Silver Staining of Axons in Subcellular Fractions of Nervous Tissue

Although methods utilizing silver salts have been the mainstay of neurohistological technique for a century, there is, as yet, no histochemical rationale behind the many empirical procedures available for staining axons. Attempts to compare the distribution of argyrophilic material with that of known protein end-groups[1,2] have been largely unsuccessful, as have attempts to identify in the electron microscope the fine structural elements upon which the silver is deposited[3]. It seems probable that it is the axoplasm, rather than the axolemma, which is stained[4], and there is some circumstantial evidence to suggest that silver may be deposited in relation to longitudinally oriented organelles[5–8] such as the neurofilaments or microtubules. The present report describes some of the properties of the argyrophilic component of the axon, as studied in subcellular fractions of cerebral tissue.

The brains of adult rats were homogenized in isotonic sucrose and subcellular fractions were prepared by density gradient centrifugation[9]. The fractions consisting of myelinated axonal fragments, axonal membranes (myelin and axolemma) and synaptosomes (nerve ending particles) were investigated. The identities of the fractions were confirmed by examination of thin sections of glutaraldehyde-fixed pellets in the electron microscope. Suspensions of the isolated subcellular particles were smeared onto slides, allowed to dry, fixed in Bodian's formol-acetic-ethanol[10] and stained by UNGEWITTER's urea-silver nitrate method[11]. This staining technique was chosen on account of its reliability and great specificity for axons when applied to conventional histological sections.

The fraction containing myelinated axonal fragments consisted of argyrophilic threads, 0.2–2.0 μ in diameter and 2–25 μ long. The membranous fraction contained no argyrophilic material and the synaptosomes were revealed as tiny argyrophilic particles and very fine threads, up to 1.5 μ in length. It was thus confirmed that stainability with silver was a property of the axoplasm.

Suspensions of the subcellular fractions were then subjected to a number of extractive and digestive procedures. It appears that the argyrophilic substance is a protein, since it is destroyed by trypsin (1%, 2 h, 37 °C, pH 8). This enzyme does not generally hydrolyse structural lipoproteins[12], but the argyrophilic material has a property in common with the lipoproteins of biological membranes in that it dissolves in 80% acetic acid and in 80% 2-chloroethanol (both for 2 h, 15 °C)[13,14]. Both these reagents completely dissolved all 3 subcellular fractions, leaving crystal-clear solutions from which no insoluble residues could be recovered by centrifugation. However, the argyrophilia was not abolished by extraction with *n*-butanol (2 h, 15 °C), which dissolves many lipoproteins[15,16], or by sodium deoxycholate (2%, 2 h, 15 °C). Neither was the argyrophilic component removed by 2:1 methanol-chloroform mixture (18 h, 60 °C), which dissolves most lipids and proteolipids[17]. If stainability with silver can be attributed to a filamentous component of the axoplasm, one might expect the responsible substance to have properties in common with other intracellular fibrous proteins such as actin[18], the neurofilament protein of squid giant axons[19,20], and the microtubular proteins of flagella[21] and of the mitotic spindle[22,23]. However, reagents which solubilize these substances (0.5 and 0.6 *M* KCl in 0.01 *M* *tris*-HCl buffer, pH 8.0; 0.1 *M* glycine; 5 *M* urea; 8 *M* urea in 34% acetic acid; 2% sodium deoxycholate; all for 2 h at 15 °C) had no effect on the argyrophilia of subcellular particles isolated from the brain of the rat. The argyrophilic moiety is insoluble in solutions of sodium chloride of various strengths, so it is unlikely to be a simple globular protein.

Solutions in 2-chloroethanol of the 3 subcellular fractions were dialyzed against excesses of water and *n*-butanol. White, floccular precipitates formed, which were smeared onto slides, fixed and stained with silver as described above. The precipitates derived from axonal fragments and from synaptosomes were argyrophilic, while that derived from the membranous fraction was not. After dialysis and removal of the precipitates, the supernatant solutions in *n*-butanol were dialyzed against water and further precipitates were formed. None of these second precipitates were stainable with silver.

It may be concluded that argyrophilia is a property of a component of the axoplasm which can be dissolved and reprecipitated. It seems likely, therefore, that a substance or group of substances, rather than a formed organelle, is responsible for stainability with silver. The properties of this proteinaceous component differ markedly from those of the known membranous and fibrous intracellular proteins[24].

Résumé. L'argyrophilie est la propriété d'une partie constituante protéique de l'axoplasme qui peut être dissoute et reprécipitée. Il est donc probable que c'est une substance, ou un groupe de substances, plutôt qu'une organelle formée, qui est responsable de la coloration de l'axone au contact de l'argent. Les qualités de cette protéine diffèrent d'une façon marquée de celles déjà connues des protéines intracellulaires membraneuses et fibreuses[25].

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