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Intracellular mucous inclusions

A feature of malignant cells in effusions in the serous cavities, particularly due to carcinoma of the breast

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SYNOPSIS Three cases of carcinoma of the breast are described, in which similar tumour cells were found in pleural or peritoneal fluid. The cells were characterized by the frequent presence of 'bull's-eye' vacuoles, in which a central spot was deeply stained by Giemsa, eosin or periodic acid-Schiff (PAS). In one of these it was shown by electron microscopy that the vacuoles are lined by microvilli, and that their mucinous content is condensed in the central area, presumably as a result of failure to discharge the secretion.

The identification of tumour cells in serous fluids generally depends upon a subjective judgement based on the observer's memory of normal and abnormal cell types. In a minority of cases the identification of tumour cells can be confirmed by objective tests. An example of this is the demonstration of intracellular mucin. Benign mesothelial cells and macrophages frequently develop vacuoles, producing the familiar 'signet-ring' shape, but these vacuoles do not give histochemical reactions for mucin, so that in the relevant context the staining reaction may be considered confirmatory evidence of malignancy, the primary growth usually being in the breast or gastrointestinal tract.

This paper describes a distinctive subgroup of tumour cells with secretory vacuoles in which there is a stained body at the centre of the vacuole, giving a 'bull's-eye' appearance. In the three cases described, the primary growth was in the breast. The nature of these bodies was elucidated by electron microscopy.

Methods

Pleural or peritoneal fluid was collected in 30 ml containers, each with 40 mg of EDTA as anticoagulant. Centrifuged deposit was used for making smears, by the methods of Spriggs and Boddington (1968).

For electron microscopy, the fluid was centrifuged in a conical tube so as to produce a cell pellet. The supernatant was replaced with cold glutaraldehyde 4%, phosphate-buffered to pH 7·4. The pellet was fragmented after one hour into small EM processing size pieces and allowed to fix at 4°C for a total of six hours; these were then washed with two changes of cold phosphate buffer and left overnight at 4°C.

Post-fixation with cold 1% OsO_4 phosphate buffered to pH 7·4 for two hours at 4°C was followed by dehydration in graded ethanol and embedding in Araldite Epoxy Resin.

Sections were cut on a LKB Ultrotome using glass knives, mounted on bare copper grids, double stained with 2% aqueous uranyl acetate and Reynolds lead citrate, and examined with a Philips EM 301.

Case Reports

CASE 1

E. B. (Hospital No. 152701), a 50-year-old woman, had a radical mastectomy performed for a carcinoma of the right breast, histologically of scirrhous type. This was followed by radiotherapy. A malignant pleural effusion developed one year later on the left side. Two pints were aspirated, and this furnished the sample examined. Treatment was by local instillation of Thiotepa. The patient died 14 months later. There was no necropsy.

The fluid contained numerous free mucus-secreting tumour cells, which accounted for almost all the cells present.

The mucous vacuoles were PAS-positive after

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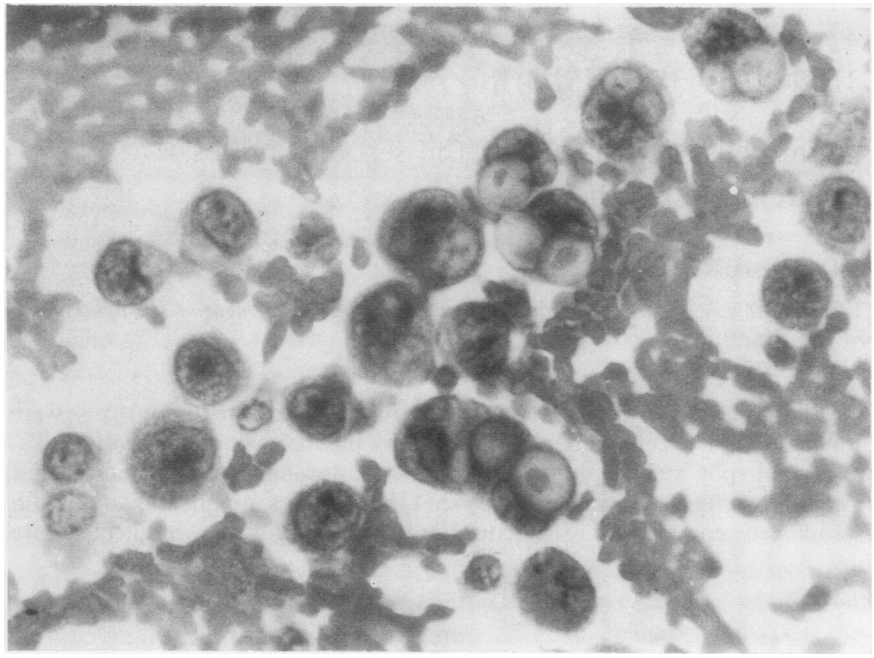

Fig 1 *Case 2 (carcinoma of breast). Malignant cells in pleural fluid deposit. Many of the cells contain one or two vacuoles, at the centre of which there is an inclusion staining with eosin. The vacuoles were PAS-positive after salivary digestion. (Papanicolaou × 1000)*

ptyalin digestion. Many of them showed a purple 'bull's-eye' with May-Grünwald-Giemsa (see fig 109 of Spriggs and Boddington (1968)); this stained orange-red in Papanicolaou preparations.

CASE 2

D. A. (Hospital No. 99827), a 66-year-old woman, had a radical mastectomy performed for a carcinoma of the left breast. Histologically this was

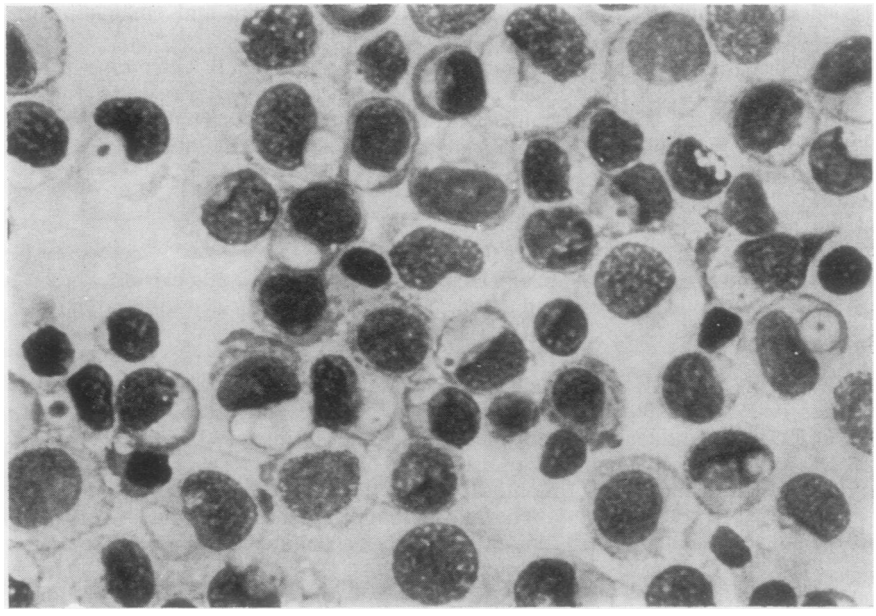

Fig 2 *Case 3 (carcinoma of breast). Air-dried deposit of peritoneal fluid. Nearly all the cells are free tumour cells, and many show a single vacuole with a purple-staining body at its centre. (May-Grünwald-Giemsa × 1000)*

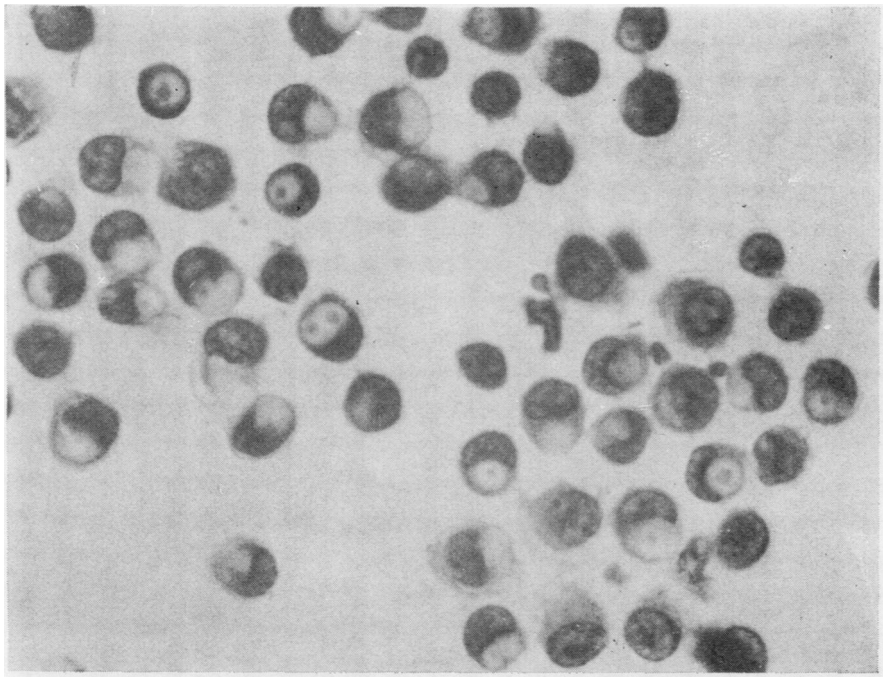

Fig 3 *Case 3. The same sample as that of fig 2, at the same magnification, but wet-fixed. Most of the tumour cells have one or two vacuoles with a central eosinophilic inclusion. (Papanicolaou × 1000)*

of scirrhous spheroidal-cell type with little tubular differentiation. Radiotherapy was given. Three and a half years later she developed a left pleural effusion and abdominal ascites, and these were both aspirated to provide the samples examined. Radioactive gold was instilled. She developed widespread bone metastases and died. No necropsy was performed.

The samples of fluid contained many red cells. Apart from a few lymphocytes, most of the nucleated cells were free mucus-secreting carcinoma cells. The mucous vacuoles often contained stainable material at the centre, which was purple with May-Grünwald-Giemsa, eosinophilic with Papanicolaou (fig 1), and PAS-positive after digestion.

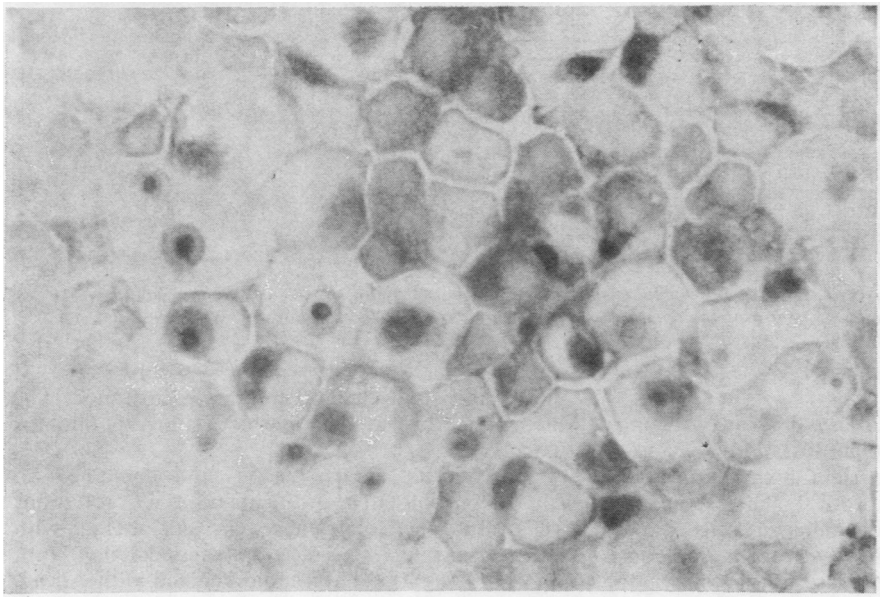

Fig 4 *Case 3. The same sample as that of figs 2 and 3, air-dried and stained with PAS. The nuclei are unstained. The PAS-positive vacuoles show a darker centre. (× 1200)*

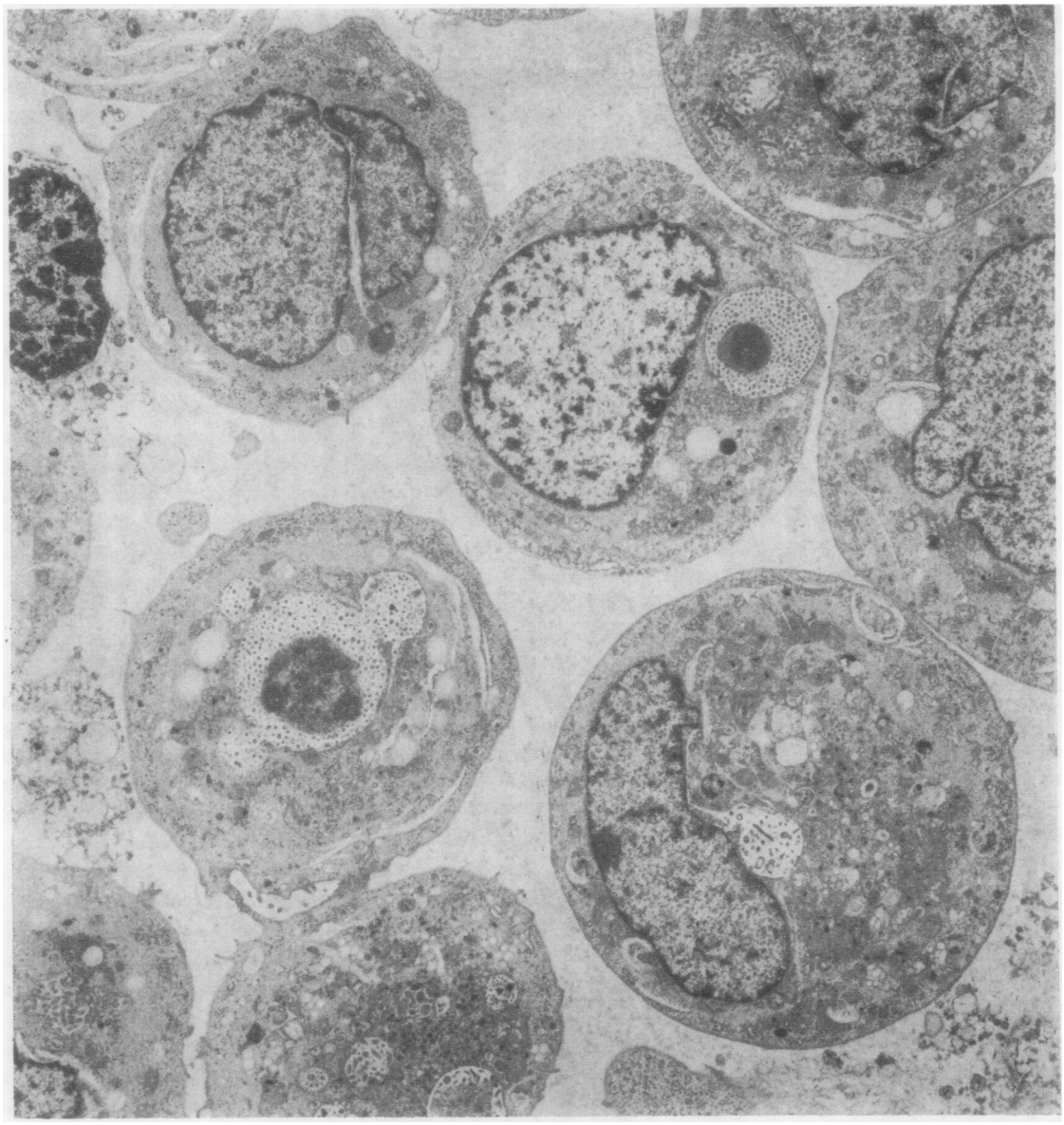

Fig 5 *Case 3. Free tumour cells, showing intracellular spaces lined with microvilli, which are mainly seen in transverse section. In two cells the centre of the vacuole is occupied by variably electron-dense structureless material. (EM × 3955)*

CASE 3

D. W. (Hospital No. 54201), a 58-year-old woman, presented with ascites, weight loss, and dyspnoea. A lump was found in the right breast. Cytological examination of the peritoneal fluid showed an almost pure population of malignant cells, showing mucus secretion. Since these were of a type consistent with a primary in the breast, biopsy was not undertaken and chemotherapy was instituted. The patient is alive at the time of writing, 11 months later.

The deposit of the ascitic fluid contained an almost pure population of mucus-secreting tumour cells. 'Bull's-eye' inclusions were very obvious with May-Grünwald-Giemsa (fig 2), Papanicolaou (fig 3), and PAS (fig 4). The vacuoles stained with Alican

Fig 6 *Case 3. A free tumour cell with a central microvillus-lined cavity. At the centre of this is a uniformly electron-dense body. The cavity is surrounded by secretion granules (barely visible), and outside these are many mitochondria. (EM × 7175)*

Blue, but this showed the inclusions as lighter staining areas.

Electron micrographs were made and are described below.

Description of Inclusions

In all three cases described, the 'bull's-eye' inclusions gave the same staining reactions. In air-dried smears stained with May-Grünwald-Giemsa, the main part of the vacuole was unstained, but the central body appeared as a purple spot or mass, sometimes resembling a phagocytosed nucleus. On the other hand, in Papanicolaou preparations the central body never took up haematoxylin but was purely eosinophilic and closely resembled a phagocytosed red cell. In

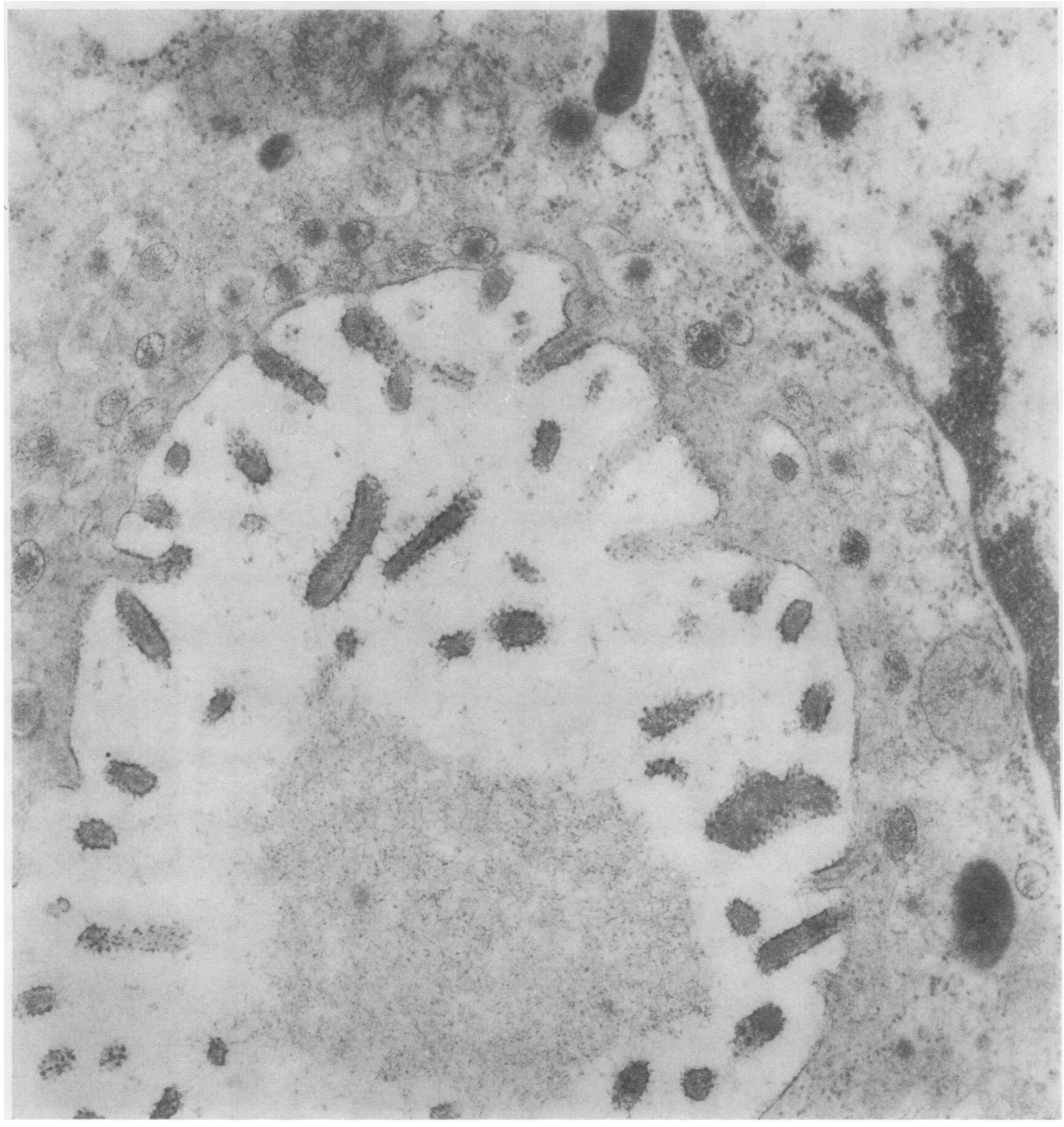

Fig 7 *Case 3. A higher magnification of part of a tumour cell. To the right is the nucleus and nuclear membrane. Most of the field is occupied by a microvillus-lined space with a central mass of secretion. Surrounding the space are many secretory granules, each with a moderately dense content variably separated from its boundary membrane. Outside this region several mitochondria are seen. (EM × 39 560)*

air-dried smears stained with PAS (with or without preliminary ptyalin digestion) the whole mucous vacuole stained pink, but the central area showed as a more intensely stained spot or ring. In case 3, Alcian Blue stained each mucous vacuole but the central area took up less stain than the rest.

Electron microscopy was performed in case 3 (see figs 5-7). The surfaces of the cells were not provided with microvilli. Many of the cells contained one, and sometimes several, vacuoles lined with microvilli, apparently corresponding with the PAS-positive structures. In many cells the larger vacuoles

contained a striking central inclusion; sometimes this showed an almost uniform density, with areas of complete electron-opacity near the centre; or two or more shades of density were present; or there were ill-defined tubule-like structures, perhaps the remains of degenerate microvilli.

Typically, these vacuoles occupied the Golgi area, and were closely surrounded or adjoined by small granules which probably represented mucous secretion due to be discharged into the vacuole (fig 7). Outside these were many mitochondria and a few fat globules; more peripherally still was an area of cytoplasm with a little rough endoplasmic reticulum, scattered ribosomes, and some clefts and vesicles of varying size.

Because of their location, and the histochemical findings, the central inclusions are interpreted as inspissated mucus in vacuoles which are unable to discharge their contents.

Discussion

A well-documented feature of carcinoma of the breast as seen in electron micrographs is the presence of intracytoplasmic 'ductules' or lumina lined by microvilli (Haguenau, 1959; Buerger and Scarpelli, 1962; Wellings and Roberts, 1963; Barton, 1964; Murad and Scarpelli, 1967; Sykes *et al*, 1968; Busch and Merker, 1968; Sagebiel, 1969; Goldenberg *et al*, 1969; Ozzello, 1971). In most of the examples illustrated, these spaces appear empty, but in some they have contained electron-dense material, apparently secretion discharged into the cavity from secretory granules (Haguenau 1959; Buerger and Scarpelli, 1962; Sykes *et al*, 1968; Goldenberg *et al*, 1969; Ozzello, 1971). The corresponding light-microscopic feature is a 'bull's-eye' intracellular inclusion, as demonstrated in histological sections of breast cancer by Sykes *et al* (1968, fig 14b). It seems likely that tumours which form large amounts of extracellular mucin consist of cells which are able to discharge their contents, while the type under discussion sequestrates the secretion in the interior of 'signet-ring' cells.

Simple intracellular microvillus-lined spaces have been previously described from free cells in serous effusions, either from carcinoma of the breast (Spriggs and Meek, 1961; fig 11) or lung (Spriggs and Boddington, 1968; figs 33-35 and 98-99), and we have seen them also in two cases of papillary adenocarcinoma of the ovary, of the type bearing tufts of microvilli on the surface (Spriggs and Jerrome, 1969). The PAS-positivity of some of these vacuoles may perhaps be explained by the large surface area of the contained microvilli, with their associated glycocalyx, rather than by retained secretion.

With the light microscope we have seen the 'bull's-eye' effect in four cases apart from the ones described above. One was a case of malignant ascites attributed to carcinoma of the stomach, but the primary site was not in fact ascertained (see fig 44 of Spriggs and Boddington, 1968). Two cases of malignant ascites due to carcinoma of the breast showed scattered mucus-secreting tumour cells, of which only a few showed the 'bull's-eye' effect. In one, electron microscopy showed microvillus-lined vacuoles of which an occasional one had dense material at its centre. The fourth case differed in that the tumour cells were not free but occurred in clusters, and the primary growth was in the lung; this is the case of Spriggs and Boddington (1968) referred to above, and the 'bull's-eye' phenomenon was seen in only a small proportion of the tumour cells.

From the standpoint of cytological diagnosis, the phenomenon described is worth recognizing because it could be mistaken for the phagocytosis of red or white cells, and without special stains its significance might then be missed. When it is recognized, a diagnosis of mucus-secreting carcinoma can be given with confidence. The breast appears to be the commonest primary site, but the relative probability of other sites will have to await further experience.

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