previous methods. Both DNA and RNA can be demonstrated by the gallocyanin–chrome alum method; the method does not separate the two nucleic acids and suitable extraction techniques must be used. The definitive, most sensitive technique for identifying DNA is that of in situ hybridization (see Chapter 26).

Feulgen reaction

The method of Feulgen and Rossenbeck (1924) is the standard technique for demonstrating deoxyribose. Mild acid hydrolysis, employing 1 M hydrochloric acid at 60°C, is used to break the purine–deoxyribose bond; the resulting 'exposed' aldehydes are then demonstrated by the use of Schiff's reagent. Elements containing DNA are stained a red–purple color. The ribose–purine bond is unaffected by the hydrolysis and RNA is not demonstrated (Fig. 13.4).

The hydrolysis is the critical part of the method; an increasingly stronger reaction is obtained as the hydrolysis time is increased until the optimum is reached. Beyond this the reaction becomes weaker, and if the hydrolysis is continued the reaction may fail completely. An important consideration in selecting the correct hydrolysis time is the fixative used. Bouin's fixative is not suitable as it causes over-hydrolysis of the nucleic acid during fixation. Bauer (1932) discussed the times of hydrolysis for various fixatives; some of these are reproduced in Table 13.1.

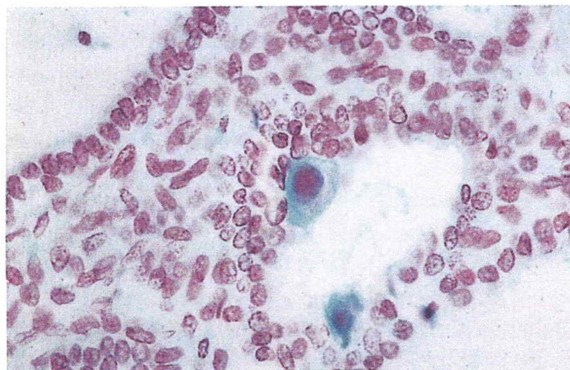

Fig. 13.4 Nuclear cytomegalovirus inclusion bodies magenta but smaller in size. Feulgen technique with light green counterstain.

Table 13.1 Hydrolysi HCl at 60°C

Fixative
Bouin
Carnoy 6.3.1
Chrome acetic
Flemming
Formaldehyde vapour
Formalin
Formal sublimate
Helly
Newcomer
Regaud
Regaud sublimate
Susa
Zenker
Zenker formal

Feulgen nuclear rec & Rossenbeck 1924)

Fixation

Not critical but not Bou

Solutions

a. *1 M hydrochloric acic*
Hydrochloric acid (cc
Distilled water

b. *Schiff reagent*
(See p. 171).

c. *Bisulfite solution*
10% potassium meta
1 M hydrochloric acic
Distilled water

Method

1. Bring all sections to
2. Rinse sections in 1 /
3. Place sections in 1 13.1).
4. Rinse in 1 M HCl at
5. Transfer sections to
6. Rinse sections in bis
7. Repeat wash in bisu
8. Repeat wash in bisu
9. Rinse well in distillec

2. Proteolytic digestion is necessary and is best achieved by using 0.1% protease type XXIV (Sigma) for 45 minutes.
3. Non-immune serum is essential, especially when polyclonal antibodies are employed.
4. Polyclonal antibodies, as described in Table 21.2, are used at high dilutions for 60 minutes followed by swine anti-rabbit peroxidase-labeled secondary antibody for 25 minutes. A high-quality DAB is employed as the chromogen (Dakocytomation DAB+).

An alternative protocol was published by Boyd SM and Ronan JE (Dakofacts Vol. 8. No. 1) using trypsin digestion with Envision reagents.

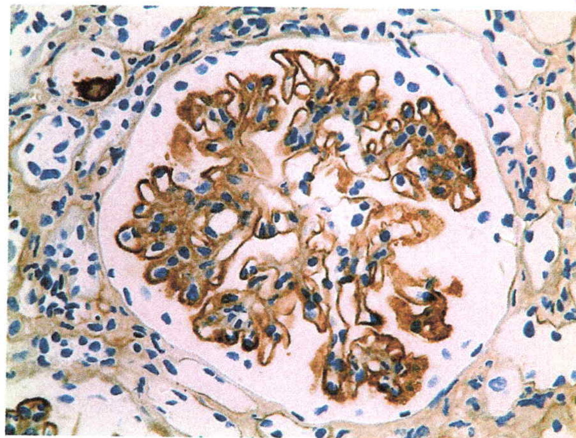

Fig. 21.19 Demonstration of IgG membranous nephropathy in a formalin-fixed paraffin-embedded renal biopsy.

Immunoperoxid formalin-fixed p skin biopsies

Reports suggest (W. tion) that this techniq tions. However, it mu capricious methods em today. Direct immunof generally preferred as i ity, compared with avid labeling of non-specific complement in the var ing immunoperoxidase munication) has, at th successful in a numb reliable.

Protocol outline

1. Fix for 3–24 hours saline, 10% neutral process to paraffin w
2. Cut sections at 3–4 and dry overnight at
3. Treat sections with Tris-buffered saline, p
4. Minimize non-specifi with 10% casein solut minutes.
5. Incubate sections in p shown in Table 21.3
6. Treat sections with C mation) reagent for 3
7. Visualise with DAB minutes.

Table 21.2 Immunocomplexes in renal biopsies

Antibody	Species	Supplier	Dilutio
IgA	Rabbit	Dakocytomation	1/2000
IgM	Rabbit	Dakocytomation	1/500
IgG	Rabbit	Dakocytomation	1/2000
C3c	Rabbit	Dakocytomation	1/800
Clq	Rabbit	Dakocytomation	1/400
Fibrinogen	Rabbit	Dakocytomation	1/3000
Kappa	Rabbit	Dakocytomation	1/2000

eimer's disease
n et al 1968,
ıal Institute on
ıp on Diagnos-
Assessment of
of tangles and
immunohisto-
ıjunction with

the accumula-
ʋy bodies. The
c (Lewy body)
ɔn's disease is
loss of neurons
of pigmented
ng neurons in
bodies, round,
ınded by a pale
of α-synuclein
Gomez-Tortosa
otein that may
ıtations in the
milial Parkin-
ıuclein in the
e, particularly
ave provided a
of Parkinson's

l features of
nson's disease
McKeith et al
term for the
's disease and
ology is 'Lewy
en et al 1990).
ar distribution
ease, but neo-
lly present in
ether (Hansen
s disease, the
loss and gross
any surviving
nany neurons

ainstem, corti-
l are therefore

more difficult to identify in routine H&E-stained sections. Lewy neurites, which are abnormal neuronal processes that occur in gray matter in several locations in the Lewy body disorders, are also a prominent feature in Lewy body variant of Alzheimer's disease. α-Synuclein antibodies are useful in demonstrating neocortical Lewy bodies and Lewy neurites (Irizarry et al 1998) (Fig. 23.13).

Frontotemporal degenerations

This is a heterogeneous group of disorders that share the clinical features of prominent language and personality manifestations (McKhann et al 2001). They have a variety of pathological lesions, but most have selective neuron loss and atrophy in the frontal and temporal lobes, and ballooned neurons in the cerebral cortex. Many fall under the heading of so-called 'tauopathies', due to the primary involvement by tau pathology or mutations in the tau gene on chromosome 17 (Spillantini et al 1998). Immunohistochemistry has played a pivotal role in their identification and differentiation.

Huntington's disease

Huntington's disease is a rare genetic disorder that is inherited in an autosomal dominant pattern. The under-

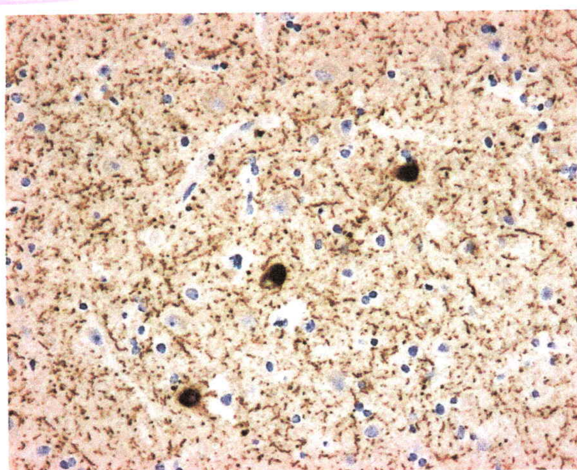

Fig. 23.13 Section of cerebral cortex in Lewy body variant of Alzheimer's disease. A monoclonal antibody to human α-synuclein demonstrates occasional Lewy bodies (larger, rounded structures) within cortical neurons, as well as a dense feltwork of fine thread-like processes of neurons, termed 'Lewy neurites'.

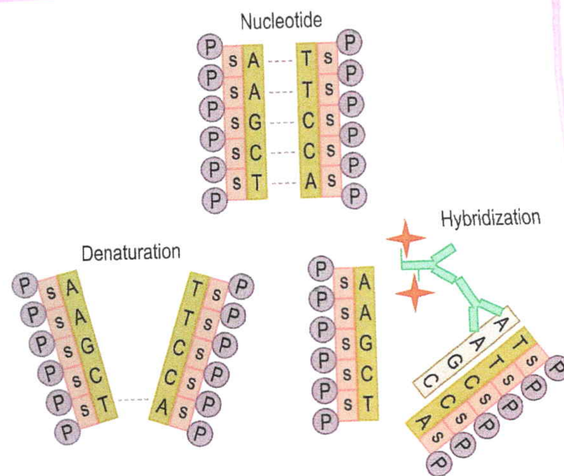

Fig. 26.1 The genetic information for humans is encoded in billions of nucleotides, the building blocks of the DNA code, arranged in a double helix molecule. Nucleotides consist of a base, a sugar (S), and a phosphate (P). The DNA code is written in an alphabet that uses four letters to represent each of the bases. (A) = adenine, (T) = thymine, (C) = cytosine, (G) = guanine. These bases will form pairs. (A) will only pair with (T). (G) will only pair with (C). Therefore, double-stranded DNA consists of two strands of homologous nucleotides. The *genetic code* in DNA is in triplets such as ATG. The base sequence of that triplet in the partner strand is therefore TAC.

ISH methods may employ radiolabeled probes that are visualized on a photographic film or photographic emulsion. However, most of these probes do not work well on routinely fixed, processed tissues, require the use of frozen sections, and take around 20–50 days' exposure before seeing results. The development of non-radiolabeled probes that perform well on routine surgical and autopsy specimens has extended the field of anatomic pathology.

Detection of mRNA is particularly useful if the protein product is quickly degraded or rapidly transported out of the target cell.

In ISH detection, immunohistochemistry (IHC)-like methods may be incorporated to detect the labeled (biotin, digoxygenin (DIG)) probe. So the question arises, why not just do IHC? It is well-established, reliable, and less time consuming than ISH. IHC has been employed in the clinical and research arenas for several decades and has become a routine procedure in the histology laboratory. IHC has pr
a close look at the prot
branes. So why do IS
IHC are:

1. high degree of speci
2. DNA and mRNA
   fixatives.
3. probe–target hybr
   antigen complex.
4. provides an alterna
   able antibodies are
5. provides a diagnosi

It is important to und
different stages in the
to result in a functio
on the 'how and why'
tissue sections.

APPLICATIONS

There are many modi
to the application nee
DNA and RNA sequ
valuable research too
(1991) and Mitchell
tically in:

- detection of abnor
- identification of vi
- tumor phenotypi

In situ hybridizatio
information on the
sequences in chrom
has been applied t
(Davis et al 1984; Lu
to be applicable to
1988; Poddighe et
preparations of tiss
cyto-genetics', as t
direct information
unselected tumor c
hybridization (CISH
tion of gene express
tional histochemica
for the detection o
gene therapy treat