0.6 $\mu\text{mol/min}$ and will consequently be underestimated by about 10%.³ More seriously, if the integrated form of the Michaelis-Menten equation is fitted to the progress curve of the reaction, the values of the Michaelis-Menten parameters estimated from the fit will be grossly wrong.^{3 5}

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Fixation and immunohistochemistry of lymphoid tissue

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The morphological distinction between reactive and malignant processes in lymph nodes may sometimes be extremely difficult, but the introduction of immunohistochemical methods may help. While a polyclonal cell proliferation may be neoplastic,¹ it is more generally reactive, but monoclonality is very suggestive of malignancy.

The identification of monoclonality is presently limited to cells of the B lymphocyte series, most simply using antisera to kappa, lambda, and heavy chains. The antigenicity of cellular immunoglobulin appears least impaired in frozen sections or imprints, but in most cases lymphoid material arriving for histopathological diagnosis has been fixed. Routine formalin fixation may not be the most suitable for many antigens, and efforts using proteolytic enzymes to 'unmask' antigenic sites² presumed concealed by formalin fixation have gone some way to overcoming this problem. When formalin fixation is unavoidable the demonstration of cellular immunoglobulin may require such manipulations, but a more logical approach might be to use a fixative that does not denature or conceal the antigenic sites to the extent whereby reaction between antibody and antigen is impaired. We decided to examine a range of common fixatives to seek any giving adequate morphological preservation of lymphoid tissue together with preservation of immunoglobulin antigenicity.

Material and methods

TISSUES

Three to four millimetre slices of both human lymph nodes and tonsils and rabbit lymph node, spleen, and gut were fixed for periods up to two weeks in the fixatives given below. The composition of the fixatives was taken from Disbrey and Rack.³ The tissues were dehydrated in ethanol, cleared in xylene, and embedded in 56°C MP paraffin wax. Four micrometre sections were dewaxed in xylene and brought to water. Staining was performed by immunoperoxidase methods.

FIXATIVES

Simple: Formalin and glutaraldehyde 1-10%.
Compound: Susa, Bouin, Zenker, Carnoy, and formol-mercury.

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Antisera

(a) Direct staining: horseradish peroxidase (HRP) conjugated antibody to human IgG and rabbit IgG was used at a 1/20 dilution (from Miles and Dakopatts).

(b) Indirect staining: rabbit antiserum to human kappa, lambda, α , γ , μ heavy chains and swine anti rabbit IgG were used as first antibody diluted 1/50 (from Dakopatts, Hoechst, and made in our department) with HRP conjugated second antibodies from Miles and Dakopatts.

(c) Peroxidase-antiperoxidase (PAP) using primary rabbit antibodies as in (b) and PAP complex from Dakopatts.

Primary antisera from three separate sources (Hoechst, Dakopatts, and raised in our department) were used since they would be unlikely to recognise identical epitopes or antigenic sites.

Endogenous tissue peroxidase activity was blocked by pretreatment in 1 part hydrogen peroxide (100 vol, 30%) in 9 parts of methanol for 30 minutes.

Horseradish peroxidase conjugates were visualised with diaminobenzidine (Sigma D 5637) dissolved in Tris buffered saline (see below) to 0.5 mg/ml, and 10 μ l of hydrogen peroxide (100 vol) was added per 1 ml of solution.

Sections were incubated in this for 5 minutes, washed, and counterstained in Mayer's haemalum. Washing of sections was performed in Tris 0.05 M, sodium chloride 0.15 M at pH 7.6.

Sections were assessed blindly (code labelled by a colleague) by both of us separately for specific staining and for non-specific or background staining, grading each from 0 (no staining) to + + + (strongly stained).

Results

The greatest number of positively staining cells was seen in human tonsils and rabbit small intestine.

The best results were found consistently with Susa and the worst with 10% neutral buffered formalin. The Table shows our results on simple dewaxed sections in order of merit.

Staining results

Fixative	Specific staining	Background
Susa	+ + +	0
Bouin's	+ +	+
Formol mercury	+ +	+
Zenker's	+ +	+ +
Carnoy	+	+
2.5% Formol saline	+	±
1% Glutaraldehyde	±	±
5% Glutaraldehyde	±	±
10% Formol saline	±	+
2.5% Neutral buffered formalin	±	+
10% Neutral buffered formalin	±	+ +

Increased times of fixation for up to two weeks did not significantly alter these findings. The primary aldehyde fixatives showed a progressive decrease in specific staining together with an increase in background staining. This was in contrast to Bouin's and Zenker's, which showed some progressive diminution of background staining and thus more conspicuous specific staining.

It appears that the most commonly used primary fixatives, glutaraldehyde and formalin, may be the worst for immunoglobulin. However, where formalin occurs together with other fixative agents, that is, in the so-called compound fixatives, its deleterious effects on the subsequent demonstration of immunoglobulin seem in some way modified. Indeed, Susa contains 20% formalin and yet consistently gave the best results. Buffered formalin appeared more damaging than unbuffered.

In terms of background staining, which varied considerably between the different fixatives used, we conclude that the degree of 'non-specific' background staining is, at least in part, a product of fixation.

It is also worthy of mention that antisera from three different sources all gave identical results, suggesting that although these different antisera were unlikely to be recognising the same epitopes or antigenic sites, such sites appeared altered to a similar degree by a specific fixative. Both heavy and light chains maintained their antigenicity to a similar degree for each fixative.

Using trypsin to expose antigenic sites, we have had the same experience as others²⁴ that the immunoglobulins in formalin and glutaraldehyde fixed tissues are much more readily stainable. Despite this we have still had considerably better results with untrypsinised Susa, achieving a lower background, particularly in postmortem tissue, and a higher dilution of primary antisera. The main reasons for high dilutions are economy and reduction in background or non-specific staining; but dilution results in a progressive diminution in the number of stainable cells. Presumably progressive dilution of heterogeneous antisera eliminates different subpopulations of cells. Increasing antibody concentration results in a plateau of specifically staining cells, and the highest dilution of antisera giving this plateau, with consequent minimal background, is used. Using PAP we find optimum dilutions of primary antisera to be in the order of 1/100 to 1/1000. The negligible background encountered with Susa would allow stronger antisera to be used if necessary, and the ready demonstration of specific staining renders empirical trypsinisation unnecessary.

In conclusion, we commend the use of Susa for the routine fixation of lymph nodes, biopsy specimens, and postmortem tissues for immuno-

globulin containing cells. We do not understand the processes involved, but this seems to be a useful procedure.

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Letters to the Editors

Antibody-coated bacteria in urine

A simple, reliable, non-invasive test to differentiate between infections of the upper and lower urinary tract would be of great value, and in the February issue Mengoli and co-workers¹ repeated the claim that a test for antibody-coated bacteria in the urine can differentiate these infections. However, their evidence is not based on direct localisation of the infection by a method such as bladder washout.² In addition, they have explicitly studied highly selected groups of patients, discarding, for example, some patients thought to have non-obstructive pyelonephritis.

We have found only three published studies that correlate the site of urinary tract infection, localised by a *direct* method, and the presence or absence of antibody-coated bacteria in the urine. Jones *et al.*³ studied 29 adults with urinary tract infections, many of them with known underlying disease such as nephrolithiasis. They found good correlation between upper tract infections and the presence of antibody-coated bacteria. A German study⁴ found similar results in 26 patients, some known to have renal disease. Hellerstein and others⁵ carried out a prospective study in children. They found antibody-coated bacteria in the urine to have no correlation with the site of urinary tract infection in 45 children.

We also performed a prospective study but in women on general medical wards, selected only by the growth from a urine sample of a coliform organism in a count of at least 10⁵ per ml. Sixteen women were

studied, with a mean age of 70 years. Their medical problems included heart failure, stroke, and uncontrolled diabetes mellitus. The site of infection was determined by a direct method² that involved drainage of the bladder through a catheter and instillation of an enzyme preparation followed by an antibiotic (we used 125 000 units of streptokinase-streptodornase in 10 ml water followed after 15 minutes by 40 mg gentamicin in 50 ml of 2.74% sodium bicarbonate, left for 30 minutes). The bladder was then drained and irrigated several times with saline. Samples of the last part of the washout fluid, and at 10, 20, and 30 minutes after the washout, were taken for viable counts. Criteria for diagnosing upper or lower urinary tract infections from these counts have been given.^{2,3,5} Antibody-coated bacteria were looked for in the patient's urine using sheep anti-human globulin conjugated with fluorescein.⁶ Controls were overnight subcultures of bacteria from the same urine treated in the same way.

We found eight infections of the upper urinary tract. Of these, five had antibody-coated bacteria. There were five lower tract infections, four having antibody-coated bacteria. Three bladder washout tests gave equivocal results, including two in which the infection was due to a mixture of a coliform and an enterococcus. The other equivocal test was associated with antibody-coated bacteria.

We concluded that the test for antibody-coated bacteria was of no value in determining the site of infection in our patients, selected not because they were known to have long-standing urinary

tract disease (as were many of those of Mengoli,¹ Jones,³ and Kohnle⁴), but only because they had bacteriuria (as were those of Hellerstein⁵). Any explanation for the discrepancy between the results of these studies must be speculative at the moment but may include factors such as duration of infection, number of previous infections, and immune competence of the patient. How the patients were selected for study is the most important factor.

We suggest that the test for antibody-coated bacteria by itself is misleading in the investigation of an individual patient with urinary tract infection.

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