

# Thiocarbohydrazide (TCH)

## Thiocarbohydrazide (TCH) is a versatile reagent for Transmission and Scanning Electron Microscopy

Reagent for

1. The histochemical and ultracytochemical demonstration of glycomacromolecules.
2. Enhancement of fine structure of cytomembranes by the osmium-thiocarbohydrazide-osmium (OTO) bridging.
3. Rendering fixed tissues electrically conductive by the OTO reaction as a simpler alternative to evaporative metal coating for SEM.

### CYTOCHEMICAL APPLICATIONS FOR GLYCOMACROMOLECULES

TCH was introduced to cytochemistry,<sup>1-3</sup> in the first adaptation of the PAS reaction, to demonstrate tissue glycomacromolecules for TEM. The reaction depends upon selective oxidation of vicinal or 1,2-glycols, ethanolamines,  $\alpha$ -hydroxyaldehydes, and  $\alpha$ -hydroxy-ketones.<sup>4</sup> During the oxidation, the C-C bond of the compound is cleaved, resulting in the formation of a dialdehyde. Upon condensation with TCH, under conditions for general acid catalysis, an osmiophilic dialdimine of Schiff's base results. Subsequent treatment with  $O_5O_4$  (or Osmeth) solution results in the formation of insoluble osmium black polymers at the tissue sites of the glycomacromolecule. These electronopaque osmium blacks are highly visible, without counter-staining, for Light Microscopy and are readily observed on 1-2 $\mu$  ("thick") plastic sections taken with a glass or diamond knife. Orientation on the "thick" plastic sections facilitates ultrathin sectioning for TEM.

Several modifications of this reaction have been published utilizing either  $O_5O_4$ <sup>5</sup> or silver proteinate<sup>6-8</sup> to react with the aldehyde thiocarbohydrazone. Pearse<sup>4</sup> lists four groups of glycomacromolecules (glycans, glycosaminoglycans, glycoproteins and glycolipid) and a group of sphingomyelin-containing lipids which give a positive PAS reaction by virtue of an ethanolamine grouping as well as specific directions for the periodate oxidation of these substances. Basically, the periodic acid-Schiff's base reaction for the particular glycomacromolecule is performed in the manner specific for that glyco compound. After rinsing, the tissues are treated with 0.25%-1.0% aqueous TCH or TCH in acetic acid. After thorough rinsing, the tissue is osmicated in 2% aqueous osmium tetroxide or with its vapor.

For details see the methods of Pearse.<sup>4</sup>

### ULTRASTRUCTURAL DELINEATION

TCH is used to render tissue-binding sites of metal ions or compounds visible for Light Microscopy and enhance contrast<sup>9-11</sup> for TEM. This is accomplished by using TCH as a reagent for bridging osmium to the ion or compound deposited in tissue.

When TCH is applied to tissue which had been fixed in osmium, profound contrast enhancement for both light and TEM results. This entire procedure, called the OTO reaction, especially enhances the demonstration of cytomembranes. Bridging osmium via TCH to uranium-labeled antibodies has been used to enhance visualization by TEM of antigen sites of embedded tissues.<sup>12</sup> Enhancement of tissue sites of cupric ferrocyanide (Hatchett's brown), deposited as a result of an enzyme histochemical reaction to demonstrate oxidoreductases, acetylcholinesterase, non-specific esterase with substrate 2-thiolacetoxybenzylidene (TAB) or acid phosphatase with substrate di(dicyclohexylammonium)-2-naphthylthiophosphate (DDNTP) may also be effected by bridging to osmium via TCH.<sup>13-16</sup> It should be noted that, in all of these reactions, recrystallized TCH gives very much superior results.

### CONDUCTIVE COATING IN SEM

The TCH bridging reaction described above as the OTO reaction has been applied in SEM to confer a conductive coating and impregnation of osmium on a variety of animal specimens before critical point drying. The procedure has been described in detail by Kelley, *et al.*<sup>17</sup> It offers a much more facile and desirable method to achieve electrical conduction of specimens when viewed by SEM than the cumbersome vacuum coating processes and results in less damage to, and distortion of, specimens. Woods and Ledbetter<sup>18</sup> have used the OTO reaction, in combination with frozen Epon cracking, for viewing organelles of corn root tip cells.

**CAUTION**

May be fatal if inhaled, swallowed, or absorbed through skin. May cause irritation. Use only in a chemical fume hood. Do not breathe dust. Avoid contact with eyes, skin, and clothing. Wear protective goggles and gloves when handling material. Wash immediately after handling. Keep container tightly closed. Store in a cool, dry place.

**STORAGE AND HANDLING****Storage**

Store at room temperature

**Handling**

Glove, chemical goggles, chemical mask or fume hood

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