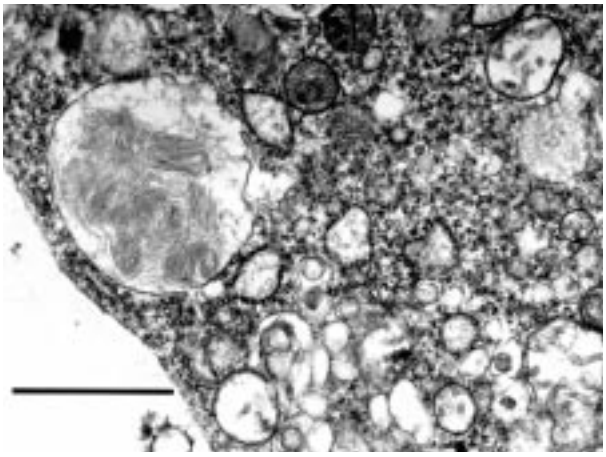

Figure 17. Confirmation of prenatal diagnosis in **juvenile Batten's disease**. Electron micrograph of part of a neuron in the spinal cord showing a membrane bound collection of lamellar inclusions similar to those found in the canine and murine models of Batten's disease. The inclusions are not of the classical fingerprint or curvilinear/fingerprint type usually considered the hallmark of juvenile Batten's disease. Similar findings were found in the liver. Scale mark 1μm.

the upper spinal cord or brain stem. It is in these sites that the neurons of the brain stem nuclei or anterior horns will exhibit the characteristic storage bodies which can be seen only by electron microscopy (Figures 16 and 17). No conclusive evidence of neuronal storage can be found by routine light microscopy until more than 20 weeks. Visceral storage disorders in which the spleen and liver are mainly affected can be confirmed by examination of the fetal bone marrow as films rather than sections. From the femur of an 11 week fetus it is possible to make at least two marrow films, and these stained with the traditional Giemsa type stain and with a method for the demonstration of acid phosphatase activity allow the identification of storage cells (see Figure 14) and thereby confirm the diagnosis within a short time. It may be difficult to identify the changes in liver, spleen, kidney, heart and nerve by routine microscopy, but cryostat sections of the snap frozen tissue stained for acid phosphatase activity will visualize any storage or storing cells (see Figure 13). The placenta often provides the best and most positive microscopical confirmation that the diagnosis of a lysosomal storage disease was correct. Enzyme assays from the cell lines derived from the products of conception will give additional confirmation.

The Future

The rapid advances in DNA technology could well open up other approaches to the prenatal diagnosis of lysosomal storage disorders, e.g. preimplantation diagnosis. This strategy has already been successfully applied to some non-lysosomal disorders where, after fetal sexing only female embryos are implanted (26). Amplification of the DNA from a single blastomere is now possible and Sermon and colleagues (71) have successfully amplified simultaneously the two most frequent mutations occurring in Tay-Sachs disease. An extension of this technique to unfertilized oocytes and the first polar bodies isolated from them, offers the possibility of detection of mutational defects even before fertilization (53).

Detection and isolation of fetal cells from the maternal circulation is being actively pursued by many groups. If this becomes a reliable technique it would provide a non-invasive method for the prenatal diagnosis, but of course will not overcome the stress of termination of an affected pregnancy.

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