

They were also stained by the indirect immunoperoxidase technique for atrial natriuretic peptide.¹² Tissue for electron microscopy was embedded in LR white resin, and ultrathin sections were stained by indirect immunocytochemistry for atrial natriuretic peptide, using goat antirabbit immunoglobulin conjugated to 15 nm colloidal gold (Janssen Pharmaceutical) as the second antibody.⁵ The antibodies to atrial natriuretic peptide were raised in rabbits using synthetic atrial natriuretic peptide conjugated to bovine thyroglobulin as the immunogen.

Results

Ultrastructural examination of the atrial tissue showed the presence of fine deposits of amyloid fibrils 7–10 nm in diameter around small blood vessels and adjacent to muscle cells. The amyloid fibrils clearly and consistently labelled for immunoreactive atrial natriuretic peptide (Fig. 1a), whereas background labelling of the surrounding connective tissue was negligible. The presence of atrial natriuretic peptide was also shown in neurosecretory granules within atrial muscle cells (Fig. 1b). Immunoperoxidase staining for atrial natriuretic peptide by light microscopy was positive in atrial muscle cells and focally positive around small blood vessels. Reaction with preabsorbed antiatrial natriuretic peptide was consistently negative. The amyloid was not detected by light microscopy, and the Congo red stain was negative.

Discussion

Amyloid has been reported in various endocrine tumours, including medullary carcinomas of the thyroid, carcinoids, pheochromocytomas, carotid body tumours, insulinomas, and gastrinomas.¹³ Endocrine associated amyloid differs from other types in that histochemical staining for tryptophan is negative. Moreover, in some cases the constituent protein of endocrine associated amyloid has been shown to be related to the native hormone product.^{3,5}

Studies on the cardiac amyloid associated with aging have subdivided it into two types, which differ both in chemical composition and distribution.¹⁴ Isolated atrial amyloid is similar to endocrine associated amyloid in that it lacks histochemically demonstrable tryptophan, whereas senile cardiac amyloid found in both atria and ventricles contains readily demonstrable tryptophan.

In this report we describe the presence of immunoreactive human atrial natriuretic

peptide in atrial amyloid fibrils, which can be seen on electron microscopy, and have also confirmed its presence within neurosecretory granules in atrial cardiocytes.¹⁵ These findings support the view that some atrial amyloid is of an endocrine type and suggest that atrial natriuretic peptide, or an atrial natriuretic related peptide, may be its primary constituent.

The amyloid was only visualised ultrastructurally and was not evident on light microscopy. This phenomenon is not uncommon if deposits are small.¹⁶ In this case it was therefore impossible to confirm the ultrastructural observations at light microscopy level, although there was a little perivascular positivity for atrial natriuretic peptide on indirect immunoperoxidase staining. As deposits of isolated atrial amyloid increase with age and the patient studied was only 64 years old, it may be possible to show amyloid atrial natriuretic peptide on light microscopy more readily in older patients. Further studies are currently being undertaken.

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References

- Skinner M, Cohen AS. Amyloidosis: clinical, pathologic and biochemical characteristics. In: Wagner BM, Fleischmajer R, Kaufman N, eds. *Connective tissue diseases*. Baltimore: Williams and Wilkins, 1983:97–119.
- Kisilevsky R. Amyloidosis; a familial problem in the light of current pathologic developments. *Lab Invest* 1983;**49**:381–90.
- Slatten K, Westermark P, Natvig JB. Characterisation of amyloid fibril protein from medullary carcinoma of the thyroid. *J Exp Med* 1976;**143**:993–8.
- Dammwich J, Ormanns W, Schaffer K. Electron microscopic demonstration of calcitonin in human medullary carcinoma of the thyroid by the immunogold staining method. *Histochemistry* 1984;**81**:369–72.
- Butler MG, Khan S. Immunoreactive calcitonin in amyloid fibrils of medullary carcinoma of

the thyroid: an immunogold technique. *Arch Pathol Lab Med* (in press).

- Pomerance A. Senile cardiac amyloidosis. *Br Heart J* 1965;**27**:711–19.
- Cornwell GB III, Murdoch WL, Kyle RA, Westermark P, Pitkanen P. Frequency and distribution of senile cardiovascular amyloid. *Am J Med* 1983;**75**:618–23.
- Cantin M, Genest J. The heart and atrial natriuretic factor. *Endocrine Reviews* 1985;**6**:107–27.
- Maack T, Camargo MJF, Kleinert HD, Laraga JH, Atlas SA. Atrial natriuretic factor: structure and functional properties. *Kidney Int* 1985;**27**:607–15.
- McLean I, Nakane PK. Periodate-lysine-parmaldehyde: a new fixative for immunocytochemistry. *J Histochem Cytochem* 1974;**22**:1077–83.
- Pomerance A, Slavin G, McWatt JJ. Experience with the sodium sulphate-alcian blue stain for amyloid in cardiac pathology. *J Clin Pathol* 1976;**29**:22–263.
- Burns J. Immunohistological methods and their application in the routine laboratory. In: Anthony PP, Woolf N, eds. *Recent advances in histopathology*. Edinburgh: Churchill Livingstone, 1978:337–50.
- Pearse AGE, Ewen SWB, Polak JM. The genesis of apudamyloid in endocrine polypeptide tumours: histochemical distinction from immunocytochemical amyloid. *Virchows Arch (Pathol Anat)* 1972;**10**:93–107.
- Westermark P, Johansson B, Natvig JB. Senile cardiac amyloidosis: evidence of two different amyloid substances in the ageing heart. *Scand J Immunol* 1979;**10**:303–8.
- Chapeau C, Gutkowska J, Schiller PW, Milne RW, Thibault G, Garcia R, Genest J, Cantin M. Localisation of immunoreactive synthetic atrial natriuretic factor (ANF) in the heart of various animal species. *J Histochem Cytochem* 1985;**33**:541–50.
- Pomerance A. Infiltrations and storage diseases. In: Pomerance A, Davies MJ, eds. *The pathology of the heart*. Oxford: Blackwell Scientific Publications, 1975:256.

Manual or radiometric method for detection of bacteraemia

We read with interest the paper by Ganguly *et al*¹ that a manual rather than a radiometric method was more rapid for the detection of bacteraemia. In this laboratory we compared the radiometric technique with the standard method of blood culture examination.² No difference between the two techniques was found. The isolation rate was 4% for each method when 5216 blood cultures were examined by the manual

method and 4503 by the radiometric technique. Indeed, the radiometric method, being less cumbersome, allowed twice daily examinations where appropriate. The species distribution of 333 isolates detected by the manual method during 1976-83 were similar to the 173 isolates detected by the radiometric method during 1984-85. The proportionate distribution of the isolates were *Escherichia Coli* 25%, *Staphylococcus aureus* 15%, *Proteus* sp 11%, *Streptococcus pneumoniae* 10%, *Staph albus* 10%, *Streptococcus* sp 7%, *Haemophilus influenzae* 6%, *Bacteroides* sp 3%, and others 12%. The antibiogram of the strains isolated since 1980 showed that 96.4% of the strains were sensitive to augmentin, 89.4% to cefuroxime, 83.4% to cephadrine, 82.4% to co-trimoxazole, and 57.5% to amoxycillin. One out of nine *Pseudomonas* sp isolates was resistant to gentamicin. The remaining strains were sensitive to aminoglycosides and uridiopencillin.

Isolates by both techniques were obtained from patients suffering from diseases of similar clinical spectra. They included 28% with fever of unknown origin, 19% with septicæmia, 9% with gastrointestinal aetiology, 12% with bronchopneumonia, 8% with meningitis, 6% with urinary tract infections, and 23% with other diagnoses. Bacteraemia occurred at all ages, being predominant at the two extremes of life (< 1 yr: 10%, and > 60 yrs: 35%). On the whole, there was no prominent seasonal variation in isolation rates. *Strep pneumoniae* isolation, however, showed a peak during November to March and a trough in June and July. Similarly, *E Coli* isolates peaked during July to September.

Blind subculture of blood cultures is time consuming, tedious, and increases the chances of contamination. The results obtained by the Bactec system were no different from the manual technique in rate of isolation. Even when seasonal variation, agent, and host factors were compared, isolation rates remained more or less identical. The manual system allowed us to increase our throughput by at least 58% without any increase in technical time.

The epidemiological analysis is of value for advice on blind treatment. It was also interesting to note that most of the strains were sensitive to cheap and currently used antibiotics.

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References

- ¹ Ganguli Leela A, Keaney Maeve GL, Hyde WA, Fraser Susan B. More rapid identification of bacteraemia by manual rather than radiometric method. *J Clin Pathol* 1985; **38**:1146-9.
- ² Gould JC, Duerden BI. Blood culture—current state and future prospects. *J Clin Pathol* 1983;**36**:963-77.

Donor Screening for Anti-HBs

Although we support English attempts to introduce large scale donor screening for antibody to hepatitis B surface antigen (anti-HBs), we feel that Lake *et al*¹ are unfair to radioimmunoassay methods.

We have been screening on a large scale for several years, and our current method is a modification of a solid phase inhibition radioimmunoassay,² standardised to detect plasma with anti-HBs content > 10 IU/ml. The labelled anti-HBs is recycled from our routine hepatitis B surface antigen testing, and the cost per test is roughly 3 pence. For daily screening overnight incubation is not possible or necessary.

Specific advantages are: that no sample dilution is required; the method suits existing radioimmunoassay technology; and an objective result is easily processed by computer. When a strong antibody is identified its content is estimated by a modification of the Bureau of Biologics Method, which gives the accuracy and precision required to ensure suitable material is forwarded for fractionation. Using the above strategy for random screening in conjunction with a plasmapheresis programme, 96 kg of suitable plasma was obtained in the year 1984-85.

Finally, we do not support the use of a modified haemagglutination method for clinical purposes, unless it is capable of detecting antibody values of 0.01 IU/ml—a value obtained by radioimmunoassay methods.

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References

- ¹ Lake SM, Puckett AY, Entwistle CC. Donor screening for antibody to hepatitis B surface antigen. *J Clin Pathol* 1985;**38**:1195-6.
- ² Hopkins R, Ross S, Barr A, McClelland DBL. Selection of plasma for hepatitis B immune globulin using a low cost radioimmunoassay. *Vox Sang* 1981;**40**:6-11.

Book reviews

Tumor Markers in Cancer Control. Ed HE Nieburgs, JH Holzner, VE Valli. (Pp 360; £52.) Alan R Liss Inc. 1985.

This ambitiously titled volume comprises the proceedings of the second international conference on tumour markers. Like its predecessor published two years ago, most of the contributions relate to one or more monoclonal antibodies raised against tumour associated proteins, none of which is in any way specific for cancer. It is perhaps important that carcinoembryonic antigen continues to figure so prominently in a book of this kind, still seeking recognition as a tumour "marker" some 20 years after its discovery. It is, however, pleasing to see a trend against this pattern, with more chapters on biochemical and chromosomal changes in cancer. Pathologists working with tumours will find this a useful book, but at £52, which represents a 20% increase in price, they might be best advised to request their library to buy it.

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Tumors and Tumorlike Conditions of the Ovary. Contemporary Issues in Surgical Pathology. Ed LM Roth, B Czernobilsky. (Pp 296; £57.) Churchill Livingstone. 1985.

The stated aim of this book, one of a north American series of texts on surgical pathology, is "to present a state of the art assessment of ovarian cancer for the practising surgical pathologist and gynaecologist." Solid workman like chapters on the various types of primary ovarian neoplasms, secondary ovarian tumours and tumour like conditions are followed by chapters devoted to aspiration cytology, ultrastructure, immunochemistry, and steroid hormone receptors. There is, unusually but refreshingly for a surgical pathology book, an excellent chapter on the treatment of ovarian cancer.

Sound and accurate throughout, the book is lifted well above the norm by a scholarly, though highly selective, historical survey of ovarian pathology. It is fascinating to learn that the Stein-Leventhal syndrome was actually reported by Irving Freiler Stein and Michael Lee Levanthal and that Fritz Brenner spent most of his life as a general practitioner in South Africa, completely unaware that there was such an entity as a Brenner tumour.