This chapter discusses aspects of methods that impregnate single neurons in a more or less unpredictable fashion. The basic methods are discussed with respect to strategies of fixation, chromation, and metal impregnation. This is followed by discussions of a possible mechanism of impregnation based on direct observations of neurons as they are impregnated and on model substrates. It is argued that silver (or mercury) chromate precipitates are not nucleated at a single point in the cell but usually begin to form throughout the cell. Some suggestions are also made about why cells rarely stain adjacently. Factors such as diffusion rates of silver ions into the tissue, permeability barriers, and reducing properties of chromated tissue are considered, and a model of stochastic impregnation is proposed (Appendix I).

The chapter also outlines various modifications of the original method and describes various combinations of chromation and impregnation that can be used on the insect central nervous system. Appendix II lists the compositions of useful fixatives and chromating solutions and describes three methods that are reliable for a range of insects.
Historical Background

The most spectacular procedures for revealing the shapes of single nerve cells are derived from a set of fortuitous experiments performed by Camillo Golgi in the 1870s. These techniques, which are now collectively known as the "Golgi methods," constitute one of the most powerful means for structural analysis of the brain.¹ They are also the least understood in terms of their chemistry.

Probably the original procedure was discovered by chance during investigations of the different affinities of fixed tissue to silver nitrate. In about 1872 or 1873, Golgi observed black anastomizations in brains that had been fixed in ammonium dichromate and sodium sulfate before immersion in a solution of silver nitrate. Similar observations were obtained from brains that had been treated with dichromates and mercuric chloride (Golgi, 1873). The use of Golgi's discovery provided the historical foundation for all contemporary knowledge about the cellular components of the central nervous system, if not all that is known about the structural relationship between neurons. It is ironic that Golgi's interpretations of his material made but little contribution to this knowledge.

The discovery of the reazione nero² within nerve cells came to the attention of only a few neurologists. However, in 1886, Simarro showed his friend Ramón y Cajal preparations made according to Golgi's original formula and brought to Cajal's attention Golgi's classic monograph describing the method and its interpretations. This account described what appeared to be supporting evidence for Gerlach and Meynert's theory of neuronal connections via a protoplasmic reticulum: Golgi's support for this doctrine was based upon observations of nerve terminals in the griseum, which he believed to be a diffuse and continuous network. He also proposed that lateral arborizations, which we now know as dendrites, were nutritive components that played no direct role in integrative processes.

According to Cajal's autobiography, Simarro was distrustful of Golgi's method. This was not because he disagreed with the reticulum theory, as did such notable neurologists as Forel and His, but because he felt that the method produced inconsistent results. These inconsistencies still hallmark the technique in that no two preparations are identical. However, Simarro was probably referring to the fragmentary nature of impregnated elements because only partial impregnation of neurons is achieved by the original method of immersing tissue in dichromate alone. Despite such reservations, Cajal immediately recognized the po-

¹ For insects, another is the resolution of neuronal assemblies by cobalt injection (Strausfeld and Obermayer, 1976; Strausfeld and Hausen, 1977).
² Black reaction.