The nucleus is bounded by two nuclear membranes that form a nuclear envelope. At certain positions, the inner and outer membrane of nucleus fuse to form *pores*. Nuclear pores provide continuity between the cytoplasm and the inner part of the nucleus. The *perinuclear space* (the space between the two membranes of the nucleus) may have access to the outer cell. The nucleus contains chromosomal DNA and also some dark granular structures called *nucleoli*, which can be seen under an electron microscope. Nucleoli are not bound by a membrane and appear to be formed by ribosomal material.

Animal cells have a *cytoskeleton* or system of protein filaments that provide cell mechanical strength, control cell shape, and guide cell movement. These elements are often critical components in controlling cell response to mechanical forces such as from fluid flow or from attachment to surfaces. The three types of filaments are *actin filaments*, *intermediate filaments*, and *microtubules*. Actin filaments are relatively thin, while microtubules have the largest diameter. Microtubules are critical in cell division and cell movement. Cell division and movement require the polymerization and depolymerization of microtubules; the anticancer agent, paclitaxel (Taxol®), works by preventing depolymerization of microtubules and thus "freezing" the cell in a nondividing step. The *centrosome* is the primary microtubule-organizing center in the cell and is a small organelle involved in the formation of spindle poles during mitosis. Some animal cells may contain *cilia*, which are used to transport substrate across the cell surface, but not for self-locomotion. Each cilium is covered by a plasma membrane and contains a specific array of microtubules.

The metabolism of nutrients by animal cells in culture is very different from that in other types of cells. A typical growth medium of an animal cell culture contains glucose, glutamine, nonessential and essential amino acids, serum (horse or calf), and mineral salts (for example, Dulbecco's modified Eagle's media, DME). The major metabolic pathways for animal cells in culture are depicted in Fig. 12.2. Glucose is converted to pyruvate by glycolysis and also is utilized for biomass synthesis through the pentose phosphate pathway. Pyruvate is converted partly to CO_2 and H_2O by the TCA cycle, partly to lactic acid, and partly to fatty acids. Glucose is used as a carbon and energy source, as is glutamine. Part of the glutamine is deaminated to yield ammonium and glutamate, which is converted to other amino acids for biosynthesis purposes. Glutamine also enters into the TCA cycle to yield carbon skeletons for other amino acids and to yield ATP, CO_2, and H_2O. Animal cells are also capable of synthesizing glucose from pyruvate by the gluconeogenesis pathway. The release of lactate and ammonia as waste products of metabolism is a major problem in high-cell-density culture systems. Both lactate and ammonia at high levels are toxic to cells, primarily by altering intracellular and lysosomal pH.

12.2. METHODS USED FOR THE CULTIVATION OF ANIMAL CELLS

The techniques used for cultivation of animal cells differ significantly from those used with bacteria, yeasts, and fungi. Tissues excised from specific organs of animals, such as lung and kidney, under aseptic conditions are transferred into a growth medium containing serum and small amounts of antibiotics in small T-flasks. These cells form a *primary* culture. Unlike plant cells, *primary* mammalian cells do not normally form aggregates, but grow in the form of monolayers on support surfaces such as glass surfaces of flasks.