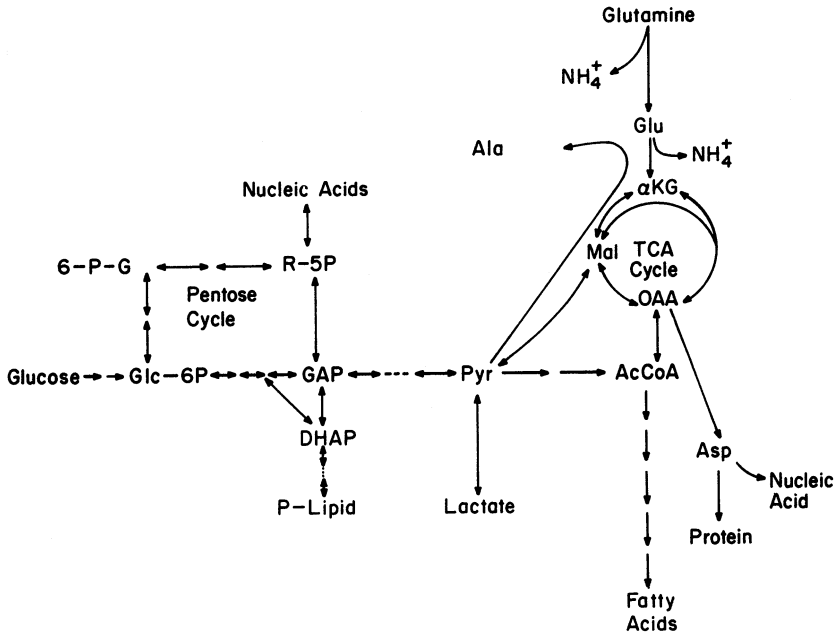

Figure 12.2. Major metabolic pathways in an animal cell. For cultured cells, glucose and glutamine are the major sources of carbon and energy. Lactate and ammonia are the primary waste products, although the release of alanine can be important. Symbols: 6-P-G is 6-P-gluconate; Glc-6P is glucose-6-phosphate; R-5P is ribose-5-phosphate; GAP is glyceraldehyde; DHAP is dihydroxyacetone; PYR is pyruvate; AcCoA is acetyl-CoA; OAA is oxaloacetate; Mal is malate; αKG is α-ketoglutarate.

Using the proteolytic enzyme trypsin, individual cells in a tissue can be separated to form single-cell cultures.

To start cultures of animal cells, excised tissues are cut into small pieces (~2 mm^3) and are placed in an agitated flask containing a dilute solution of trypsin (~0.25% w/v) in buffered saline for 120 min at 37°C. The cell suspension is passed through a presterilized filter to clear the solution, and cells are washed in the centrifuge. The cells are then resuspended in growth medium and placed in T-flasks or roller bottles. Cells usually attach onto the glass surface of the bottle and grow to form a monolayer. The cells growing on support surfaces are known as *anchorage-dependent* cells. Surface attachment is necessary for these cells to assume the three-dimensional shape necessary for the alignment of internal structures in a manner allowing growth. However, some cells grow in suspension culture and are known to be *nonanchorage-dependent* cells.

The cells directly derived from excised tissues are known as the primary culture. A cell line obtained from the primary culture is known as the *secondary* culture. Cells are removed from the surfaces of flasks using a solution of EDTA, trypsin, collagenase, or pronase. The exposure time for cell removal is 5 to 30 min at 37°C. After cells are removed from surfaces, serum is added to the culture bottle. The serum-containing suspension is centrifuged, washed with buffered isotonic saline solution, and used to inoculate