

CHAPTER 2

Methods of Histology

Technological progress in various scientific disciplines affords histologists many ways to study cells and tissues. The information desired usually dictates the use of one approach over another or the use of combined approaches. The maximal information attainable with any technique requires familiarity with the technique, an understanding of information output, and an appreciation of its advantages and disadvantages. The following will acquaint you with diverse techniques available to examine living cells, dead cells, and cell components. Histologists use these methods to solve histological problems.

Cell culture permits direct observation of living cells. It is routine because of the use of antibiotics. Cell culture also allows the continual observation, manipulation, and testing of explanted cells without donor jeopardy (Figs. 2-1 and 2-2). It facilitates studying cell differentiation, cell transformations, cytogenetics, cellular metabolism, cell-to-cell interactions, host-parasite relationships, and other biological processes. Cell culture is indispensable in diagnostic virology (Figs. 2-3 and 2-4), vaccine development, and vaccine production.



Figure 2-1. Low-power micrograph of a monolayer of normal bovine fetal spleen cells. Contact inhibition results in the formation of a monolayer of stellate- and spindle-shaped cells (arrows). ($\times 40$, Giemsa stain.) (Courtesy H. Storz.)

Implantation of transparent viewing chambers and the exteriorization and transillumination of organs and tissues complement direct observation. Although exteriorization techniques limit observation time, insights about microcirculation and the response of tissues and organs to chemotherapeutic agents have been obtained. Transparent viewing chambers extend the observation period. Neovascularization, cellular differentiation and movement, and other vital processes are studied by these techniques. The anterior chamber of the eye is a naturally occurring viewing chamber used in this approach.

The selective uptake of vital and supravital stains has added to our understanding of cell function, cell structure, and extracellular matrix materials. These stains have low toxicity; they may be injected into a living organism (vital) or used on living cells removed from the organism (supravital).

Oxytetracycline, a broad-spectrum antibiotic, is also a vital stain. At therapeutic dosages, this drug selectively stains all surfaces where bone, cementum, and dentin form (Figs. 2-5 and 2-6). Insights

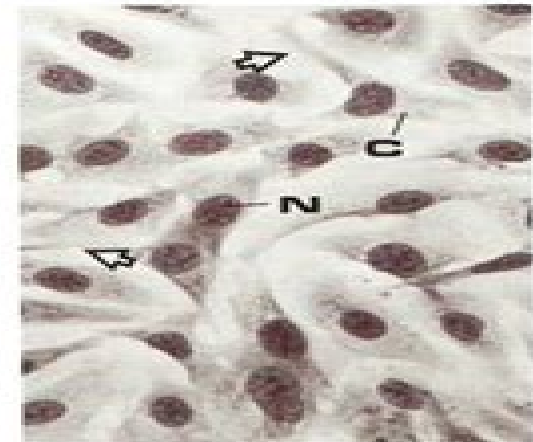


Figure 2-2. Medium-power micrograph of a monolayer of normal bovine fetal spleen cells. The nuclei (N) are vesicular and the foamy and granular cytoplasm (C) has processes (open arrows) imparting a stellate or spindle appearance. ($\times 100$, Giemsa stain.) (Courtesy H. Storz.)

concerning rates of bone formation, turnover, and maintenance have been obtained through carefully timed, spaced, serial administration of the drug.

Lithium carmine and trypan blue are vital dyes for studying phagocytosis. Janus green B and neutral red are supravital stains used to study mitochondria and lysosomes.

The separation and purification of cell fractions through differential centrifugation and density gradient centrifugation are aids for studying biochemical and metabolic phenomena.

The simplest method for the direct observation of living cells is to remove them from the organism, place them on a slide, and examine them with a phase-contrast microscope (Fig. 3-4) or dark-field microscope.

Examining living cells and tissues is difficult microscopically, because they are

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