and concentration in which they are active in different tissues.

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Use of Deoxyribonuclease Inhibitors in the Isolation of Deoxyribonucleic Acids

In recent methods[1] for the isolation of deoxyribonucleic acids from mammalian tissues, the use of buffered (*p*H 7) solvents containing low concentrations of sodium arsenate, sodium citrate or sodium borate has been advocated in order to prevent enzymic depolymerization during the initial extraction of the deoxyribonucleohistone. As these salts are known to inhibit the action of pancreas deoxyribonuclease[2], their use in this connexion presumably rests upon the tacit assumption that the intracellular deoxypentosenucleases of mammalian tissues[3] have properties similar to those of the pancreas enzyme.

A study of the deoxypentosenucleases isolated from various animal tissues, however, shows that this assumption is not justified. Thus, the properties of the purified enzymes from, for example, calf thymus, rat liver, mouse tumour tissue and rat embryo, while closely similar among themselves, differ appreciably from those of the deoxyribonuclease of pancreas. The intracellular tissue enzymes require ions for activation, and are reversibly inactivated by dialysis against distilled water. They have optimal activity at *p*H 5·2 and are almost inactive at *p*H 7–7·5. In marked contrast to pancreas deoxyribonuclease, enzyme activity is not inhibited by sodium citrate, sodium fluoride, sodium arsenate or sodium borate. Indeed, in solutions of favourable *p*H but low ionic strength, these salts may even activate the enzymes.

The *p*H of tissue homogenates prepared in unbuffered aqueous solvents usually lies between 5·6 and 6·2, and is favourable for the action of the intracellular deoxypentosenucleases. The activity of these enzymes, however, is considerably reduced when homogenates are prepared in solvents buffered at *p*H 7–7·5. The fact that little or no depolymerization occurs when deoxyribonucleohistones are isolated in buffered citrate or arsenate solutions[1] thus appears to be due to the maintenance of a *p*H unfavourable for enzyme activity, rather than to the inhibition of the enzymes themselves.

An investigation of the nuclear and cytoplasmic fractions separated from tissue homogenates according to well-established methods[4] shows that the deoxypentosenucleases are confined to the cytoplasm of the cells, and are possibly associated with some particulate fraction (for example, mitochondria). As the intracellular substrate for the cytoplasmic deoxypentosenuclease is located entirely within the cell nucleus, it is essential in these studies of enzyme distribution to carry out all operations as rapidly as possible and to avoid undue damage to the nuclei, otherwise artefacts, due to redistribution by the combination of the enzyme with its substrate as well as by adsorption, are particularly liable to occur. It may be mentioned that the cytoplasmic enzyme is almost invariably adsorbed to a greater or less extent by the nuclei when the latter are isolated in 30 per cent sucrose[5]. In ionic solvents, adsorption of the deoxypentosenuclease is less likely to occur.

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Effect of Trichloracetic Acid on Nuclear Proteins

The demonstration of the property of hot trichloracetic acid in bringing about the dissociation of nucleic acid from nucleoproteins by Schneider[1] has led many authors in recent years to use it in different cytochemical experiments[2–4]. The protein framework of the chromosomes thus liberated provides a useful substrate for several biochemical procedures. Both ribo- and deoxyribo-nucleoproteins have been found to be affected by such treatments.

It has been recently demonstrated by Kaufmann and his associates[4] that, if cells with their nuclei are treated with hot trichloracetic acid at low concentration followed by staining with Bhaduri's[5] Feulgen and light green technique, an increase in the intensity of coloration in contrast to the control results for both chromosomes and nucleoli, as a result of reaction with acid dyes. The green colour, which is strongly taken up by both the nucleoli and chromosomes in such treated materials, shows that in all probability the increase in staining effect is referable to the reaction of acid dyes with released protein. Following enzyme treatment, Kaufmann *et al.* have also indicated the presence of a histone type of protein in chromosomes and possibly tryptophane in nucleoli.

We have been studying for some time, in different plant materials, the effect of different concentrations of trichloracetic acid on both chromosomes and nucleoli, and their demonstration by staining with both acidic and basic dyes; though our results are parallel to those of Kaufmann *et al.*, they have provided additional information.

The materials for the present investigation consisted of different plant materials (*Zephyranthes*, *Allium*, etc.), where mainly the long-chromosome types have been chosen, as there the effect on the chromosome structure could be indicated precisely. The experiments, involving a series of trials in different concentrations of the acid applied for different times at different temperatures, show that while hot trichloracetic acid in low concentration brings about an increase in the intensity of staining with acidic dyes, interesting results come out if the concentration of the acid is gradually raised. The duration of treatment, too, has a marked effect.