dissolving 25 mg L-cystine-di-β-naphthylamide in 10 ml. hot 0·012 N hydrochloric acid and adding 10 ml. distilled water. The substrate is stable and may be stored at 4° C. The incubation period lasts 1 h at room temperature. The final reaction product is diffusely and evenly deposited along the outer margin of the syncytium. Background staining does not occur and sections incubated in the presence of trichloroacetic acid do not show enzyme activity.

The distribution of enzyme activity may reflect oxytocinase activity. Alternatively, another enzyme may be hydrolysing L-cystine-di-β-naphthylamide and the final reaction product represents the localization of this enzyme. Therefore, the trivial name cystine aminopeptidase should not be applied to histochemical activity observed with this substrate, as confusion may result. A similar situation already exists with leucine aminopeptidase (*E.C.* 3.4.1.1). Leucine aminopeptidase hydrolyses leucineamide more rapidly than L-leucine-di-β-naphthylamide. At least one other enzyme, however, hydrolyses L-leucine-β-naphthylamide more rapidly than leucineamide[3]. The histochemical activity with respect to leucine-β-naphthylamide represents a system of enzymes and does not reflect leucine aminopeptidase activity. In addition, the present biochemical nomenclature covering peptide hydrolases is inadequate due to overlapping specificities, and it has been suggested that the trivial names based on particular substrates should be abandoned[4]. The enzyme that hydrolyses the histochemical substrate L-cystine-di-β-naphthylamide is a cystine-di-β-naphthylamide hydrolase. If it is shown, however, that this enzyme specifically hydrolyses the cystine–tyrosine bond of oxytocin, then the term oxytocinase may be retained for histochemical use.

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NIGEL T. JAMES*

Department of Pathology,
Radcliffe Infirmary, Oxford.

* Present address: University of Oxford Medical School, Osler House, Woodstock Road, Oxford.

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Preferential Staining of Collagen by Acid Dyes in Methanol

THE effects of dye solvents on the selectivity of histological staining have not been extensively investigated. Robards and Purvis[1] have reported preferential staining of the gelatinous fibres of tension wood by chlorazol black *E* when dissolved in methyl cellosolve. Earlier the influence of solvents on the staining reactions of this dye was discussed by Cannon[2]. Puchtler and Sweat[3] reported a method for selectively staining haemoglobin in sections with amido black in a methanol solution, but not in water. Investigation of phospholipid staining by Luxol fast blue *G* revealed a changing selectivity dependent on the solvent used[4]. This dye in methanol solution was later found to stain only collagen and elastin[5].

When other sulphonated acid dyes were later used as methanolic solutions they preferentially stained connective tissue elements. This selective staining did not occur with aqueous solutions of the dyes. Among the dyes successfully used in this manner were acid fuchsin, aniline blue, methyl blue, fast green *FCF*, chlorazol black *E*, and pontamine fast turquoise 8*GLS*. Most of these dyes also selectively stain collagen when used as mixtures in saturated picric solutions, such as the Van Gieson stain.

Methylation, nitrosation and acetylation of tissue sections did not prevent the selective staining[5]. The staining was reduced by tanning with tannic acid, which affects the physical nature of collagen by the introduction of cross-linkages. Although the mechanism of this collagen staining has not been finally elucidated, it would seem to be of a physical nature rather than an affinity for specific chemical groupings in the collagen macromolecule.

It was considered that the most likely explanation for the preferential staining was a difference in the type of micelle or molecular arrangement of the dyes in methanol and water. The molecular form of the dye in methanol might conceivably have a greater affinity for collagen. However, as the molecular arrangement of the dyes in the two solutions appears to be comparable when examined in the analytical ultracentrifuge[6], this explanation is unlikely. It may be that methanol affects the permeability of the various proteins present in tissue sections, allowing only the collagen to stain. Collagen has a maximum solvation coefficient of 218 g/g, which is much higher than that for other non-globular protein polymers likely to be present in mammalian tissue sections, such as myosin (49 g/g) and tropomyosin (23 g/g)[7]; possibly this particular property of collagen is associated in some manner with its preferential staining.

As the collagen-staining reaction can be used after other histochemical and staining procedures in which the dyes used are not removable by methanol, it may be of value in histochemical investigations of connective tissue.

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T. N. SALTHOUSE

Department of Pathology,
The Squibb Institute for Medical Research,
New Brunswick, New Jersey.

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Histochemical Distribution of Alcohol Dehydrogenases in Endocrine Tissue

HISTOCHEMICAL investigations of alcohol dehydrogenase in rodents using various alcohols[1] revealed no obvious differences in the sites of alcohol utilization. Primary and secondary alcohols in general tissues were utilized at the same sites; the present communication deals with the histochemical variations in sites of utilization of different alcohols in steroid-producing endocrine glands.

Human term placenta, obtained at Caesarean section, and rat (Royal Wistar) adrenal glands, testes and ovaries were frozen on solid carbon dioxide and the tissues were prepared and incubated as described by Ferguson[1], using the following alcohols (final concentration 2·5 per cent): methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol, nonanol, decanol, undecanol, dodecanol, tridecanol, phenol, hydroquinone, 2-ethoxyethanol, allyl alcohol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, nonyl alcohol, tertiary butanol, tertiary butyl carbinol, *iso*-propanol, secondary butanol, pentan-2-ol, pentan-3-ol, octan-2-ol, *cyclo*hexanol, ethylene glycol, propylene glycol, glycerol, butan-1 : 3-diol, butan-1 : 4-diol, pinacol and furfuryl alcohol.

Control sections were concurrently incubated in the buffered medium containing no alcohol.

The results are summarized in Table 1.

Since Bonnichsen[2] isolated alcohol dehydrogenase from horse liver, the biochemical properties and mechanism of action of this enzyme have been widely described[3–14].