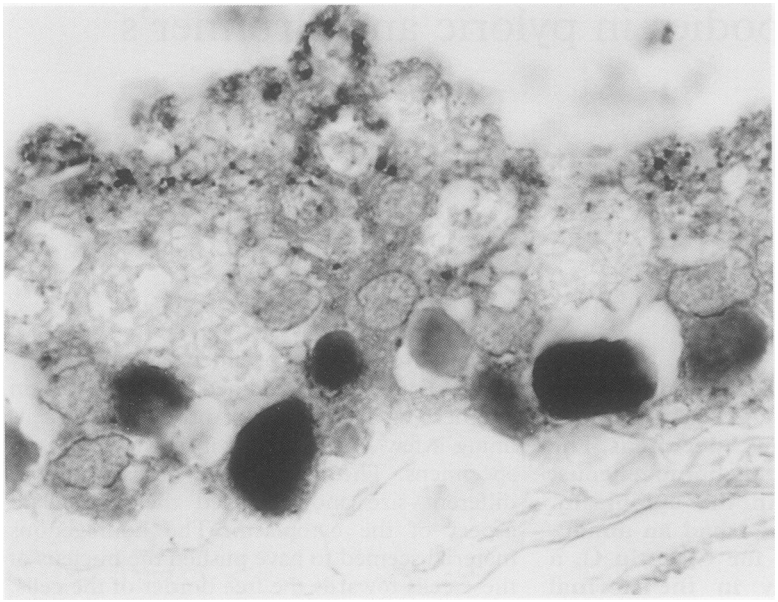

Figure 2 Strong positivity of the intracytoplasmic eosinophilic material following immunohistochemical staining with cystatin C.

reported before.[8-10] Further studies are necessary to assess the true prevalance of mucous gastroduodenal cells with eosinophilic bodies in Japanese nationals and to clarify the functional importance of the Cystatin C material retained in these cells.

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Reassessment of the rate of fixative diffusion

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Abstract

The diffusion of fixatives is slow. Early work using plasma gels and animal tissues showed the distance penetrated by a fixative to be a simple function of the fixation time but this relation has not been established in human tissues. The rates of diffusion into whole human spleens were measured for three primary fixatives over periods ranging from one to 25 days. A positive correlation was demonstrated between penetration distance (mm) and fixation time (hours). The diffusion rates were slower than those in previous studies. These results have possible implications for the handling of surgical specimens.

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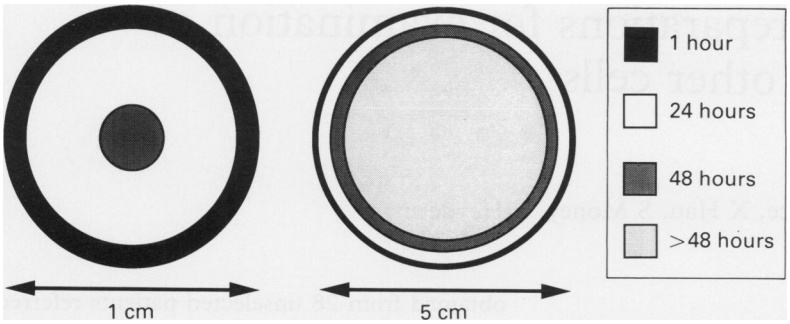
Diffusibility coefficients of fixatives

Fixative	*K**	*r (standard error)*
4% formaldehyde solution	0·55 (0·78)	0·88 ($p < 0·001$)
Saturated picric acid solution	0·40 (0·50)	0·79 ($0·001 < p < 0·01$)
5% acetic acid solution	0·90 (1·20)	0·82 ($0·001 < p < 0·01$)

*Figures in parentheses are the values of K from Medawar, 1941[2]

Tissues are usally fixed before microscopic examination and effective fixation is essential for accurate histological interpretation. Fixation depends not only on the rate at which a fixative reacts with tissue components, but also on the ability of the fixative to diffuse into tissues. An important rate limiting factor within the complex process of fixation is the slow speed of fixative diffusion into tissues.

Our understanding of this subject is largely based on early studies which examined the diffusion of fixatives into plasma gels and animal tissues.[1-3] These studies indicated that fixatives obey the laws of diffusion and that the distance penetrated by a fixative depends on a simple function of fixation time. This distance in millimetres is equal to the square root of the fixation time in hours, multiplied by a coefficient of diffusibility (K) for that fixative.[2,3] More complicated formulae have been proposed,[4] but no further studies have tested this relation nor examined the diffusion of fixatives into human tissues. This study was designed to examine the rate of fixative diffusion into a human tissue in the context of routine surgical specimens using necropsy material.



The predicted diffusion of 4% formaldehyde solution into 10 mm and 50 mm diameter specimens.

Methods

The spleen was chosen because of the relatively homogenous solid structure, convenient size, and availability. Ten human spleens were immersed whole in 4% formaldehyde solution for periods of between one and 25 days after removal at necropsy. The hilar vessels were ligated before immersion and the specimens were kept at a constant temperature of 20°C which approximates to the average constant temperature in our laboratory. Three complete cross-sections were then taken from the central portion of the long axis of each spleen and immersed in 0·02% chromic acid solution for 14 days to macerate any unfixed tissue.[1] This produced a sharp line of demarcation between the fixed outer rim and the rest of the tissue. The depth of penetration, as represented by the thickness of the outer rim, was calculated from the mean of 100 Vernier micrometer measurements on each slice after a simple correction for tissue shrinkage.[1] Each measurement was made in a horizontal plane towards the central point of the slice. Similar fixation experiments were performed using saturated picric acid solution and 5% acetic acid solution. The correlation between the depth of penetration (mm) and the square root of the fixation time (hours) was assessed by linear analysis of raw data from which the correlation coefficient (r) and its standard error based on 10 data points were calculated for each fixative. The coefficients of diffusibility were determined by regression analysis.

Results

The results are presented in the table. A positive correlation was demonstrated between the depth of penetration and the square root of the fixation time for each of the fixatives. The predicted diffusion of 4% formaldehyde solution into hypothetical, solid 10 mm and 50 mm diameter specimens is shown in the figure.

Discussion

The diffusion of fixatives is usually measured on uniform coagulants such as gelatine or plasma gels.[2 3] The rates obtained using animal tissue are generally lower because of barriers such as cell membranes.[1] The diffusion rates of the primary fixatives in our human tissue model are slower than in these previous studies and may result from differences in tissue structure together with less controllable factors such as impedance by the splenic capsule. The presence of fixed tissue is also believed to slow subsequent inward diffusion of fixatives.[5] Within the limitations of a simple model, our results do not support this theory and are more consistent with earlier studies which showed that fixatives obey the laws of diffusion and appear to neither retard nor facilitate their own penetration.[2 3]

Primary fixation can affect histological interpretation.[5] Delayed fixation has been shown to influence the number of observable mitotic figures in tissues,[6 7] and fixation may influence the immunoreactivity of tissue antigens.[8] The commonest primary fixative is 4% formaldehyde solution which will penetrate just 2·4 mm in 24 hours. Small specimens measuring less than 10 mm in diameter will fix by simple diffusion. Larger specimens should be thinly sliced, partly dissected or perfused to facilitate rapid and even fixation. Although this study has only investigated the diffusion of a limited number of fixatives into a single human tissue, the observations emphasize the need for prompt and efficient fixation of surgical specimens.

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