The study of metabolic disorders in children has provided significant insight into normal physiological pathways. Following these studies, methods of diagnosis have altered over the years, with the result that diagnostic histopathological examinations are less frequently performed. Education of the biochemist by the histopathologist, and vice versa, has led to the development of diagnostic techniques dependent on the study of urinary excretion products, serum metabolites, enzyme assay of white blood cells, or microscopy of blood or bone marrow. In some disorders biopsies are still necessary, but these now make up a minor proportion of cases.

This chapter is devoted to conditions in which microscopy (light and/or electron) can provide a specific diagnosis or permit assignment of a metabolic defect to a particular group. Conditions that show non-specific changes on microscopy and require biochemical analysis (e.g. maple syrup urine disease, phenylketonuria etc.) are beyond the scope of this chapter and are well covered in the standard texts (Stanbury et al. 1983).

Since the first edition two main areas of metabolic interest have become important. Disorders of oxidation of fats have been recognized; in particular a defect in medium chain fatty acid CoA dehydrogenase has been reported, and multiple defects of several fatty acid CoA dehydrogenases have been found in glutaric aciduria II. Defects in fatty acid oxidation give rise to the symptoms of Reye's syndrome and account for many of the patients with the so-called recurrent Reye's syndrome. The other disorders which have been recognized are related to peroxisomes and peroxisomal enzymes. An absence of peroxisomes was reported in liver and kidney in Zellweger's cerebrohepato-renal syndrome (Goldfischer et al. 1973), and a similar lack of peroxisomes is now recognized in infantile Refsum's disease. Although peroxisomes are present in reduced numbers in neonatal adrenoleucodystrophy but normal in X-linked adrenoleucodystrophy, defective peroxisomal enzyme activity is recognized in both forms of adrenoleucodystrophy, rhizomelic chondrodysplasia punctata, pseudo-Zellweger syndrome, primarily hyperoxaluria type I and probably also in cerebrotendinous xanthomatosis (Goldfischer et al. 1986; Schutgens et al. 1986). The peroxisomal disorders are now at the same stage as the lysosomal disorders were in the 1960s, and the recognition of further defects and syndromes can be expected.

The simplest possible approach to the problem will often lead to a diagnosis in the shortest time with least discomfort to the patient and minimal involvement of the laboratory. As in all branches of medicine, there is no substitute for a well-informed clinical appraisal, and it is most important to establish a dialogue between the paediatrician and pathologist to ensure adequate investigation. Any surgeon to be involved should also be aware of the reasons for the biopsy and, more importantly, of what should and should not be done to it.

It must be remembered that metabolic disorders affect only a very small proportion of children; thus each disorder is rare and few paediatricians will be familiar with the whole range. It may be better to refer the patient to a specialist centre rather than to attempt a diagnosis with limited experience and expertise. However, some tests which can confirm or exclude the presence of metabolic disease can be performed in most laboratories.

Occasionally a biopsy will arrive in the laboratory without prior warning or consultation, even in the best-regulated centres. Under these circumstances, provided the specimen has arrived fresh and without undue delay, part should be fixed in formalin for routine histopathology, part should be frozen by the most rapid means available for
histochemistry and biochemistry, and part should be fixed for electron microscopy. The two best methods for freezing tissue are (1) freezing in isopentane cooled to \(-160^\circ C\) in liquid nitrogen; and (2) freezing in hexane cooled to \(-79^\circ C\) in an acetone/solid carbon dioxide bath. An adequate, but less desirable method is freezing directly in liquid nitrogen, although if the tissue is well dusted with starch powder (Biosorb glove powder), perfect freezing can be obtained even in inexperienced hands. If these methods are not practicable, freezing on solid carbon dioxide, or in the last resort by placing in a deep freeze, will preserve the tissue for biochemical analysis but may leave it in a state unsuitable for sectioning. Whichever freezing technique is used, the frozen tissue should be carefully wrapped in aluminium cooking foil or parafilm to prevent drying, and stored in a precooled small bottle at \(-20^\circ C\) or below. It is a useful principle to “fix some, freeze some, and take some for electron microscopy” from most biopsies, whether or not they are from patients suspected of having a metabolic disorder.

The sections below set out the principal tests that can be performed in the majority of hospital laboratories. The number of special stains required is not large. Considerable information can be derived from:

1. A periodic acid-Schiff (PAS) reaction to detect glycogen and compounds containing 1:2 glycol groups (e.g. oligosaccharides, gangliosides).
2. Sudan black for lipids. Some indication of the presence of neutral fat or complex lipids can be inferred from the colour of the stained section.
3. A reaction to show acid phosphatase activity. A change in the intensity of the reaction, or of its distribution within the cell, indicates altered cellular function.

Other methods will be necessary from time to time, but these three and a haematoxylin and eosin preparation will allow the general nature of the disorder to be appreciated. Figure 14.1 shows a rectal suction biopsy stained to reveal acid phosphatase activity.

The enzyme defects and substances stored in various types of lysosomal storage disease are shown in Table 14.1.

**Blood Film Examination**

**General**

The examination of blood films by light microscopy can help either in making a specific diagnosis or in suggesting a particular direction for further investigation. In many instances a confident diagnosis can be made and confirmed by a specific enzyme assay. In this way valuable time and expensive reagents—some of which have to be prepared and radiolabelled in the laboratory—can be saved. In contrast