500–1000, and eight >1000. Our subjects are members of a well established aboriginal community whose facial and footwear habits have not changed substantially over the years. Intermittent random sampling of stools has shown a high prevalence of stool parasites, especially of hookworm. Malaria, however, was eradicated from this community in the late fifties.

Although our investigation was not as rigorously controlled as that of Bain et al., these figures and those of others such as Vaterlaws et al. working with Papua New Guinea highlanders suggest that blacks other than those of African origin have total white cell and neutrophil counts which are more akin to Caucasian rather than black African values.

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References


Two further alkaline phosphatase staining methods for immunohistochemistry

Alkaline phosphatase labelled antibodies may be used as alternative reagents to peroxidase-antiperoxidase (PAP) reagents in the immunohistochemical method of Sternberger. Visualisation of alkaline phosphatase can be achieved by various azo coupling reactions. We report two novel staining protocols for alkaline phosphatase, one resulting in a green and the other a brown-black final reaction product. The new methods are described in relation to the staining of human synovium.

Material and methods

Human synovial tissue was fixed in 4% non-buffered formalin. The specimens were processed by routine methods and embedded in paraffin plus.

All chemicals used were of analytical quality and were obtained from Sigma (München, FRG). Antibodies, if not stated otherwise, were provided by Dakopatt (Bohringer, FRG).

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An indirect alkaline phosphatase method was compared with a standard PAP technique. The first antibody was a heavy chain specific rabbit antihuman immunoglobulin diluted 1/50 (final protein concentration 0·2 mg/ml; alkaline phosphatase activity/slide = 50 units).

Alkaline phosphatase was coupled to a swine antirabbit IgG by the method of Müller.

Standard staining methods for alkaline phosphatase were employed. The final reaction products comprised: fast blue BB combined with naphtol-AS-MX-phosphate and fast red ITR with the coupling reagent naphtol-AS-TR-phosphate.

PAP staining was performed by standard methods. The first antibody was used at the same dilution as in the alkaline phosphatase methods. The bridging antibody was a swine antirabbit IgG diluted 1/100 (protein concentration 0·1 mg/ml) followed by rabbit PAP complex diluted 1/100. The final reaction substrates used were 3-amino-9-ethylcarbazole, diaminobenzidine 4-chlor-naphtol, or Harker Yates reagent.

Novel alkaline phosphatase techniques

1 Fifteen milligrams of naphtol-AS-GR-phosphate was dissolved in 25 ml 2% sodium 5, 5-diethylbarbiturate (pH 9·2); 0·2 ml 10% MgCl₂ was added to the incubation medium. Twenty five milligrams of varianime blue sale was added to this solution, which was filtered and used immediately after preparation. Incubation time was 15–30 min at 20–22°C. A one minute wash was performed in 1% acetic acid before counterstaining.

A second staining protocol originally used by McGadey for demonstrating alkaline phosphatase in enzyme histochemistry was adapted for this study. Incubation medium consisted of 3 mg tetranitro-blue-tetrazolium (TNBT) or neotetrazolium chloride (NBT) dissolved in 10 ml 0·2 M TRIS HCL (pH 9, 2–9, 4), to which 2 mg 5-bromo-4-chloro-3-indoxylphosphate toluidine salt was added. Incubation time was 30 min at 20–22°C.

Results

By the standard alkaline phosphatase methods either blue or red final reaction products are achieved. The blue final reaction product cannot be combined with haematoxylin counterstaining, whereas the fast red ITR is well differentiated against the blue background. The azo dye coupling method described here for alkaline phosphatase based on varianime blue salt and naphtol-AS-GR-phosphate yields a distinct green product, which, as far as we know, has not yet been used in immunohistochemistry or enzyme histochemistry. The McGadey technique for demonstrating alkaline phosphatase in enzyme histochemistry was adapted for this study. Incubation medium consisted of 3 mg tetranitro-blue-tetrazolium (TNBT) or neotetrazolium chloride (NBT) dissolved in 10 ml 0·2 M TRIS HCL (pH 9, 2–9, 4), to which 2 mg 5-bromo-4-chloro-3-indoxylphosphate toluidine salt was added. Incubation time was 30 min at 20–22°C.

Methodological comparison of five different immunohistochemical results

<table>
<thead>
<tr>
<th>Method</th>
<th>Peroxidase-antiperoxidase</th>
<th>Alkaline phosphatase</th>
<th>Variamine blue salt</th>
<th>TNBT + 5-bromo-4-chloro-indoxyl-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of incubation steps</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Colour of reaction product</td>
<td>Red</td>
<td>Blue</td>
<td>Red</td>
<td>Green</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Very good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Background staining</td>
<td>Minimal</td>
<td>Slight</td>
<td>Slight</td>
<td>Slight</td>
</tr>
<tr>
<td>Counterstaining with haematoxylin possible</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Letters to the Editor
for enzyme histochemistry is easily adapted to immunohistochemistry. With TNBT a
distinct black precipitate was obtained, whereas NBT did not result in an accept-
able product. TNBT staining was somewhat less distinct than azo dye coupling
reactions.

With both newly introduced methods plasma cells and extravasated deposited
immunoglobulins were easily demonstrated in human synovial tissue. When the
sensitivity of the standard methods for alkaline phosphatase were compared with
either the new azo coupling method with varianime blue salt or the McGadey tech-
nique, no differences in the sensitivity could be seen (Table). But the four
methods demonstrating alkaline phosphatase labelled antibodies were less sensitive
than the PAP technique in identifying endogenous IgG within conventionally
processed tissue sections of human synovium.

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False positive results with a tube pregnancy test

We have recently compared a slide pregnancy test (Roche Diagnostika) with the
Pregnosticon tube pregnancy test (Orga-
on Teknika). Both tests were performed as described in the manufacturer’s instruc-
tions except that initial screening with the tube method was at one in three for spec-
imens from hospital patients (to detect 3000 IU/l of human chorionic gonado-
trophin) and one in five for specimens from other sources (to detect 5000 IU/l of
human chorionic gonadotrophin).

Altogether 232 urine samples were tested; the two tests agreed in all but 13
instances (5-6%). Three specimens were slide positive but tube negative. This could
be explained by the greater sensitivity of the slide test compared with the tube test at
the dilutions being used. Indeed, in one case, for which a subsequent specimen was
received, positive results were obtained by both tests. Two specimens were negative
by the slide test and equivocal by the tube test. This was not considered significant
because in practice repeat specimens would have been requested in cases where the
tube test alone was used.

A more important observation was that eight specimens were negative by the slide
test but positive by the tube test. On repeat testing later the same day or after storage
overnight at 4°C, all became negative by both tests. The explanation for these ini-
tial false positive results by the tube test is unclear. Vibration is known to affect the
tube test in this way, causing the edge of the red blood cell mat to roll down the
tube. But this was well known to our staff, who took great care to avoid such vibra-
tion. Another explanation could be that compounds were present in the urine which
inhibited haemagglutination and which rapidly became inactive. We were con-
cerned about these false positive results, which were detected only because the slide
test was being performed in parallel. Laboratories relying solely on the tube test
should be aware of the problem and be careful that they are not reporting false
positive results.

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References


2 Heydermann E. Immunoperoxidase technique in histopathology: applications, methods and

3 Mason DY, Sammons RE. Alkaline phosphatase and peroxidase for double immunoen-

4 Mason DY, Woolston RE. Double immunoen-

5 Chayen J, Bitensky L, Butcher RG, His-

6 Müller J, Pfleiderer G. A new method of conjugation of protein for the enzyme immuno-

7 McGadey J. A tetrazolium method for non-
specific alkaline phosphatase. Histochemie
Two further alkaline phosphatase staining methods for immunohistochemistry.

P Fritz, H V Tuczek, J G Saal and G Wegner

*J Clin Pathol* 1984 37: 1078-1079
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