

## ORIGINAL PAPER

## Reduction of nitroblue tetrazolium to formazan by folic acid

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The reduction of nitroblue tetrazolium (NBT) to formazan by folic acid, *N*-(4-aminobenzoyl) glutamic acid, and other amino acids was studied in this paper. The reduction involves only one of the two tetrazolium rings of NBT. The reaction is considerably more rapid with folic acid and *N*-(4-aminobenzoyl) glutamic acid than with the other amino acids under study. The electron donor moiety appears to be the carboxylic acid in the alpha position. *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide notably increases the rate of the reaction and promotes the reduction of both tetrazolium rings.

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## Introduction

Many tetrazolium salts, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS), 4-(3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolium)-1,3-benzene disulphonate (WST-1), and 2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT), are used to investigate cell proliferation and viability in colorimetric assays based on reductive cleavage of the tetrazolium ring by metabolically active cells, resulting in the production of water-soluble or water-insoluble dark-blue formazans (Berridge et al., 2005). Furthermore, 2,2'-bis(4-nitrophenyl)-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene) ditetrazolium (nitroblue tetrazolium, NBT) can be reduced to water-insoluble monoformazan or diformazan by superoxide anions (Fig. 1); for this reason, it is widely used in colorimetric assays designed to measure the superoxide ions released during the activation of phagocytic cells (granulocytes, macrophages) (Berridge et al., 2005).

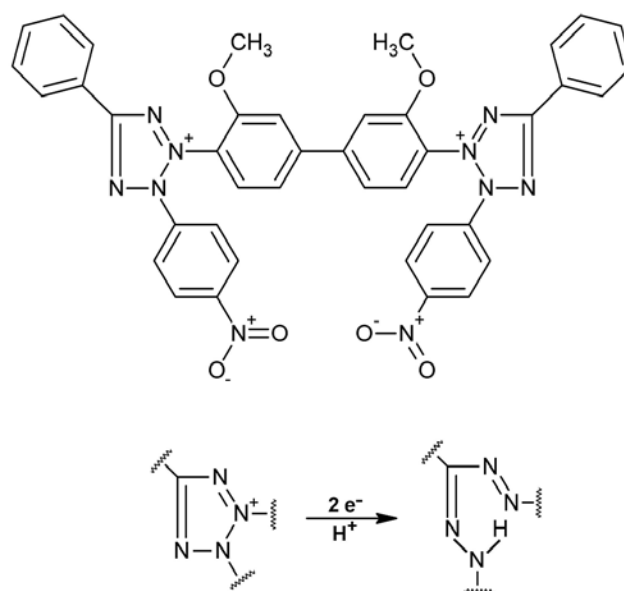


Fig. 1. Structure of NBT and reduction of tetrazolium ring to formazan.

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