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.1,2 Visualisation of alkaline phosphatase can be achieved by various azo coupling reactions.3–5 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 (Böhringer, 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.6

Standard staining methods for alkaline phosphatase were employed.7,8 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 Hanker 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 variamine 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.

2 A second staining protocol originally used by McGadey7 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 red and ITR is well differentiated against the blue background. The azo dye coupling method described here for alkaline phosphatase based on variamine 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 technique7 originally described...
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 extravasal 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 variamine blue salt or the McGadiey tech-
tique, 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.

**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-
non 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 speci-
mens from hospital patients (to detect
3000 IU/l of human chorionic gonado-
trphin) 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
initial 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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**Book reviews**


There is an increasing use of quantitative methods in histopathology. They are most
easily applied in the tightly controlled experimental situation but perusal of any
journal with a component of clinical pathology shows that there are also many
uses in this field either diagnostic, or simply trying to analyse the tissue processes.

Dr Baak and Oort have been prominent in the field during the past decade and have
produced a short monograph which is an excellent introduction to the subject and
covers both older grid-type techniques as well as developments based on computer
assisted microscopy. The reference lists to work already accomplished are most valu-
able.

Most people who buy this book will have at least an incipient interest in the subject
but it is well worth buying for the departmental library and will stimulate those who
hitherto have regarded diagnostic histopathology as necessarily subjective.

**Basic and Clinical Tumor Immunology.**
*Cancer Treatment and Research Series*. Ed
Ronald B Herberman. (Pp 348; D fl
180-00; US$72-00.) Martinus Nijhoff.
1983.

This is a timely and useful review of the state of the art of selected topics in tumour
immunology. It deals with various aspects of host resistance to neoplasms and discus-
ses current thoughts in the ways that such responses could be augmented or modified.

For pathologists, however, the chapter on monoclonal antibodies to human
tumours will be of most value. Lloyd has reviewed the wide range of such reagents
indicating their specificities and potential utility in the biological and pathological
classification of human neoplasms.

Overall, the book is a useful and timely contribution to the literature.

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