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Staining of neuroendocrine cells by Linder's argyrophil method

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INTRODUCTION

Argyrophil techniques are currently used in the histochemical study of neuroendocrine (APUD) cells because of their property of impregnating amine- and/or peptide-containing secretory granules. The pioneer of these techniques was, at the beginning of this century, the Golgi 'black reaction' capable of staining nervous structures. The techniques utilise a silver salt (nitrate or proteinate) as the impregnating substance, followed by various reducing agents as 'developers'. Grimelius' technique (1968) produces widely diffused positive cells and its mechanism of action seems to be closely related to the presence of some acidic proteins in the secretory granules known as chromogranins (Lloyd *et al.* 1984; Rindi *et al.* 1986).

The extension to neuroendocrine cells of an argyrophil technique, recently developed by Linder (1978) to stain nerves, is presented here.

MATERIALS AND METHODS

Samples from the proventriculus, duodenum and rectum of the Japanese quail, the stomach, pylorus, duodenum and colon of the rabbit, and the abomasum, pylorus, duodenum, colon and urethra of the sheep were fixed both in Bouin's fluid and in 4% formaldehyde in phosphate buffer (0·1 M, pH 7·3). Samples from the pancreas and urethra of rabbits and from hyperplastic non-neoplastic prostate of human subjects were fixed in Bouin's fluid. The material was routinely embedded in Paraplast and serially cut at 5–6 μm. Sections were stained by the argyrophil techniques of Linder (1978) and Grimelius (1968) and by the argentaffin method of Masson–Hamperl (modified by Singh, 1964). Sometimes the argyrophil staining was bleached by potassium cyanide, 0·3%, and the argentaffin staining by sodium thiosulphate, 1%, in order to restain single sections by different techniques. The sections were photographed before bleaching and after restaining.

Some samples from the rectum of quails, the stomach of rabbits and the human prostate were double-fixed in glutaraldehyde and osmium tetroxide (2·5% and 2·0% respectively in cacodylate buffer 0·1 M, pH 7·3), dehydrated and embedded in Epon 812. Semithin sections (1 μm) were stained by Linder's method after resin removal in sodium methoxide (Mayor, Hampton & Rosario, 1961). Consecutive thin sections were cut, routinely stained and observed in a Philips EM 201 to obtain ultrastructural features of the argyrophil cells.

The brief variant of Linder's method was always used (for technical details see Linder, 1978) omitting the celloidin step and the subsequent wash in tap water.

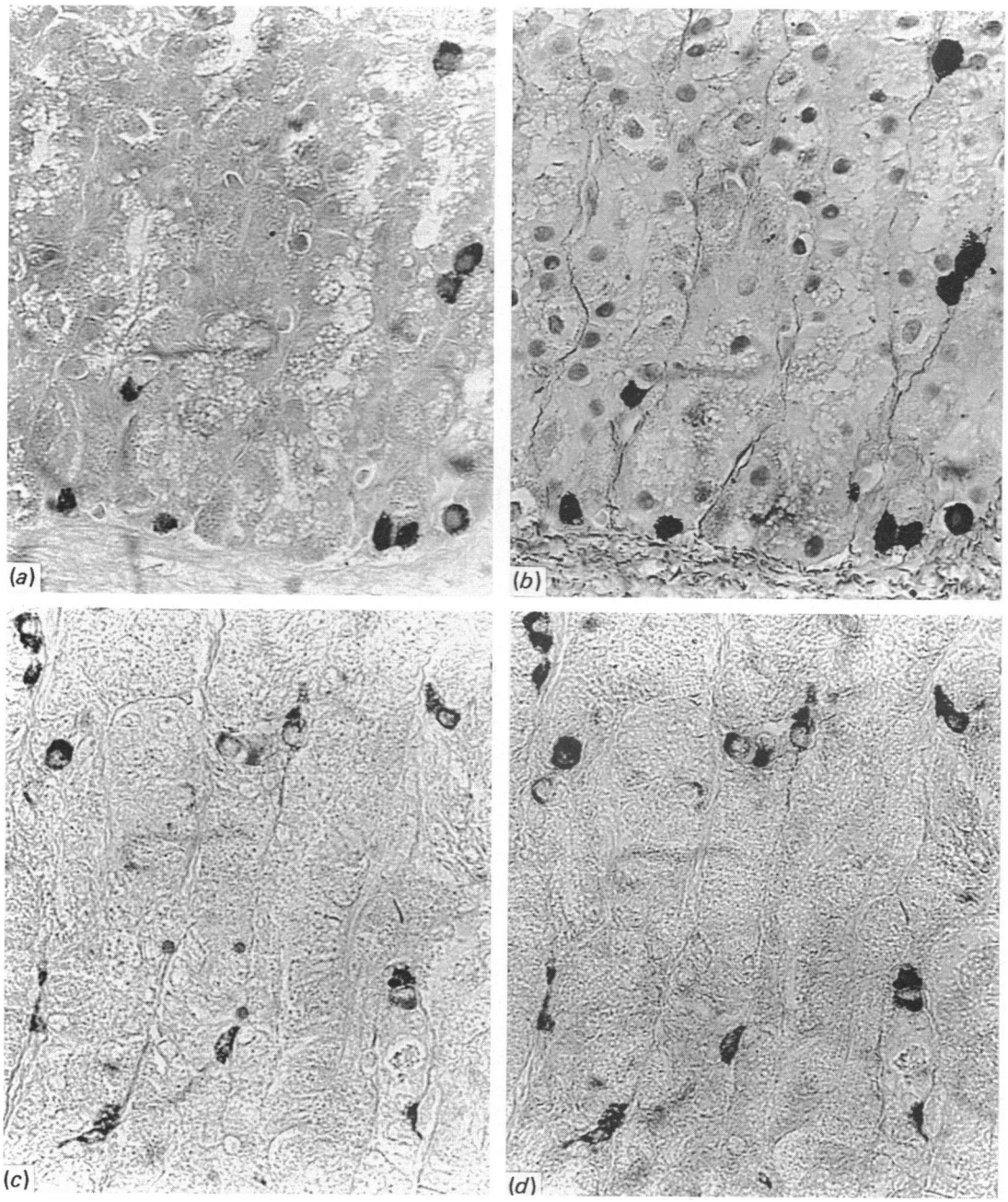

Fig. 1 (*a–d*). Two staining sequences on single paraplast sections of rabbit gastric mucosa. (*a*, *b*) Masson–Hamperl, Linder. (*c*, *d*) Grimelius, Linder. ×450.

RESULTS

Paraplast sections

Sections from all organs examined showed Linder-positive cells with the exception of those from formaldehyde-fixed quail proventriculus. Positively stained cells were much more numerous in sections from Bouin-fixed material. Two or three cell types

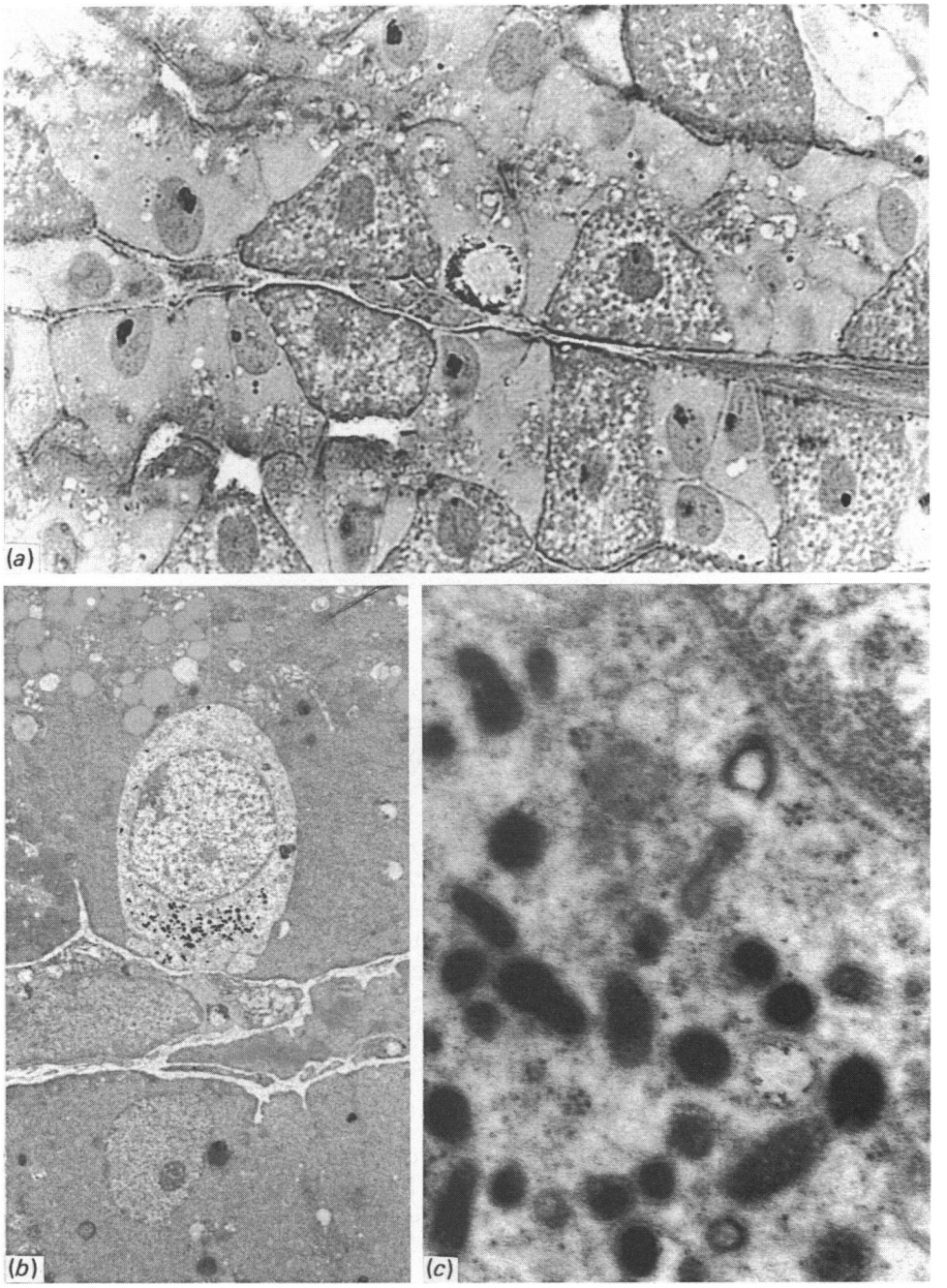

Fig. 2 (*a–c*). Consecutive Epon semithin (*a*), thin (*b*, *c*) sections of rabbit stomach showing a Linder-positive endocrine cell (*a*) × 1280, distribution and morphology of its secretory granules (*b*) × 3700 (*c*) × 50000; uranyl acetate, lead citrate.

were recognisable in the same tissue, after Bouin fixation, on the basis of their size, location and the staining properties of the cytoplasm (yellow, red-brownish, black). The development time of Linder's impregnation was found to be slightly longer for nerves than for neuroendocrine cells.

Moreover, samples from the gastric mucosa of the rabbit were employed to study the effect of formaldehyde fixation on the staining of neuroendocrine cells by Linder's method and to compare this technique with that of Grimelius. In restained sections of formaldehyde-fixed material Linder's method showed, with rare exceptions, only Masson–Hamperl-positive endocrine cells (Fig. 1*a*, *b*). Accurate examinations of many microphotographs of restained sections from Bouin-fixed mucosa led us to conclude that Grimelius and Linder results were comparable (Fig. 1*c*, *d*). However, Linder-positive cells were generally more heavily impregnated.

Epon sections

The staining of neuroendocrine cells on deplasticised sections resulted in rapid and optimal results for the human prostate and quail rectum. In these species the impregnation (~20 min) and development (~3–5 min) times were short, and good contrast was obtained between black cytoplasmic granules and pale yellow background.

It was always possible to obtain consecutive thin sections which, routinely treated, showed the ultrastructural features of argyrophil cells (Fig. 2*a–c*).

DISCUSSION AND CONCLUSIONS

Linder's method differs from other argyrophil techniques used in the study of neuroendocrine cells mainly in the use of silver cyanate as impregnating salt instead of the usual nitrate or proteinate. In our opinion silver cyanate has a good affinity for neuroendrocrine cells resulting in strongly impregnated cytoplasmic granules. It was even possible to stain intensely neuroendocrine cells that had been embedded in paraffin wax for four years.

Suitably performed, Linder's method can stain both nervous structures and endocrine cells. Its use is thus suggested for the study of the contacts between the two systems, since 'fine terminal fibrils' are also clearly impregnated (Linder, 1978).

Bouin fixation is much more suitable than formaldehyde for staining neuroendocrine cells by Linder's technique. Formaldehyde fixation of rabbit gastric mucosa allowed us to stain only argentaffin Masson–Hamperl-positive cells, and these are less numerous than the argyrophil cells evident in sections from Bouin-fixed mucosa. Moreover, formaldehyde enhanced the staining of connective tissue fibres and chromatin, thus reducing the contrast between the background and positive cells.

The time required by the short variant of Linder's method to obtain optimal staining of neuroendocrine cells ranged from 35 to 75 minutes; this compares well with the argyrophil methods currently being used. Its stock solutions remain stable for several months. Linder's method is more effective and consistent than Grimelius' on tissues from different taxonomic classes.

In conclusion, Linder's argyrophil method can be suitably applied to the study of the neuroendocrine system for the following reasons.

(1) It is rapidly performed.

(2) It impregnates intensely both nerve fibres and neuroendocrine cells.

(3) It demonstrates (as does Grimelius' technique) different types of neuroendocrine cells.

(4) It gives reproducible results on tissues from different classes of animals.

(5) It is particularly useful in routine staining because its stock solutions are stable for a long time.

SUMMARY

Linder's argyrophil method, recently developed to stain nervous structures, is useful in the histochemical study of amine- and/or peptide-producing neuroendocrine (APUD) cells. On sections from various organs of four animal species Linder's method worked well and rapidly stained the neuroendocrine cells yellow, red or black; it stained black nervous structures against a pale yellow background. Double staining of single sections from Bouin-fixed gastric mucosa of rabbits demonstrated the correspondence of both Linder- and Grimelius-positive cells. Rapidity of application, intensity of impregnation and reproducibility in results are the best features of Linder's method when applied to the study of the neuroendocrine system.

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