

lines require different serum-free media composition. Not all cell lines have been adapted to serum-free media.

A number of defined media have been developed. Eagle's minimal essential medium (MEM), Dulbecco's enriched (modified) Eagle's medium (DMEM), and rather complex media, such as Ham's F12, CMRL 1066, and RPMI 1640, are commonly used. Media such as Eagle's MEM are often supplemented with 5% to 10% (by volume) of serum when used. For serum-free media formulations, often a 1:1 (v/v) mixture of DMEM (nutrient rich) and F12 (rich in trace elements and vitamins) is used. More specialized media (e.g., MCDB 170MDS) may be used for serum-free growth of specific cell lines. A simpler serum-free medium may contain insulin, transferrin, and selenium as serum replacement components, in addition to glucose, glutamine, other amino acids, and salts. Filtered whole lymph from a cow has been used as a growth medium for some mammalian cells.

Mammalian cells grow at  $37^{\circ}\text{C}$  and  $\text{pH} \approx 7.3$ . Typical doubling times are 12 to 20 h. Usually, 5%  $\text{CO}_2$ -enriched air is used to buffer the medium pH around  $\text{pH} = 7.3$ . A carbonate buffer ( $\text{HCO}_3^{2-}/\text{H}_2\text{CO}_3^-$ ) is used to control pH around 7.3. Since bicarbonate is used up by the cells,  $\text{CO}_2$ -enriched air is provided to balance carbonate equilibrium to keep  $\text{pH} \approx 7.3$ . The culture medium needs to be gently aerated and agitated. Other buffers such as HEPES (*N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid]) are also used to keep  $\text{pH} \approx 7.3 \pm 0.1$ . Insect cells grow best at about  $28^{\circ}\text{C}$  and a pH of about 6.2. Fish cell lines tolerate a wide range of temperature, although temperatures below  $37^{\circ}\text{C}$  are usually preferred ( $25^{\circ}$  to  $35^{\circ}\text{C}$ ). Values for pH typical of mammalian cell cultures are usually satisfactory for fish cells; pH values between 7.0 and 7.5 usually give good growth.

The kinetics of the growth of mammalian cells are similar to microbial growth. Usually, the stationary phase is relatively short, and the concentration of viable cells drops sharply thereafter, as a result of the accumulation of toxic metabolic products such as lactate and ammonium. A high level of ammonium is usually the result of glutamine metabolism, and lactate is usually a product of glucose metabolism. Cell concentration reaches a peak value within three to five days. However, product formation, such as monoclonal antibody formation by hybridoma cells, can continue under nongrowth conditions. Most of the products of mammalian cell cultures are mixed-growth associated, and product formation takes place both during the growth phase and after growth ceases. Figure 12.4 depicts a typical variation of growth, product (MAb's) formation, and glucose utilization by hybridoma cells.

The kinetics of product formation (e.g., MAb formation by hybridoma cells) can be described by a Luedeking–Piret equation:

$$\frac{1}{X} \frac{dp}{dt} = q_p = \alpha \mu + \beta \quad (6.18)$$

where  $\mu$  is the specific growth rate. The first term in eq. 6.18 is for growth-associated production and the second term is nongrowth-associated. The doubling time of mammalian cells varies between 10 and 50 h, and a typical value is 20 h. As expected, the growth rate varies depending on cell type, medium composition (including growth factors), and other environmental conditions (dissolved oxygen, carbon dioxide levels, pH, ionic strength).