in Japanese nationals and to clarify the functional importance of the Cystatin C material retained in these cells.

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1 Helander HF. The cells of the gastric mucosa. Int Rev Cytol 1981;70:217-9

Reassessment of the rate of fixative diffusion

R D Start, C M Layton, S S Cross, J H F Smith

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.

(J Clin Pathol 1992;45:1120–1121)

Diffusibility coefficients of fixatives

<table>
<thead>
<tr>
<th>Fixative</th>
<th>K*</th>
<th>r (standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% formaldehyde solution</td>
<td>0.55 (0.78)</td>
<td>0.88 (p &lt; 0.001)</td>
</tr>
<tr>
<td>Saturated picric acid solution</td>
<td>0.40 (0.50)</td>
<td>0.79 (0.001 &lt; p &lt; 0.01)</td>
</tr>
<tr>
<td>5% acetic acid solution</td>
<td>0.90 (1.20)</td>
<td>0.82 (0.001 &lt; p &lt; 0.01)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are the values of K from Medawar, 1941*
Reassessment of the rate of fixative diffusion

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. 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. 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. The rates obtained using animal tissue are generally lower because of barriers such as cell membranes. 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 impendence by the splenic capsule. The presence of fixed tissue is also believed to slow subsequent inward diffusion of fixatives. 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.

Primary fixation can affect histological interpretation. Delayed fixation has been shown to influence the number of observable mitotic figures in tissues, and fixation may influence the immunoreactivity of tissue antigens. 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.

2 Medawar PB. The rate of penetration of fixatives. JR Microsc Soc 1941;81:46-57.
Reassessment of the rate of fixative diffusion.

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