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An ultrastructural morphometric analysis on ultrathin epon and ultrathin cryosections of normal human gastric tissue and human gastric cancer

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Summary. An ultrastructural morphometric study on ultrathin epon, and cryosections of normal and neoplastic, adult, human, gastric tissue is presented. The data show a characteristic numerical pattern for the different neoplastic types of gastric tissues.

Key words: Gastric carcinoma staging – Electron microscopy – Ultrathin cryosections – Morphometric investigations

The ultrastructure of gastric tumours has often been described qualitatively (Lillibridge 1964; Yamashiro and Sazuki 1977; Nevalainen and Järvi 1976; Helander 1964; Yeonaus 1974; Ito 1967). Through the description of variations in subcellular patterns between normal and tumour cells on the one hand, and between different types of tumour cells on the other, helpful quantitative techniques can be developed. One such simple method is morphometry with "scanning screens", especially in conjunction with an intelligent data comparison system for rapid processing (Wolf 1983a, b; Wolf and Schimassek 1983). By applying this technique to the analysis of electron micrographs of different normal and tumour tissues, subcellular variables can be reliably registered in a numerical manner, thus allowing analytical comparisons (Wolf 1984a, b).

In the present paper, we have compared electron micrographs of tumour cells from different gastric carcinomas (WHO No. 18), with the mucoid neck cells of the normal gastric mucosa, analytically. Carcinomas usually originate from undifferentiated cells with a high mitotic rate (Elster 1970; Laurén 1965). Cells showing a high mitotic rate in the normal stomach mucosa are generally found in the mucoid neck area, where the mucoid neck cells predominate. The main function of these cells is the regeneration of other stomach mucosal cells. Therefore, one must assume that these mucoid neck cells represent a relatively undifferentiated precursor cell type.

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This means that they show all the necessary characteristics to qualify as forerunners of the various types of transformed stomach cells. For this reason, these cells have been used for morphometric comparisons with the various types of stomach carcinoma cells.

The data correlate so well with the established classification, that the development of a corresponding diagnostic programme appears feasible. For this purpose, ultrathin cryosections are of particular value because of their rapidity in preparation and examination.

Material and methods

For this study, surgically gained material from 5 normal and 10 neoplastic tissues was investigated. The samples were classified in the Pathology Institute of the University. For the Epon-technique, tissues were fixed in cold 2.5% glutaraldehyde in 150 mM cacodylate buffer at pH 7.4 followed by post fixation in 2% osmium tetroxide, dehydration and embedding, according to Luft (1961). The samples were sectioned on an ultramicrotome with a thickness of 40–50 nm (Hama 1982).

In case of the cryo-technique, tissues were fixed in cold 2.5% glutaraldehyde in 100 mM phosphate buffer at pH 7.4, washed in the same buffer and incubated for cryo-protection in 1.1 M saccharose in 100 mM phosphate buffer, pH 7.4. The following specimen preparation was carried out after published methods by Tokuyasu (1980). By using a self-constructed apparatus for rapid freezing of surgically gained samples, the formation of ice crystals at the time of freezing was prevented (Wolf 1984c). Frozen samples were sectioned according to Sitte (1967, 1982). Light microscopical analysis of semithin sections from epon embedded samples and cryostat sections (see Wolf 1985), allowed localization of the areas of interest prior to ultrathin sectioning. Ultrathin sections were examined with a Zeiss EM 109 R, or, Siemens E 102, where an "on-line" morphometric apparatus was applied (Wolf 1983a).

Morphometric investigations were carried out using a newly, self-developed, interactive and intelligent morphometric system (DBP. 3121727.3) for cytological and histological studies on biological samples (Wolf 1983a, 1984b). The theoretical background was according to Weibel and Haug (Weibel and Paumgartner 1978; Weibel 1969; Haug 1980). The applied mode of data sampling and the method of classification has already been described in detail elsewhere (Wolf 1984a, b, 1985). In brief, the relevant cells (between 51 and 85 cells per sample, i.e. mucoid neck cells in normal tissues and various stages of tumour cells) were analysed morphometrically according to Wolf (1983). The mean values of the volume to surface area relationships of cellular and subcellular components within a given sample were determined. The class specific data shown in Table 1 were obtained from the mean values for different samples of the same cell type.

The statistical calculations as well as the selection of representative cells were carried out automatically by an interactive dialogue system (Wolf 1984b).

Results

The figures show representative cells and tissues of normal mucosa as well as various types of neoplasia of the stomach, as determined with the morphometric analysis data, classified by computer (cf. Figs. 1–5). Semithin and ultrathin sections, and in some cases cryo-sections, from the same region are grouped together. The corresponding morphometric data are shown in Table 1. The morphometric ratios presented allow a differentiation between the given tumour types after previous selection of the corresponding semithin sections for localization of the tumour (cf. Fig. 1a, 2a, 3a, 4a, 5a).

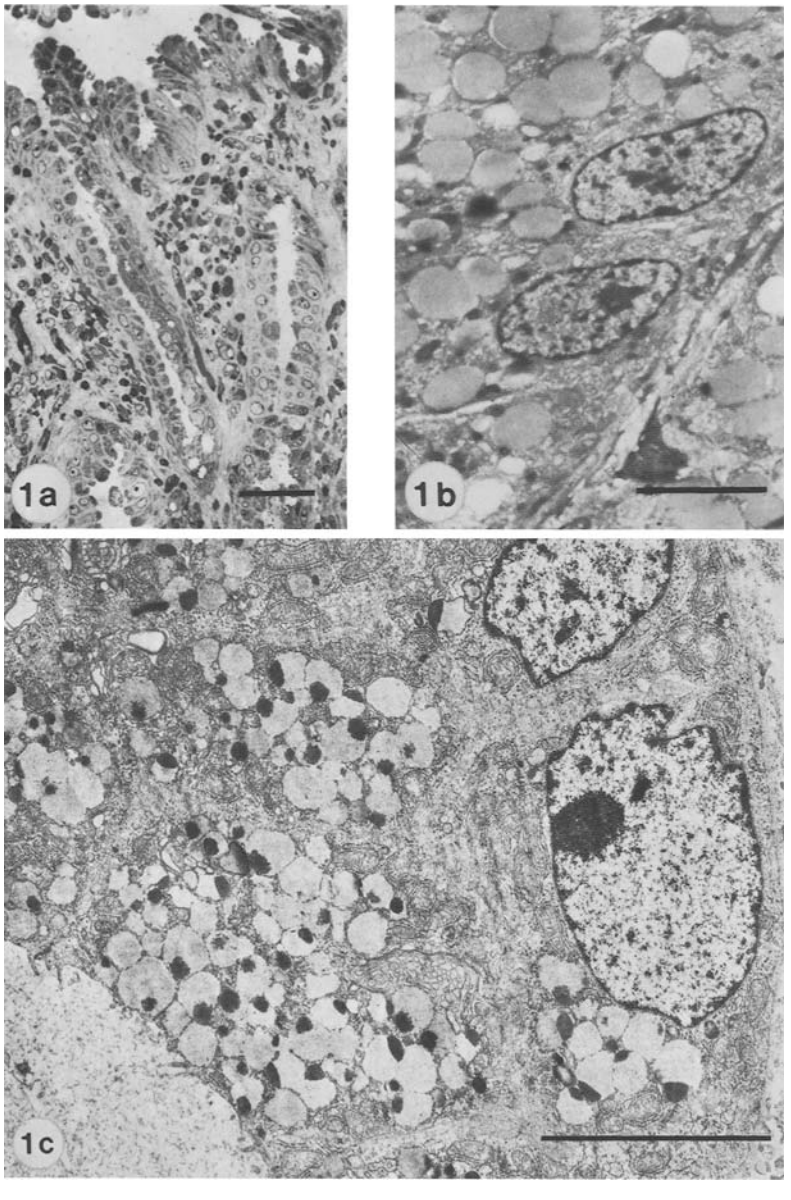

Fig. 1. a Semi-thin section of a pit and a gland of normal gastric mucosa. Bar 20 μm. **b** Ultrathin cryosection of typical mucoid neck cells. Bar 5 μm. **c** Typical electron micrograph of a normal mucoid neck cell, selected by the computer as characteristic. Bar 5 μm

In particular, the ratios volume-nucleus (V_n)/volume cell (V_c), surface area-cell (S_c)/volume-cell (V_c) and surface area-nucleus (S_n)/ volume nucleus (V_n), show remarkable differences between different tumour types and when comparing each type with the mucoid neck cells. All gastric tumour cells, with the exception of the signet ring cell carcinomas, reveal significantly

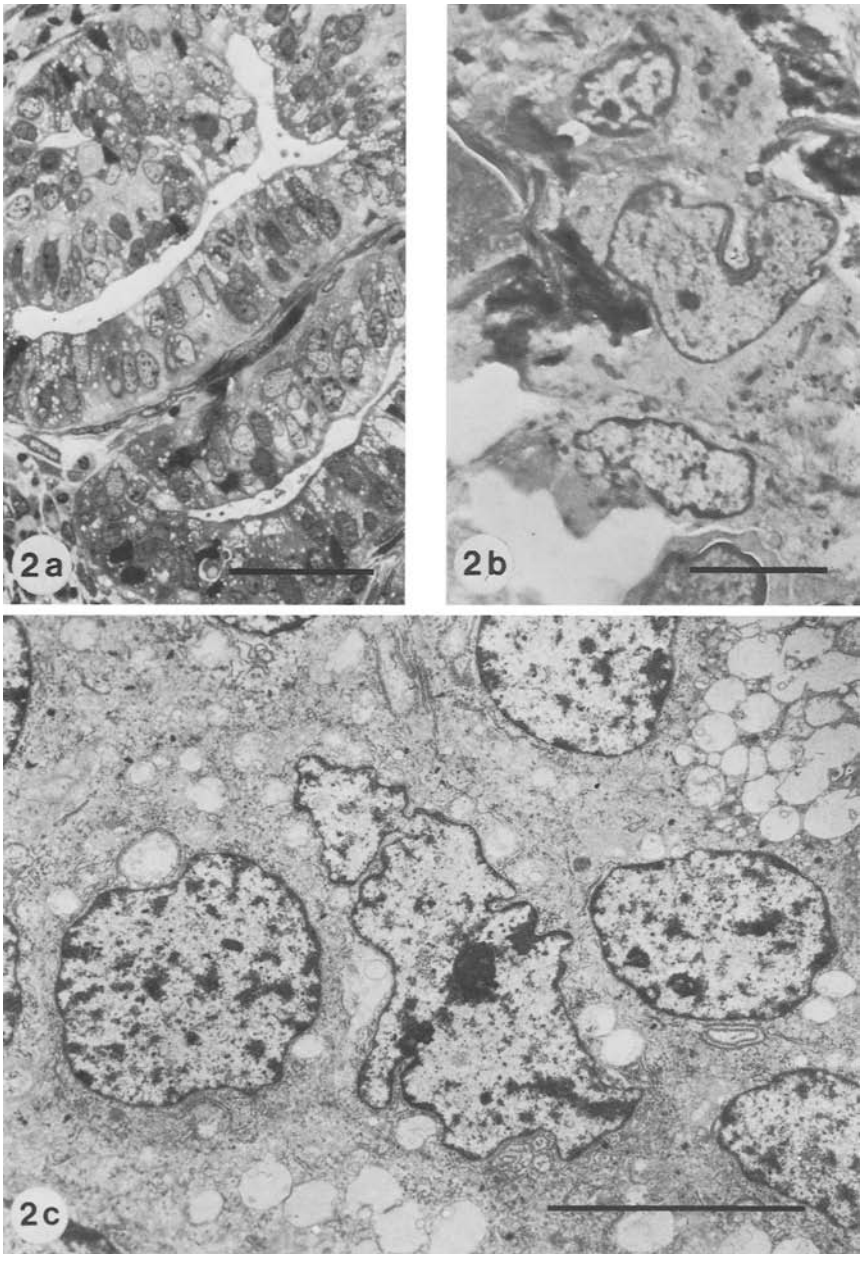

Fig. 2. a Semi-thin section of a tubular adenocarcinoma (G2). Bar 20 μm. **b** Ultra-thin cryosection of the same tumour. Bar 5 μm. **c** Electron micrograph of a typical tumour cell from a tubular adenocarcinoma (G2). Bar 5μm

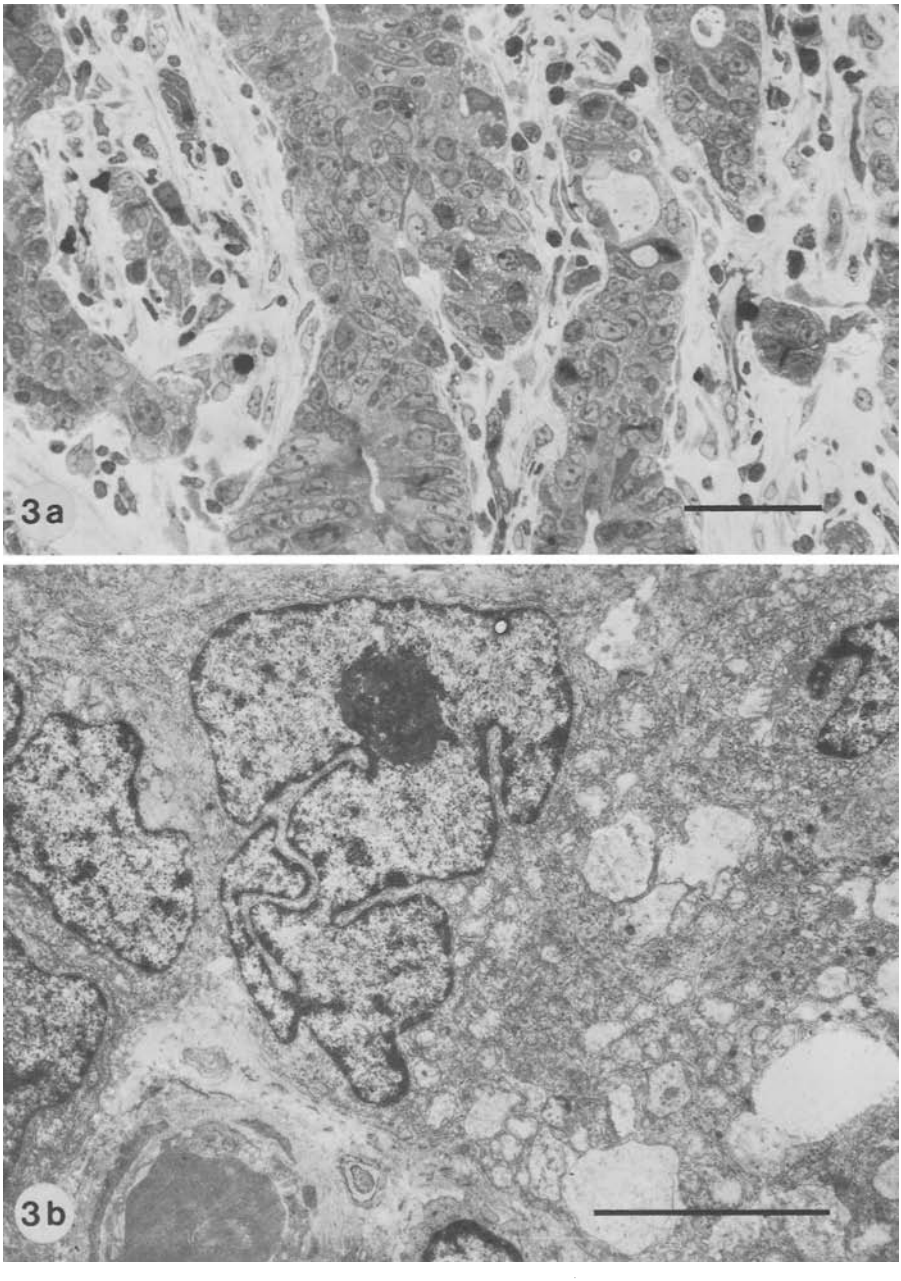

Fig. 3. **a** Semi-thin section from a tubular adenocarcinoma (G3) Bar 20 μm. **b** Electron micrograph of a typical cell of the corresponding tumour. Bar 5 μm

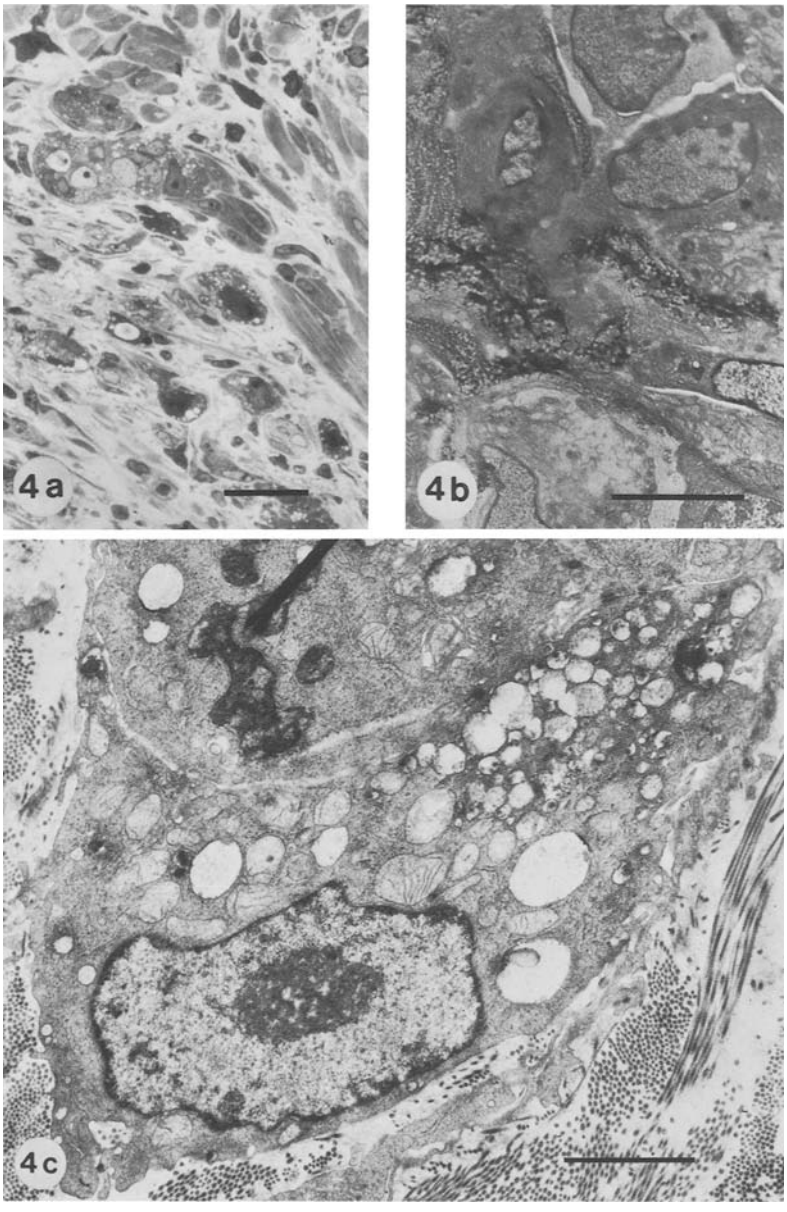

Fig. 4a–c. Reproduction of representative micrographs of an undifferentiated carcinoma selected by the computer. **a** Semi-thin epon section. Bar 20 μm; **b** ultra-thin cryosection. Bar 5 μm; **c** ultra-thin epon section. Bar 2 μm

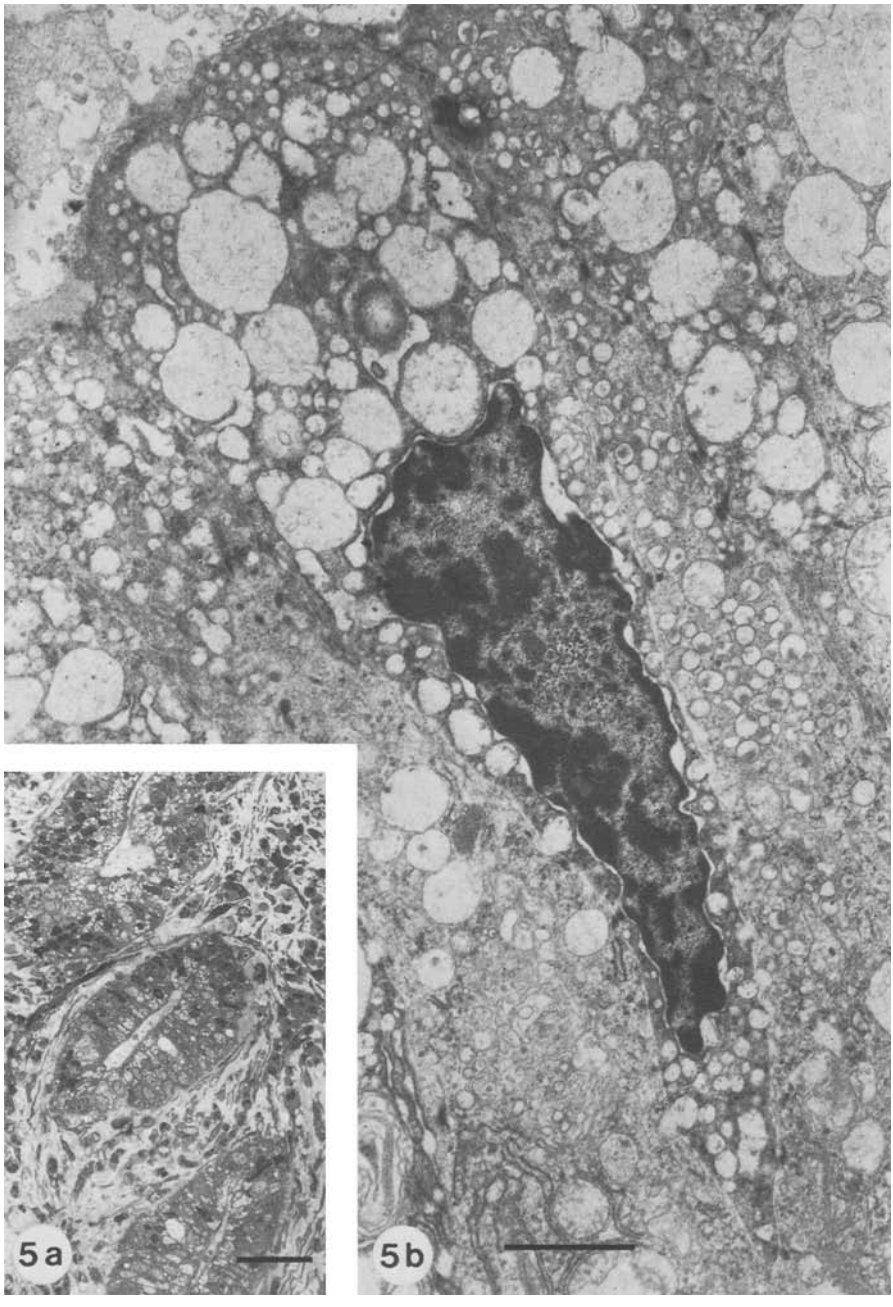

Fig. 5. a Semi-thin section of gastric carcinoma containing signet ring cells. Bar 20 μm. **b** Electron micrograph of a typical signet ring cell. Bar 2 μm

Table 1. Ratios of cell parameters for different types of gastric carcinoma and mucoid neck cells

WHO classification	Laurén classification	No. of patients	V_n/V_c	V_m/V_c	$V_{r\text{-}ER}/V_c$	V_s/V_c	S_c/V_c ($\mu m^2/\mu m^3$)	S_n/V_n ($\mu m^2/\mu m^3$)
Mucoid neck cell	–	5	0.22 ±0.028	0.12 ±0.03	0.12 ±0.04	0.28 ±0.11	0.79 ±0.07	1.01 ±0.06
Tubular adenocarcinoma G2	Intestinal type	3	0.51 ±0.06	0.07 ±0.029	0.047 ±0.024	0.053 ±0.049	0.98 ±0.12	1.20 ±0.12
Tubular adenocarcinoma G3	Intestinal type	3	0.58 ±0.07	0.12 ±0.04	0.087 ±0.035	0.061 ±0.077	0.74 ±0.10	1.36 ±0.12
Total of intestinal types G2+G3	Intestinal type	6	0.54 ±0.07	0.095 ±0.038	0.067 ±0.030	0.057 ±0.063	0.85 ±0.11	1.28 ±0.13
Undifferentiated carcinoma	Diffuse type	4	0.40 ±0.08	0.083 ±0.031	0.034 ±0.022	0.057 ±0.48	0.54 ±0.07	0.94 ±0.14
Signet ring cell-carcinoma	–	3	0.26 ±0.08	0.032 ±0.025	0.045 ±0.030	0.37 ±0.10	0.73 ±0.13	1.75 ±0.36

V_n = Volume-nucleus, V_c = Volume-cell, V_m = Volume-mitochondria, V_{r-ER} = Volume-rough endoplasmic reticulum, V_s = Volume-secretion granules, S_c = Surface area-cell, S_n = Surface area-nucleus

larger nuclei in relation to the cell volume (V_n/V_c) than the mucoid neck cells. S_c/V_c show a shift in favour of the cell volume for the cells of the diffuse type when compared with mucoid neck cells, which is in contrast to the cells of the intestinal type (especially G 2). This result may be explained by the fact that the cells of the intestinal type carcinomas, as well as the mucoid neck cells, show a higher degree of differentiation i.e. more microvilli than diffuse type carcinoma cells. However, cells of diffuse type carcinomas are mainly found in isolated, small, cell clusters with a negligible, or totally lacking, microvilli border (cf. Fig. 4a, c).

When considering S_n/V_n, it is obvious that the nuclear surface area is increased in the intestinal type and signet ring cell carcinomas. For this reason, scalloped nuclei are more prevalent and are more distinct in the former than in the mucoid neck cells (cf. Fig. 2c, 2b, 3b, 5b). The cells of the diffuse type carcinoma normally possess rounded nuclei, which are comparable in all respects to these of the mucoid neck cells (cf. Fig. 1c, 1b, 4c). Therefore, in this latter case, variations in the surface area relationships have not been found.

The ratios; V_{r-ER}/V_c, mitochondrial volume $(V_m)/V_c$, as well as secretion granule volume $(V_s)/V_c$, show lower values for all stomach neoplastic cell types than for the mucoid neck cells, with the exception of the signet ring cells which possess more slime than the mucoid neck cells. Obviously, the obtained structural relationships reflect the chosen classifications very well. These findings have been gained with epon-embedded material. When comparing cryosections of stomach carcinomas or normal stomach mucosa with

epon embedded sections from the same source (cf. 1b, 1c, 2b, 2c, 4b, 4c), it becomes obvious that the calculation of some parameters, eg. V_{r-ER}, V_m, V_s, is only possible on epon sections. This is because mitochondria, r-ER and some other subcellular structures are difficult to recognize on cryosections. Morphometry on cryosections is therefore limited to calculating V_c, S_c and V_n.

However, as Table 1 shows, these three parameters are sufficient for an adequate classification of the different stomach carcinoma types. It is of interest, that no important differences in the relationships V_n/V_c, S_c/V_c and S_n/V_n have been found when comparing ultrathin, epon with the corresponding cryosections (Wolf 1985). The application of the cryo-technique would thus allow a drastic shortening of the times between receiving the material to be investigated, to the time of diagnosis, from up to a week or more for the epon embedding technique, down to a few hours.

Discussion

Many qualitative findings have been published on ultrastructural investigations of normal and neoplastic gastric tissue (Lillibridge 1964; Yamashiro and Suzuki 1977; Nevalainen and Järvi 1976; Helander 1964; Yeonaus 1974; Ito 1967).

All of these investigations have yielded useful information regarding the ultrastructure of different carcinomas, but without any numerical quantification of the data being possible. In principle, it may be possible to quantify subjectively chosen criteria subsequently in the form of an evaluation scale, but specification of discrete values for each tumour type seems, in our opinion, to be more advantageous for future investigations. As the results for both ultrathin epon sections and ultrathin cryosections show, quantification of at least 3 structural relationships is easily achieved and the values of these allow a clear cut categorization into the different types of neoplasm. This means that different tumour types appear to be classifiable, and their data could be used as a reference for the categorization of future samples. The greater the number of samples processed in optimizing the programme, the more reliable the system, which in turn could favour the development of a computer aided diagnosis system (Wolf 1984b). Absolute values for volume or surface area are not taken into consideration here because variations are to be found between preparations.

A possible practical application of these results seems to be feasible for the future. Electron microscopic diagnoses of biopsies may help to clarify cases which cannot be satisfactorily diagnosed with light microscopical methods. For electron microscope investigations, samples must be deep frozen in propane and sectioned on an ultra-cryomicrotome prior to analysis in the electron microscope using an on-line morphometer. The ratios V_n/V_c, S_c/V_c and S_n/V_n could be ascertained and fed into a computer where they would be compared with the values achieved in this study. Present experiments support the feasibility of the application of such a system, although more data must be collected.

The application of ultrathin cryosections is further advantageous for such investigations, because they not only allow a rapid determination of ultrastructural variables, but also ultraimmunohistological investigations in the diagnosis of the malignancy or benignity of the biopsies. Such controls have been carried out by our group for the tumour marker, CEA (Wolf et al. 1984).

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