The methods currently used to impregnate nerve tissue with reduced silver fall broadly into two classes: methods of block impregnation and the so-called silver-on-the-slide techniques. In the earlier block impregnation procedures, such as those of Golgi, Cajal, and Bielschowsky, and their modifications, whole pieces of tissue are treated with silver solutions and only afterward sectioned and mounted. In the silver-on-the-slide methods the material is embedded and sectioned first, and then the sections are stained after they have been mounted on slides. Block-impregnation methods are discussed elsewhere in this volume (see Chap. 9) and need not be considered further here. Procedures for impregnating mounted sections, usually of paraffin wax-embedded material, are numerous, but they can be subdivided roughly according to the type of silver compound used as the impregnator. This is generally either an inorganic silver salt, most often silver nitrate, or a silver-protein complex, such as protargol.

Protargol was first used to stain mounted paraffin sections of fish (bullhead) brain by Bartelmez and Hoerr (1933), who substituted it in a modified Bielschowsky method—a useful historical survey of earlier reduced silver techniques is given by Silver (1942a). Bodian (1936), working in the same laboratory on the brain of the opossum, found that staining
could be speeded up and made more reliable by adding a small quantity of metallic copper or mercury, or nitric acid, to the protargol impregnating bath, and afterward reducing the silver with a hydroquinone/sodium sulfite solution. This method was subsequently much used on vertebrate tissue, and many writers reported the effects of variations in the procedure. Bodian himself (1937) studied the influence of different fixatives, and Davenport and his co-workers, in an extended series of investigations (Davenport and Kline, 1938; Davenport et al., 1939, 1947; Bank and Davenport, 1940; MacFarland and Davenport, 1941), tested numerous other methods of fixation. They also examined the effects of changes in most of the stages in the procedure. Romanes (1950), too, analyzed some of the variables in his investigation of the mechanism of silver staining. However, Holmes (1942, 1943) criticized the Bodian method on the grounds that protargol was "a substance of uncertain and variable composition," samples of which, from different sources, varied greatly in their effectiveness. He proposed instead the use of an ammoniacal solution of silver nitrate for impregnation, later (Holmes, 1947) modifying this to a silver nitrate solution buffered with boric acid/borax. This technique was the forerunner of a variety of silver nitrate methods, together with concomitant studies of variations in procedure and of the mechanism of silver staining in general, such as those by Palmgren (1948), Romanes (1950), Samuel (1953a–d), Peters (1955a–e), Wolman (1955a,b), Blest (1961), and Rowell (1963). Chapter 7 in this volume deals with the use of silver nitrate in more detail.

Other silver-protein impregnators have been tried. Glassner et al. (1954) reported the effects of varying the steps in the technique using a German product (from Serumvertrieb, Marburg, West Germany), and Polley (1956) tested another, of French origin (Laboratoire Roques, 36 Rue St. Croix de la Bretonnaire, Paris, France), and found it to have many similarities to protargol.

A combination of protargol and silver nitrate was used by Davenport et al. (1939) in their much-shortened (2-h) staining method, and FitzGerald (1964) recommended a similar double impregnation in his method. Various modified procedures have been devised for impregnating frozen or celloidin sections (Silver, 1942b; Foley, 1943; FitzGerald, 1963; Moshkowitz, 1967).

Bodian's original method was adapted for staining insect nerve tissue by Power (1943), who used it first to show the general neuroanatomy of the brain of the fruit fly Drosophila and later more detailed aspects of the brain (Power, 1946) and the neuroanatomy of the thoracicoabdominal ganglia (Power, 1948). He employed double impregnation in two baths of protargol and copper, each followed by reduction in a hydroquinonone/sodium sulfite solution. Subsequent workers (Hess, 1958a,b; Pipa et al., 1959; Chen and Chen, 1969) followed this procedure with only minor