Virchows Arch. Abt. A, Path. Anat. 357, 19—28 (1972)

Mastocytes, Autofluorescent and Silver Reducing Elements in Bronchial Carcinoids

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Received April 25, 1972

Summary. We report observations of three bronchial carcinoids, using optical, UV fluorescent and electron microscopy. The scattered autofluorescent and silver reducing cells observed in these tumours are mastocytes and not tumour cells.

Zusammenfassung. Bericht über 3 bioptisch gewonnene Bronchus-Carcinoide, welche lichtmikroskopisch, UV-Fluorescenz-mikroskopisch und elektronenoptisch untersucht werden. In 2 Fällen können zahlreiche, im Geschwulstgewebe verteilte, granulierte Zellen nachgewiesen werden, deren Granula eine positive Reaktion nach Masson-Hamperl zeigen. Dieselben Zellen lassen außerdem eine wechselnd ausgeprägte Autofluorescenz im UV-Licht erkennen. Nach Entfernung der Silbergranula und Färbung der gleichen Präparate mit metachromatischen Lösungen sind diese Zellen vorwiegend orthochromatisch. Die Ultrastruktur der Granula unterscheidet sich eindeutig von den Einschlüssen typischer Carcinoid-Zellen; es handelt sich um teils homogene, teils lamellär gestreifte Strukturen. An Hand der lichtoptischen, fluorescenzmikroskopischen und ultrastrukturellen Befunde werden die Elemente als Mastzellen bezeichnet, die in einer „unreifen" (orthochromatische, homogene Granula) und einer „reifen" (metachromatische, differenzierte Granula) Form vorliegen. Es kann angenommen werden, daß diese Zellen eine Beziehung zur Serotonin-Produktion in den Carcinoid-Zellen haben und deshalb nicht als eigentliche Geschwulstzellen interpretiert werden dürfen.

Introduction

During a study of three cases of bronchial carcinoids, we were struck by the elevated number of mastocytes in 2 of these tumours, there being fewer in the third. We also noticed several isolated fluorescent and silver reducing cells. We wondered if these elements belonged to the tumour cells, or if they represented mastocytes, which were often observed.

Material

The material studied comes from three pulmonary resections for bronchial carcinoids:

Case No. 1. (T 632/71): Male, 30 years. Left pneumonectomy for a polypoid carcinoid of the bronchial origin.

Case No. 2. (T 3209/71): Male, 64 years. Right median lobectomy for carcinoid.

Case No. 3. (E 5986/71): Male, 58 years. Left inferior lobectomy for carcinoid of the anterio-basal, segmental bronchus.

Not one of these three cases shows mediastinal lymph node metastasis nor any other clinically discernible metastases. No carcinoid or endocrine syndrome has been found.

Methods

a) Optical Microscopy. The material designated for optical microscopy was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin, toluidine blue, alcian blue-cresyl violet (Selye, 1965) and silvered according to the Masson-Hamperl method (Roulet, 1948). Semi-thin sections in Epon from material reserved for electron microscopy were stained with azure blue-methylene blue and silvered according the Masson-Hamperl technique.

b) Fluorescence Microscopy. We used a Leitz Orthoplan microscope with a Leitz equipment for UV fluorescence: a high pressure mercury vapour lamp of 200 W, a diffuse N heat filter, an excitation filter UG 1 of 2 mm, a red absorption filter BG 38 of 4 mm and a stop filter K 430.

Several series of non-stained sections were photographed, first with UV fluorescence then a second time after silver impregnation according to the Masson-Hamperl method. The silver was then removed by Lugol solution followed by a 2.5% solution of sodium thiosulphate; the same slides underwent restaining with toluidine blue or alcian blue-cresyl violet and were photographed a third time.

c) Electron Microscopy. Portions of the tumours from cases 1 and 3 were immediately fixed in 2.3% glutaraldehyde and postfixed with 2% osmium tetroxide. The material, included in Epon, was sectioned with a Reichert OMU 2 or a Sorvall MT 2 ultratome. Thin sections, contrasted with uranyl acetate and lead citrate, were examined with a microscope Philips EM 300 at 80 kV with a metal contrast diaphragm 40 μ thick.

The second case was fixed with 10% buffered formalin, then treated and examined as above.

As for the paraffin sections, some semi-thin sections in Epon, from material intended for electron microscopy were silver impregnated according to the Masson-Hamperl method and photographed. After removal of silver and restaining with azure blue-methylene blue, the same sections were photographed a second time.

To establish a closer link between observations from optical and electron microscopy, the semi-thin sections were taken immediately adjacent to the thin sections. These semi-thin sections were treated by the Masson-Hamperl method, then stained with azure blue-methylene blue. The thin sections were contrasted and examined as usual.

Results

a) Optical Microscopy. The three cases present the well-known characteristics of bronchial carcinoids: the tumour tissue is arranged in islets, in epithelial strands, surrounded by a thin stroma, well vascularized with hyalinized areas. Occasionally, in case no. 2, tubular formations can be seen. There are no mitoses. The nuclei have a regular size, are rounded or oval, the chromatin is not very dense. The nucleoli are clearly seen. Between the tumoural strands, frequently in the neighbourhood of small blood vessels, mastocytes in elevated number in cases 1 and 2, less in case 3, can be observed.

b) Fluorescence. In the paraffin sections, de-paraffinated or not, we notice isolated cells, frequently in cases 1 and 2, rarely in case 3, containing coarse granula emitting a bright yellow fluorescence, whereas the adjacent tumour cells do not fluoresce, with the exception of the third case where several intracytoplasmic granula in tumour cells emit a pale yellow fluorescence.

c) Histochemistry. For sections embedded in paraffin, the Masson-Hamperl reaction reveals isolated cells containing numerous coarse silver reducing granula. These elements are frequent in cases 1 and 2 but rare in case 3. Their form is very variable, sometimes globular, sometimes elongated or star-shaped. The

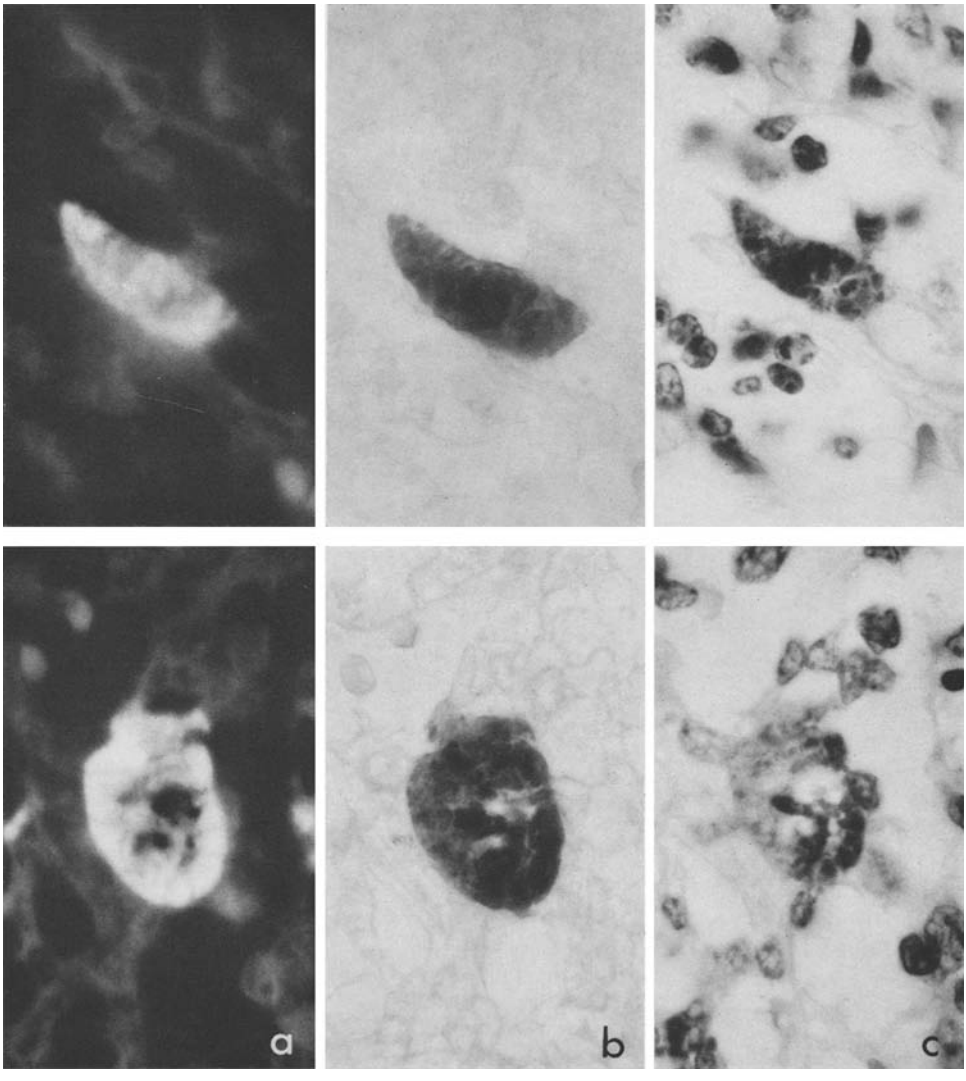

Fig. 1. Case No. 2. Two mastocytes photographed successively with UV fluorescence (*a*), silvered according to Masson-Hamperl (*b*), then, after silver removal, stained with alcian blue-cresyl violet (*c*). 930:1

neighbouring tumour cells do not show silver affinity in any of the three cases. In the Epon-sections, the Masson-Hamperl reaction reveals scattered cells between the tumour islets whose cytoplasm contains silver reducing granula. After silver removal, the same granula appeared orthochromatic with azure blue-methylene blue. Photographs, taken successively from the same section, demonstrate (Fig. 1) that the granula of the isolated cells are at the same time autofluorescent, silver reducing and essentially orthochromatic.

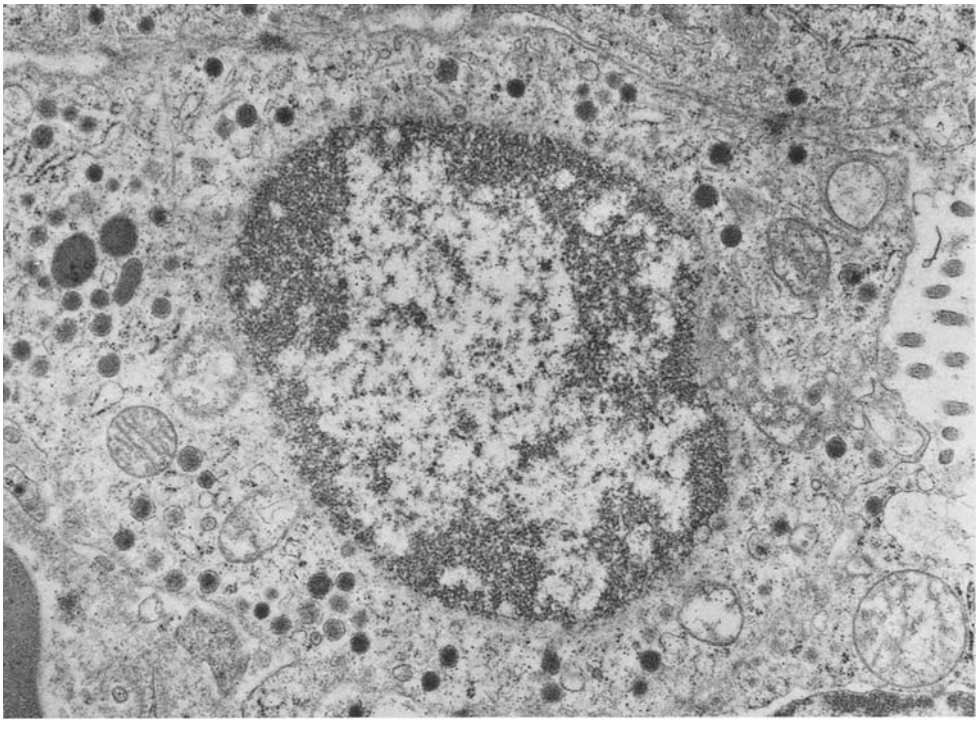

Fig. 2. Case No. 1. Carcinoid tumour cell containing characteristic granula. Right, outlines of microvilli. 18000 : 1

d) Electron Microscopy. The ultrastructural appearance of the three cases is comparable, with several slight differences. One deals with polygonal cells, whose membranes often form microvilli, to varying degrees, at one pole. The cytoplasm contains dense granula, 100 to 300 mμ in diameter (Fig. 2), surrounded by a unit membrane; a clear narrow band exists between the central dense body and the membrane. Otherwise, lysosomes of an inhomogeneous structure are observed, partly hollowed out with vacuoles, especially in case no. 3 where they attain up to 2.5 μ in diameter.

The combined study of the immediately adjacent semi-thin and thin sections allows identification and examination of the same silver reducing cells with both optical and electron microscopy. Their cytoplasmic granula, which optically reduce silver by the Masson-Hamperl method and remain orthochromatic in the azure blue-methylene blue reaction (Fig. 3), differ completely by electron microscopy from the typical granulations of the carcinoid tumour cells, as they do from their original cell, the clear bronchial cell as well as the intestinal Kultschitzky cells. These granula, mostly homogeneous and osmophilic, attain 400 to 600 mμ in diameter. In the neighbourhood of the cell membrane, the contents of several granula are differentiated into parallel lamellar formations or in rolls resembling, in places, fingerprints (Fig. 4).

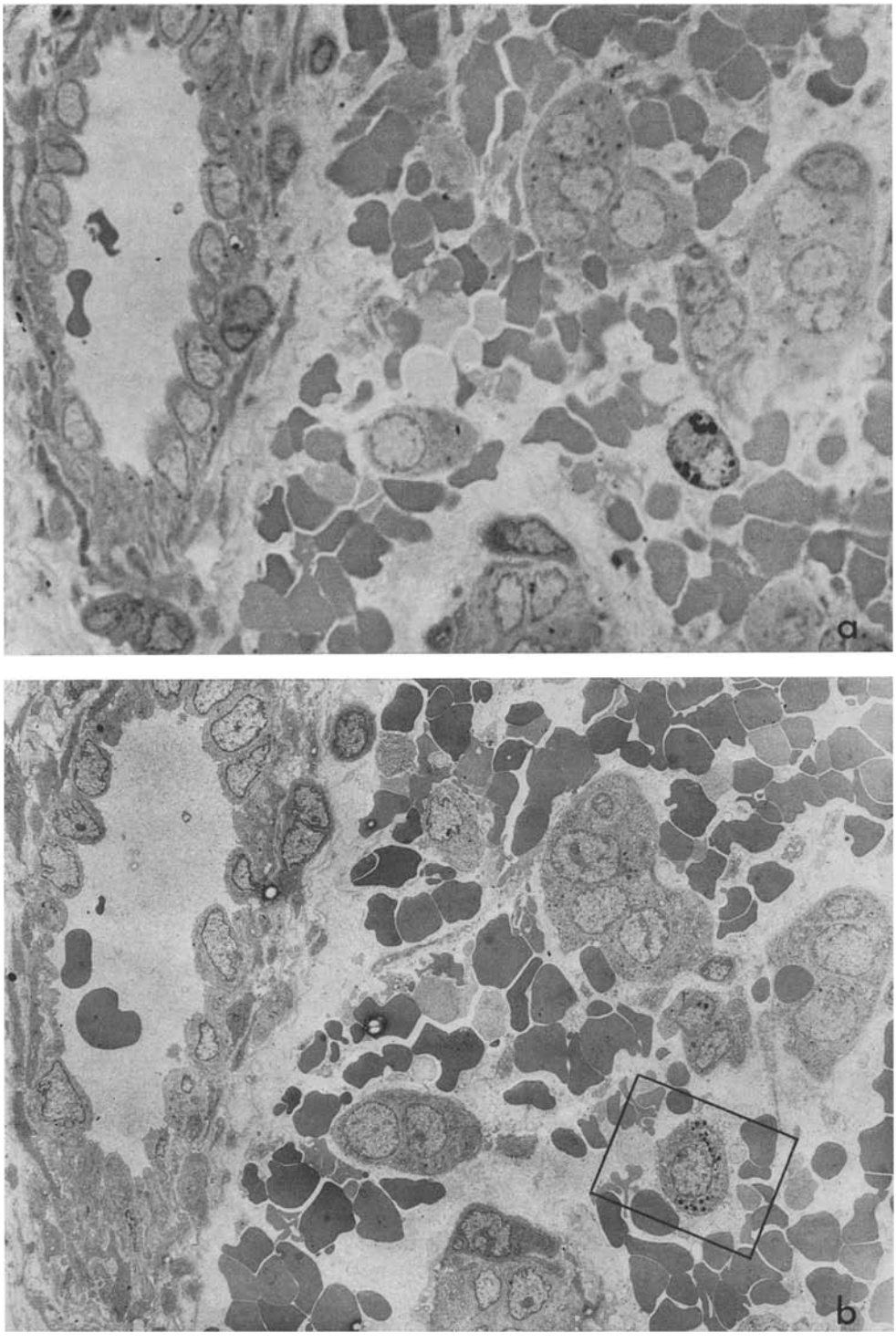

Fig. 3. a Case No. 1. Semi-thin section silvered according to Masson-Hamperl. Right, a cell containing silver reducing granula. 1300:1. b Thin section adjacent to the semi-thin section reproduced above (Fig. a). The framed cell, illustrated in detail by Fig. 4a and b, is the same as that containing silver reducing granula shown in Fig. a. 1300:1

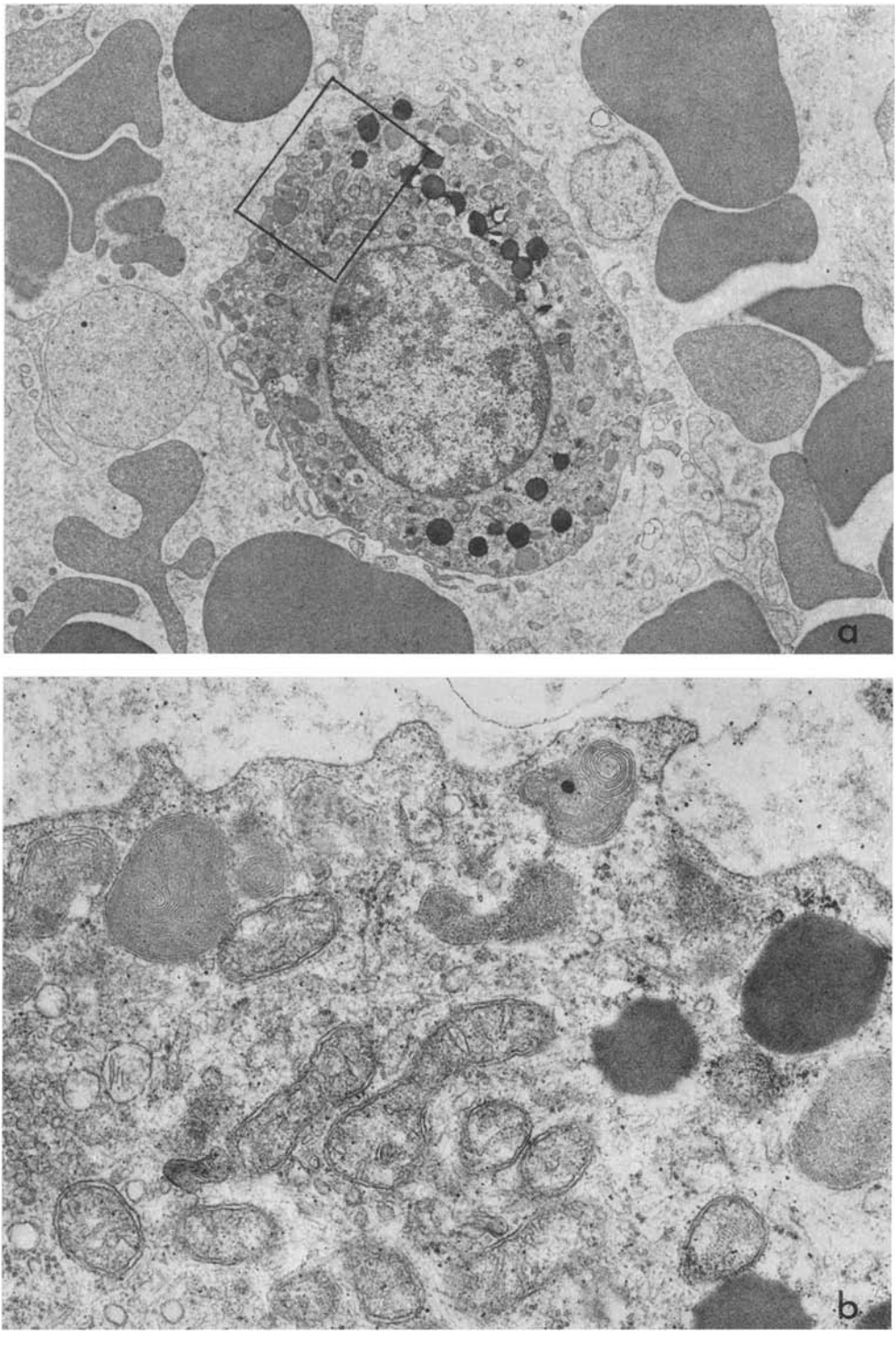

Fig. 4. a Cell framed in Fig. 3b. 7100:1. b Area outlined in Fig. a. In the neighbourhood of the cell membrane, granula with lamellary structures (in "fingerprints"), characteristic of mastocytes. 43000:1

Discussion

It must be pointed out that mastocytes escape current examination in sections stained by routine methods such as haematoxylin and eosin. Moreover, stains with metachromatic power such as azure blue, methylene blue, toluidine blue or cresyl violet reveal only the mastocytes called "mature", that is to say those which contain polysulphate esters of heparin (Selye, 1965). The "immature" mastocytes are orthochromatic, but show affinity for alcian blue. This idea of "mature" or "immature" mastocytes, depending on their different reactions to metachromatic stains (Selye, 1965), corresponds to their particular ultrastructural characteristics (Hibbs, 1960; Thiéry, 1963). The "mature" type of mastocytes contains the granula with parallel lamellar formations found towards the cell surface resembling fingerprints, which could be responsible for the metachromasia (Thiéry, 1964). The other "immature" type contains essentially homogeneous granula of a larger size.

The cytoplasmic granula of the isolated cells which we have studied, correspond closely to the granula of mastocytes and not to those of the neighbouring tumour cells: the autofluorescent and silver reducing cells scattered throughout our three bronchial carcinoid cases are, in fact, mastocytes. During our study, we were able to observe all the intermediate stages between "mature" and "immature" mastocytes. Curiously, the silver reducing and autofluorescent properties seem to be the prerogative of the "immature" and orthochromatic mastocytes, which show very few ultrastructural lamellar formations in their cytoplasmic granula.

It is known that bronchial carcinoids are not, generally, silver reducing, just as their original cell, the clear bronchial cell derived from the primitive anterior intestine (Altmann *et al.*, 1959; Bensch *et al.*, 1965b, c; Black, 1968; Jones *et al.*, 1969; Soga *et al.*, 1971; Spencer, 1969; Toker, 1966; Williams *et al.*, 1963). However, several observations of bronchial carcinoids mention the presence of silver reducing cells after different variants of the Masson reaction (Bensch *et al.*, 1965a; Bernheimer *et al.*, 1960; Delarue *et al.*, 1960; Dube, 1970; Feyrter, 1959; Hosoda *et al.*, 1970; Stanford *et al.*, 1958; Weiss *et al.*, 1961; Williams *et al.*, 1960). Other authors, using reactions for argentaffinity, such as that of Bodian (Williams *et al.*, 1960), of Gros-Bielschowsky (Bernheimer *et al.*, 1960; Delarue *et al.*, 1960; Feyrter, 1959) or of Gros-Schulze (Hamperl, 1937) report the existence of similar cells. On closer examination, we realise that a) the cells illustrated by these authors (Delarue *et al.*, 1960; Feyrter, 1959; Hosoda *et al.*, 1970; Weiss *et al.*, 1961; Williams *et al.*, 1960) as silver reducing or argentaffin tumoural cells are scattered; b) they often have a larger size than the neighbouring tumour cells; c) they are sometimes provided with prolongations and d) are frequently situated in the neighbourhood of blood vessels. These cells, therefore, present characteristics closely agreeing with those of the cells which we have observed in our three cases and demonstrated to be of mastocytic nature and not tumoural. Almost none of the authors consulted (Altmann, *et al.* 1959; Bernheimer *et al.*, 1960; Cohen *et al.*, 1960; Delarue *et al.*, 1960; Dube, 1970; Feyrter, 1959; Hamperl, 1937; Haupt *et al.*, 1967; Holley, 1946; Hosoda *et al.*, 1970; Jones *et al.*, 1969; Kreyberg, 1967; Langer, 1960; Larsen, 1970; de Paredes *et al.*, 1970; Pariente

et al., 1967a, b; Pieron *et al.*, 1970; Shames *et al.*, 1968; Spencer, 1969; Stanford *et al.*, 1958; Toker, 1966; Verley *et al.*, 1965; Weiss *et al.*, 1961; Williams *et al.*, 1960) mentions the presence of mastocytes in bronchial carcinoids. Only Bensch *et al.* (1965a) describe and illustrate mastocytes, which they often observed in close relationship to the tumoural buds. Several authors mention, in their observations of bronchial carcinoids, cells producing a yellow UV autofluorescence (Bensch *et al.*, 1965a; Delarue *et al.*, 1960; Feyrter, 1959; Williams *et al.*, 1960). This phenomenon is attributed to a complex formed between serotonin and formaldehyde, called beta-carbolin (Adams-Ray *et al.*, 1964; Corrodi *et al.*, 1963; Delarue *et al.*, 1960). Such a fluorescence, if it has been observed in intestinal carcinoids, for example, has also been precisely pointed out in healthy and pathological human and animal mastocytes, which may contain serotonin (Benditt *et al.*, 1955; Rice *et al.*, 1961).

How can the abundance of mastocytes in two of our examples of bronchial carcinoids be explained ? It is known that these tumours are capable of producing serotonin or perhaps its precursor, 5-hydroxi-tryptophan, if the necessary decarboxylase is lacking (Black, 1968). We also know that mastocytes are able to transport and liberate serotonin, transforming the 5-hydroxi-tryptophan into 5-hydroxi-tryptamin (= serotonin). It can be thought, therefore, that mastocytes are responsible for the taking up and evacuation of the secretory product of these tumours. In this connection, the observation of an elevated number of mastocytes in the skin and endocardium of patients affected by carcinoids should be mentioned (Selye, 1965).

Conclusion

Our study shows that isolated silver reducing and autofluorescent cells observed in our three examples of bronchial carcinoids are not tumour cells, but mastocytes. It is therefore important to investigate meta- and, especially, orthochromatic mastocytes and to pay attention to the interpretation of cells presenting argentaffinity.

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