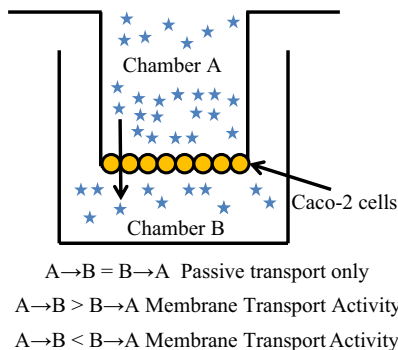


FIGURE 6.46 Measuring transporter protein activity can be accomplished by measuring the relative rate of migration of compounds across a monolayer of cells (Caco-2 or MDCK). Transporter activity is indicated if there is a difference between “A to B” and “B to A” transfer of compound.



(Figure 6.46). In this case, the artificial membrane is replaced by a monolayer of cells that are known to possess transporters. The most commonly used cells for this purpose are CACO-2 cells, a cell line developed from human epithelial colorectal adenocarcinoma cells,⁷⁰ and the Madin–Darby canine kidney epithelial cell line, also referred to as MDCK cells.⁷¹ Both of these cell lines contain membrane transport proteins and can be employed to assess a compound’s susceptibility to active transport. These assays use the same two chamber arrangement designed to measure passive permeability, but in this case, two experiments are required. The migration of a compound moving across a monolayer of cells is measured from both sides of the monolayer in two independent experiments. If the rate of migration across the monolayer is the same irrespective of the starting point of the test compound, then compound permeability is not significantly impacted by transporter activity. If, however, the rates of migration are different, then active transport is occurring, and the difference in migration rates provides insight into the impact of membrane transporter activity on compound permeability.

In vivo metabolism can also be predicted with *in vitro* models.⁷² Liver microsomes, vesicles formed from pieces of the endoplasmic reticulum (ER) of liver cells via differential centrifugation, from a variety of species are commercially available and contain the majority of enzymes responsible for xenobiotic metabolism. Incubation of a known concentration of a test compound with liver microsomes can be used as an initial assessment of a compound’s susceptibility to phase 1 metabolism. Compound concentration measurements over a defined time course provides a liver microsomal half-life. In addition, metabolic by-products can be identified from the incubation, and this information can be used to design compounds that are less likely to be metabolized. Phase 2 metabolism can be predicted in similar assays using additional cellular components generated during the differential centrifugation or fully intact hepatocytes which contain all of the metabolic machinery of the liver. Metabolic half-lives determined