OAT-CELL CARCINOMA OF THE BRONCHUS

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(PLATES CXVI AND CXVII)

Carcinoma of the bronchus is usually subdivided into three main types with the addition by some authors of a "mixed" group, a "carcinoma simplex" and other less well defined types. In the course of investigating a consecutive series of 280 bronchial carcinomata, the limits of variation within the oat-cell carcinoma group were studied with particular attention to the feasibility of separating it from other varieties of bronchial carcinoma. Certain histochenical features came to light and suggested a new interpretation of a previously observed phenomenon.

MATERIALS AND METHODS

The paraffin blocks from 100 cases of oat-cell carcinoma were available. These included 16 surgical and 84 necropsy specimens. In the latter there was an average of 6 blocks of tumour-bearing tissue per case. Hæmalum-and-eosin preparations were studied, and a mucin stain, diastase-P.A.S., Southgate mucicarmine or both, carried out on all cases. In some cases blocks were cut serially at 5 µ to allow comparison of staining reactions for mucin, calcium, etc. The extractive methods for nucleic acids are those given by Pearse (1953), unless otherwise stated.

RESULTS

The small oat-shaped cell is the best known feature of this tumour and is a characteristic finding in this series. However, there is considerable cytological variation in both size and shape in different blocks from the same case and between different cases. The shape varies from the nearly round cell which resembles a lymphocyte to a sometimes quite fine spindle-cell. Again, though small size is a characteristic feature, cell size is not a reliable diagnostic criterion, as a few of the undifferentiated adenocarcinomas contain areas of rather small spheroidal cells either lining poorly formed tubules or in structureless sheets and cords. At least as characteristic of the oat-cell tumours as their cytology is their histological pattern. Though they are commonly regarded as structureless, they do in fact show structural variations which taken in conjunction with the cytology often enable a diagnosis to be made on a small biopsy specimen. These are best described under the following headings:—

(a) Streams. Even the most undifferentiated oat-cell tumour...
shows this pattern in which cell clumps, small or large, are made up of cells with a parallel orientation of their long axes; often several "streams" are present within the same neoplastic clump giving the impression of intersecting bundles.

(b) Ribbons. This pattern is also a more or less constant feature of oat-cell tumours (fig. 1). The tumour cells are aligned in ribbons, one or a few cells in width. Adjacent cells usually over-ride slightly, often interlocking in a curiously neat manner (fig. 2). Where this ribbon arrangement is prominent, a festooned appearance is often produced (fig. 1).

Some minor variations of this basic pattern deserve mention. Giant-cells are sometimes seen, singly or in small groups, with a large, irregular, often grotesque, very dark nucleus. Clear-cell changes, frequent in the squamous carcinomas and bronchial adenocarcinomas, are not often found here. Frank squamous metaplasia was seen in only one case, but squamoid changes are not so infrequent and may lead to errors of classification if material is limited. Many oat-cell tumours contain groups of larger, pink-staining polygonal cells (fig. 3). These sometimes form small rounded foci which have been regarded as indicating squamoid changes (McKeown, 1952); an alternative explanation is offered below.

(c) Pseudo-rosettes and rosettes. Pseudo-rosettes formed by perivascular mantles of surviving cells are conspicuous in some of this material. A more important finding is the presence of true rosettes in variable number. Neoplastic clumps sometimes consist of aggregates of compactly arranged rosettes with an eosinophilic nucleus-free zone in the centre of each rosette (fig. 4). These rosettes merge insensibly into tubular structures.

(d) Tubules and ductules. These are seen in thirty cases (7 surgical and 23 necropsy). They often consist of empty spaces surrounded by wedge-shaped cells with well defined glandular orientation. Others have a lining of cuboidal cells with eosinophilic cytoplasm and a less deeply staining nucleus than the surrounding oat-cells (fig. 5). The small solid foci of pink-staining cells referred to above, regarded by some as squamoid, are largely primitive attempts at such ductule differentiation. These ductular spaces are not usually numerous or conspicuous. Many of them appear empty, but close scrutiny reveals that a certain number contain a finely granular eosinophilic secretion. This secretory product is found only in relation to the ductules and is present in metastases in sites like the mediastinal lymph-glands, adrenals and bone-marrow where the origin of the material is not open to question (fig. 5). The appearance suggests an eosinophilic mucinous secretion such as is known to occur in ductules in adnexal tumours (Lennox et al., 1952; Azzopardi and Smith, 1959). This is confirmed most convincingly in periodic acid-Schiff preparations after diastase treatment, but Southgate's mucicarmine also gives a positive result. This material stains only faintly with alcian blue and shows no metachromasia with toluidine blue; it does not stain with Sudan black B. In metastases of three oat-cell tumours in adrenal, bone-marrow and
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Fig. 1.—Showing the ribbon-like structure of the tumour. Hæmalum and eosin. ×95.

Fig. 2.—The over-riding of adjacent cells in a ribbon of oat-cells. H. and E. ×550.

Fig. 3.—Sharply defined foci of large cells with more copious cytoplasm. H. and E. ×320.

Fig. 4.—Numerous rosette-like structures in an oat-cell carcinoma. H. and E. ×460.

Fig. 5.—Ductules containing mucin in a vertebral metastasis of oat-cell carcinoma. Periodic acid-Schiff and hæmalum. ×270.

Fig. 7.—Hæmatoxyphil sheaths around vessels in and around necrotic focus of oat-cell carcinoma. H. and E. ×230.
lymph-gland, the secretion in the ductules is non-granular and haematoxyphil and there is not much doubt about its mucinous nature even in routine sections (fig. 6). The secretion in these three cases actually shows β-metachromasia with toluidine blue. No intracellular mucin is seen in any of the oat-cell tumours. The staining reactions of the mucins studied are unaltered after 18 hours' treatment with hyaluronidase at 37°C. The eosinophilic material in the ductules of these oat-cell tumours is an epithelial mucin probably of the nature of a neutral mucopolysaccharide. The stroma of the oat-cell tumour is occasionally rather mucinous and haematoxyphil. This mucin, which is only very weakly P.A.S.-positive and is hyaluronidase-labile, is clearly a stromal mucin and not indicative of tumour differentiation; it is easily distinguished from the ductule secretion.

The connective-tissue framework of the oat-cell carcinoma is in general rather finer than that of the squamous carcinoma and adeno-carcinoma of the bronchus, occasionally assuming a fir-tree distribution. A feature seldom referred to in the literature is a peculiar vascular alteration seen in greater or less degree in 32 cases (3 surgical and 29 necropsy). In sections stained with haemalum the change consists of a peculiar bluish-grey to bluish-black coloration of the walls of small venules in the tumour stroma. It is seen in the primary tumour as well as in metastases in lymph-glands, myocardium, spleen, pancreas, adrenals, etc. In a striking example most of the small vessels in an area of tissue may be stained almost black (fig. 7). In the most heavily affected vessels, this "incrustation" may appear homogeneous or it may appear to consist of coarse agglomerated material; in less affected vessels the material appears more definitely granular or fragmented. Sometimes the haematoxyphil substance appears to coat individual collagen fibres near the blood-vessels and so assumes a fibrillary appearance. The walls of the vessels do not show any other constant changes which could be interpreted as preceding and being connected with the haematoxyphilic change. There is a striking association between necrosis in the tumour and the presence of this change (fig. 7), and it is partly for this reason that it has been attributed to calcification in vessel walls. Staining with v. Kossa's method gives completely negative results and the more specific alizarin red method for calcium is also negative. Staining with 0.5 to 1 per cent. napthochrome green for 1 hr produces negative results. As haematoxyphilia in sections is not caused by calcium itself, stains were employed to identify substances which might have been responsible for the altered staining reaction and with which calcium salts are often associated. Staining with periodic acid-Schiff reveals no increase of mucopolysaccharide ground-substance and Perls's reaction is negative. Because the vascular "incrustation" is related to areas of tumour necrosis and since the material in the vessel wall shows in some areas a distant resemblance to the haematoxyphil bodies of disseminated lupus erythematosus, the possibility of the material being DNA was considered. The Feulgen
reaction, hydrolysing with NHCl at 60° C. for 8 min., is positive in the altered vessel walls, the location and intensity of the positive reaction corresponding precisely with the hæmatoxyphilia in routine sections (fig. 8). Solochrome cyanine R.S. stains the altered vessels a dark blue colour, but where the material is abundant and very coarse, there is sometimes a patchy reddish staining in addition to the blue coloration.

Confirmation that the hæmatoxyphil material is DNA was sought by nucleic acid extraction. The material is removed by treatment with 5 per cent. perchloric acid at 60° C. for 25-30 min. It is not removed by treatment with 10 per cent. perchloric acid at 4° C. for 16 hr. It is extracted also by treating sections with 4 per cent. trichloracetic acid at 90° C. (Schneider's method) for 15 min. A beef pancreas deoxyribonuclease prepared by Dr J. C. White by the McCarty process was also used. A solution containing 1.0 mg./ml in 1 per cent. gelatin was used with a final concentration of 0.02 \( M \) MgCl₂. The nuclei of normal invaded structures and of the well preserved tumour cells are digested by 3 hours' incubation at 37° C. However, there is greater difficulty in digesting the pyknotic nuclear material fringing areas of frank tumour necrosis in which nuclear staining has been lost. Similar difficulty is encountered in digesting the hæmatoxyphil material deposited in the vessel walls. Resistance to digestion in these two sites may be due to inaccessibility to the enzyme; it can only be partly overcome by incubation for periods of 24-36 hr. Controls consisted of enzyme solutions without the activator.

**DISCUSSION**

The name “oat-cell carcinoma” is widely used in this country; “small-cell carcinoma” is often used by American authors (e.g. McBurney et al., 1951). Alternatively this tumour type is often labelled “anaplastic” or “undifferentiated”, an unfortunate usage since some bronchial squamous carcinomas and adenocarcinomas are also anaplastic and the term loses significance. Oat-cell carcinoma is neither anaplastic squamous carcinoma nor anaplastic adenocarcinoma, but a distinctive type of neoplasm with histological, cytological and histochemical features that stamp it as a separate entity. It is these features that enable its recognition in the very rare event of an extrabronchial origin (McKeown, 1952). The designation of small-cell carcinoma has the disadvantage previously mentioned and lacks individuality.

The streams and ribbons that form the basic pattern of this tumour have not been sufficiently stressed. The presence of tubular structures was noted by Barnard (1926, 1938) and more recently by Walter and Pryce (1955). Some authors (Rienhoff, 1947; O'Keefe, 1948) have not separated oat-cell tumours from adenocarcinomata, and Walter and Pryce have drawn attention to the fact that differentiation in oat-cell tumours with the production of rosettes and tubules has resulted in difficulty in separating them from the adenocarcinomas. This is undoubtedly one of the major pitfalls in the classification of bronchial epithelial neoplasms. The production of mucin in these tumours has not been previously described. It is seen in some of my material although it must be emphasised that when mucin is present
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Fig. 6.—Adrenal metastasis showing ductules containing mucin. P.A.S. and hemalum. ×95.

Fig. 8.—Feulgen-positive material in vessel walls. Feulgen. ×400.
it is scanty, and as most of it is eosinophilic, it can be missed unless carefully sought for, or unless a P.A.S.-stained section is examined. Before we can accept the fact that mucin may be secreted by oat-cell tumours, two requirements must be adequately fulfilled. There must be no doubt that the tumours studied are in fact oat-cell tumours and it must be conclusively shown that the tumour contains epithelial mucin which is not derived from included structures. There is no doubt that by the most rigid criteria the tumours under consideration would be accepted as oat-cell tumours by all those who use the term. The presence of an epithelial mucin has been shown. That this could conceivably be derived from invaded normal tissues has been excluded by the examination of metastatic growth in sites where such mucin is not normally present. The relation of the mucin to the ductular spaces and its absence elsewhere leaves no other explanation than that this is a secretory product of the oat-cell carcinoma. It may be felt that the demonstration of mucin secretion in oat-cell tumours renders their separation from the adenocarcinomata impracticable. In the material examined this is not true. The oat-cell tumour can be distinguished from the adenocarcinoma in its variant forms. The well differentiated adenocarcinoma with copious extracellular and intracellular mucin offers no difficulty. The less well differentiated types with cords and sheets of large, polygonal, finely foamy or powdery cells, often with intracellular mucin, offer little difficulty. All grades of differentiation are seen down to the tumour containing sheets of more or less spheroidal cells. These lack the patterns seen in the oat-cell tumour and have rather vesicular nuclei, the nucleoli of which are conspicuous at a low magnification. Moreover, prolonged search will usually reveal some areas in which the tumour cells are arranged in imperfect, poorly formed, elongated and sometimes ramifying tubular formations. In such varieties of undifferentiated bronchial adenocarcinoma mucin may be very scanty or absent. The ability to distinguish between a relatively well differentiated oat-cell carcinoma and a poorly differentiated adenocarcinoma may be difficult to understand, especially if one follows Halpert, quoted by Ochsner and DeBakey (1941), in the belief that all bronchial carcinomas arise from the undifferentiated basal cell of the bronchial mucosa. It seems equally, perhaps more, likely that the adenocarcinomas arise from bronchial and bronchiolar columnar epithelium or bronchial mucous glands, but histological studies on this point are not conclusive. Whatever the theoretical explanation, the fact remains that in the material studied oat-cell carcinoma and adenocarcinoma appear to be separable.

The oat-cell tumour is distinct from the squamous carcinoma and, as Willis (1953) states, there is nothing to suggest that it is any more closely related to squamous carcinoma than it is to adenocarcinoma. Squamoid change in oat-cell tumours may cause difficulty in diagnosis if material is limited. The squamoid change may be restricted to some foci and in one case squamoid metaplasia in a cerebellar metastasis had resulted in a previous diagnosis of

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undifferentiated squamous tumour. In none of the squamous carcinomas of bronchus studied are there areas of oat-cell pattern. In one surgical specimen two separate apparently independent tumours were found, each connected with a major bronchus. Histological examination shows that one is a squamous, the other an oat-cell tumour. This is regarded as an example of a double primary carcinoma; a similar case is recorded by Bryson and Spencer (1951). In this connexion it is worth recalling that the primary tumour in an oat-cell carcinoma is sometimes minute in the presence of numerous large metastases. In one such case in this series the primary tumour was only 3 mm. in diameter. Most pathologists are familiar with the very occasional case of oat-cell carcinoma in which necropsy reveals widespread metastases and no primary tumour can be identified. A case of this type, not included in this series, was seen elsewhere in which careful necropsy, with prior knowledge of the nature of the tumour from a lymph-gland biopsy, failed to reveal the seat of the primary growth. This has a bearing on one other problem case in this series. In this case necropsy revealed a large mass connected with a major bronchus and thought to be the primary tumour, together with numerous nodules of growth in both lungs and many distant metastases. Of the neoplastic foci studied, all but one showed moderately differentiated squamous carcinoma; the exception was a metastatic focus in the liver of oat-cell carcinoma of typical morphology; multiple sections through this focus showed a uniform pattern with nothing to suggest transition to or from squamous carcinoma. This case is interpreted as a bronchial squamous carcinoma with metastases, along with a hepatic metastasis from an oat-cell tumour of the lung which was not identified.

The peculiar vascular change described in these tumours is referred to by Ogilvie (1957) and McKeown and attributed to calcification. The calcification is said to follow hyaline change in the vessel walls. Cameron (1930) showed that calcium salts are not hematoxyphil whilst inorganic iron deposits are, and that most of the hematoxyphil reaction at sites of normal and pathological calcification is due to a special ground-substance which has an intimate relationship with the deposition of calcium salts. This ground-substance is now known to be a mucopolysaccharide. The abnormal vessel walls in the oat-cell tumour do not show an excess of mucopolysaccharide when stained with P.A.S. Since hematoxylin is a test for calcification only in so far as it demonstrates the accompanying mucopolysaccharide, there is no reliable evidence for regarding the vascular change as calcification. The negative tests for calcium confirm this interpretation. The positive Feulgen reaction is remarkable and consistent with the histological and other histochemical findings. DNA, like inorganic iron salts and mucopolysaccharides, may be responsible in diseased tissues for a hematoxyphil reaction that has often been erroneously attributed to pathological calcification.

This deposition of DNA in the vessel walls is presumably the result of liberation of nucleic acids in large amount from degenerating neoplastic tissue. The intensity of the deposition must reflect a balance between the rate of formation and the rate of removal or destruction. In the material examined, this change was not seen in bronchial adenocarcinoma and squamous carcinoma, though in hematoxylin-stained sections of these tumours as well as of oat-cell tumours a slaty
blue-grey colour in areas of tumour necrosis was associated occasionally with varying, usually slight, degrees of calcification. A mild degree of vascular "coating" with DNA was seen, however, in an undifferentiated spheroidal-cell carcinoma which did not show the characteristic features of an oat-cell tumour.

**Summary**

Oat-cell carcinoma of the bronchus has positive structural features that identify it: streams, ribbons, rosettes and ductules. It sometimes produces an epithelial mucin which is not derived from invaded structures and is found in metastases as well as in the primary tumour. A peculiar haematoxyphil staining of blood-vessel walls is attributable to deposition of DNA and not to calcification.

Oat-cell carcinoma is a special type of bronchial carcinoma which is sharply separable from squamous tumours and generally separable from adenocarcinoma. Its confusion with the other two chief histological types is due in part to the finding in it of nests of larger epithelial cells and of ductules, and in part to the failure to recognise squamoid change as a secondary modification of a basic pattern. Double primary carcinomas of different histological type may also cause difficulty. The term "oat-cell carcinoma" is preferable to "anaplastic" and similar terms; squamous carcinoma and adenocarcinoma of the bronchi are often anaplastic, at least in part, and yet identifiable.

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**References**