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Minute and small early gastric carcinoma with special reference to eosinophil infiltration

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Summary. The authors examined 25 minute early gastric cancers (EGC) and 13 small EGC in order to investigate the incidence and possible causes for the infiltration of eosinophils. The degree of eosinophil infiltration was higher in tumour stroma than in adjacent normal-appearing mucosa; this correlation was statistically significant ($P < 0.001$). Tumour-associated tissue eosinophilia (TATE) was not correlated with size, histological type, necrosis of the tumour nor gastritis activity in adjacent non-tumoral mucosa. Electron microscopy, performed in 4 cases of small EGC, showed tumour stromal eosinophils with morphological evidence of activation similar to those described for tissue eosinophils in various disorders. Some tumour cells in intimate contact with activated eosinophils exhibited focal cytopathic changes. TATE represents local inflammation probably leading to tumour cell damage. The immunological role of the eosinophils against tumour cells *in vivo* deserves further investigation.

Key words: Gastric carcinoma, Eosinophil infiltration

Introduction

In the gastrointestinal tract, benign or malignant tumours whether epithelial, lymphoid or mesenchymal may on occasion show tumour-associated tissue eosinophilia (TATE) (Yoon, 1959; Lowe et al., 1981; Pretlow et al., 1983; Iwasaki et al., 1986; Shepherd et al., 1987; Fisher et al., 1989). The prognostic significance of this TATE has received much attention in recent years, because of the accumulating data in experimental animals (Vaage et al., 1986; Tepper et al., 1989) and *in vitro* systems (Jong and Klebanoff, 1980) suggesting an immune reaction, involving these reactive eosinophils against tumour. However, the influence on prognosis of TATE appears controversial because the exact nature of the infiltration is usually difficult to evaluate. In other words, it is difficult to determine in man whether the TATE is directly antitumoral in nature or whether it was induced by secondary effects, such as necrosis and/or inflammation.

The current study refers to minute (diameter ≤5 mm) and small (diameter >5 ≤10 mm) early gastric cancer (EGC). In this paper small and minute EGC are studied to correlate the eventual TATE with the presence or absence of necrosis in the tumour tissue; in addition, the ultrastructural features of eosinophils, observed in the tumour stroma of 4 small EGC, are also described, in order to discuss pathogenetical factors for TATE.

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Materials and methods

From 1980 to 1991, 92 patients with EGC were included in the files in the Department of Human Pathology of the University of Messina and in the Pathology Service of Cremona Hospital (Italy). None of the patients had undergone preoperative irradiation or immunochemotherapy. 25 lesions from 25 patients were minute gastric carcinomas and 13 lesions from 13 patients were small gastric carcinomas. Of the 25 minute EGC, 7 were diagnosed by endoscopic biopsy specimens before surgical resection, while the other 18 minute EGC were found incidentally in stomachs that were resected for another larger carcinoma. On the other hand, 10 small EGC were diagnosed as carcinoma before surgery, while the other 3 were found incidentally in stomachs that were resected for another carcinoma. All lesions were measured, photographed and macroscopically classified according to the general rules for gastric carcinoma from the Japanese Research Society for Gastric Cancer (JRSGC, 1981). The tumour was removed and sectioned in tissue fragments; additional tissue samples were taken at random from areas of irregular architecture as well as from apparently normal areas and surgical borders of the specimens.

For light microscopy, the specimens were fixed in 10% formalin for 24 hrs at room temperature and embedded in paraffin. Sections (4-5 μm thick) were

stained with haematoxylin-eosin (H&E). A micrometer eyepiece was used to measure any EGC microfoci, unobserved on gross examination. The identification of the minute or small secondary tumours depended on the discontinuity of the neoplastic lesions. All EGC could be classified into intestinal or diffuse types, according to Laurèn (1965) classification. The activity and type of gastritis in the mucosa adjacent to carcinoma were recorded. The numbers of eosinophils within the tumour and the adjacent mucosa were counted in 20 nonoverlapping high power fields (HPF) (0.08 mm^2) by using a x40 objective and a square grid mounted in a x10 microscopic eyepiece. The median value and the range of the number of eosinophils, expressed per 20 HPF, were determined for each case. The degree of eosinophilia was quantified as slight (≤10 eosinophils/HPF), moderate (>10≤20 eosinophils/HPF) and marked (>20 eosinophils/HPF). The degree of eosinophil infiltrate within the tumour was correlated with the following: gross appearance; size; histological type; and necrosis in tumour as well as the activity of gastritis and the eosinophil infiltrate in the adjacent non-tumoral mucosa. Statistical evaluation was performed using the chi-square test.

For electron microscopy, fresh fragments were available at the time of surgery in only 4 out of 13 cases of small EGC. 1 mm minced pieces were fixed in 3% phosphate-buffered glutaraldehyde (pH 7.4) and post-fixed in 1% osmium tetroxide. Semi-thin Giemsa-stained araldite-embedded sections were reviewed. Areas selected for study by electron microscopy included those with eosinophil infiltration in the tumours. Thin sections, double-stained with uranyl acetate and lead citrate, were examined with a Siemens 101 electron microscope.

Results

The main clinicopathological findings of the 38 cases

Table 1. Pathological features observed in 38 cases of minute-small EGC.

CASE	SEX	AGE	SITE	GROSS	SIZE	HISTOLOGY	GASTRITIS	ACTIVITY	RE/HPF-T	MVE/HPF-T	RE/HPF-NTM	MVE/HPF-NTM
1	F	73	LP	I	Mn	Intestinal	CSG	Q	0-2	0.50	0-2	1.50
2	M	71	LP	I	Sm	Intestinal	CAG	Q	0-8	0	0-7	1
3	F	77	LP	I	Sm	Intestinal	CSG	Q	0-6	1	0-1	0
4	M	50	MP	IIb	Mn	Intestinal	CAG	Q	1-13	2	1-25	8.50
5	F	57	MP	IIb	Mn	Intestinal	CAG	A	0-13	3	0-7	1
6	F	76	LP	IIb	Mn	Intestinal	CAG	Q	3-21	5.50	0-8	3.50
7	M	72	LP	IIb	Mn	Intestinal	CAG	Q	1-25	6.50	1-10	4
8	M	78	LP	IIb	Mn	Intestinal	CAG	Q	2-15	7	0-9	2
9	M	28	LP	IIb	Mn	Diffuse	CSG	Q	1-32	8	0-8	2
10	M	68	UP	IIb	Mn	Intestinal	CAG	A	2-26	8	1-9	3
11	M	72	MP	IIb	Mn	Intestinal	CAG	Q	4-29	14.50	0-10	3
12	M	32	MP	IIb	Mn	Intestinal	CAG	Q	14-28	17.50	1-12	5.50
13	F	39	LP	IIb	Mn	Diffuse	CSG	Q	4-22	18.50	0-22	7.50
14	M	78	LP	IIb	Mn	Diffuse	CAG	Q	6-44	19	0-14	1
15	M	67	MP	IIb	Mn	Intestinal	CAG	Q	10-46	21.50	0-13	2.50
16	F	73	LP	IIb	Sm	Intestinal	CSG	O	0-15	2	0-8	1
17	M	67	UP	IIb	Sm	Intestinal	CAG	Q	1-17	4.50	0-16	1
18	M	34	MP	IIb	Sm	Intestinal	CAG	Q	16-32	22	2-13	9
19	M	63	LP	IIc	Mn	Intestinal	CSG	A	0-16	1	0-13	4.50
20	M	55	LP	IIc	Mn	Intestinal	CSG	Q	0-13	2	0-7	1
21	M	70	MP	IIc	Mn	Intestinal	CAG	Q	1-12	7	1-8	1.50
22	F	48	LP	IIc	Mn	Diffuse	CAG	Q	5-30	11.50	1-9	3.50
23	M	54	LP	IIc	Mn	Intestinal	CAG	Q	6-66	14	0-10	2
24	F	65	LP	IIc	Mn	Intestinal	CAG	Q	1-60	20	1-20	5
25	F	83	LP	IIc	Mn	Intestinal	CAG	Q	5-60	22.50	0-18	3.50
26	M	48	LP	IIc	Mn	Diffuse	CSG	Q	17-50	36.50	1-12	6.50
27	M	49	MP	IIc	Sm	Intestinal	CAG	Q	2-10	3	1-13	5
28	F	69	LP	IIc	Sm	Intestinal	CAG	Q	1-14	8	0-10	4.50
29	F	70	LP	IIc	Sm	Diffuse	CAG	Q	1-43	12.50	0-13	1
30	F	68	MP	iic	Sm	Intestinal	CAG	Q	4-38	13.50	0-5	1
31	F	70	LP	IIc	Sm	Intestinal	CAG	Q	2-52	21	0-26	7.50
32	M	54	LP	III	Mn	Intestinal	CAG	Q	0-2	0	0-1	0
33	M	66	MP	III	Mn	Intestinal	CAG	Q	1-17	3.50	1-25	5
34	F	52	LP	III	Mn	Intestinal	CSG	Q	1-16	4	2-14	6.50
35	M	58	LP	III	Mn	Intestinal	CAG	Q	1-30	10.50	0-16	2.50
36	M	77	LP	III	Sm	Diffuse	CAG	Q	0-2	0	0-8	1
37	F	71	LP	III	Sm	Intestinal	CSG	Q	0-15	3.50	0-8	1
38	M	61	LP	III	Sm	Intestinal	CAG	Q	16-26	19.50	3-22	9.50

RE/HPF-T: range eosinophils per high power field in tumour; MVE/HPF-T: median value eosinophils per high power field in tumour; RE/HPF-NTM: range eosinophils per high power field in non-tumoral mucosa; MVE/HPF-NTM: medial value eosinophils per high power field in non-tumoral mucosa; M: male; F: female; Mn: minute; Sm: small, LP: lower portion; MP: middle portion; UP: upper portion; CSG: chronic superficial gastritis; CAG: chronic atrophic gastritis; Q: quiescent; A: active.

of minute and small EGC are summarized in Table 1. The 25 patients with minute EGC included 17 men and 8 women; the ages ranged from 28 to 83 years, with a median age of 63 years. The 13 patients with small EGC included 6 men and 7 women; the ages ranged from 34 to 77 years, with a median age of 70 years.

According to the rules of JRSGC (1981), the carcinoma location in the stomach was the following: 17 minute EGC were in the lower portion; 7 in the middle; and 1 in the upper; 9 small EGC were present in the lower portion; 3 in the middle; and 1 in the upper (Table 1).

By light microscopy, eosinophils were either diffusely scattered as single cells or clustered as 2 or more cells in the tumoral stroma (Fig. 1). Size and histological types of minute-small EGC were not correlated with the tumour eosinophilia (Tables 2, 3).

Table 2. Correlation between degree of eosinophilia and size of EGC.

	TISSUE EOSINOPHILIA		
	<10	≥10<20	≥20
MINUTE	14	7	4
SMALL	8	3	2

χ^2: 0.126. P: N.S.

Table 3. Correlation between degree of eosinophilia and histological types of EGC.

	TISSUE EOSINOPHILIA		
	<10	≥10<20	≥20
INTESTINAL	20	6	5
DIFFUSE	2	4	1

χ^2: 4.385. P: N.S.

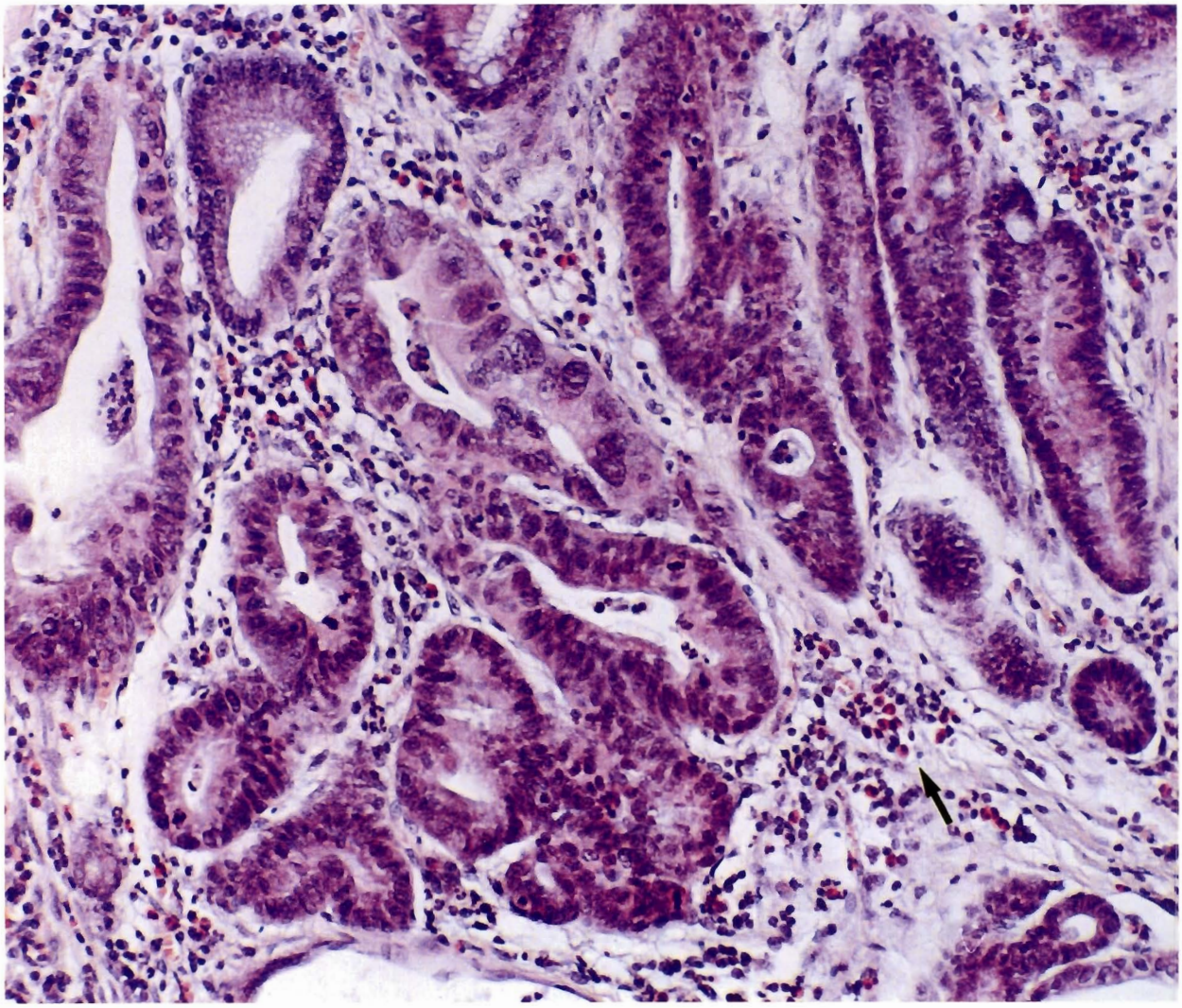


Fig. 1. IIb type minute EGC. Eosinophil clusters near adenocarcinoma glands (arrow). H&E. x 272

There was no correlation between degree of eosinophilia in minute-small EGC and gastritis activity in adjacent non-tumoral mucosa (Table 4). The tissue eosinophilia was usually greater in the tumour than in the adjacent non-tumoral mucosa (Fig. 2); this correlation was statistically significant ($P < 0.001$) (Table 5).

Of the 13 cases of small EGC, slight tissue eosinophilia was present in 8 cases (2 in type I, 2 in type IIb, 2 in type IIc and 2 in type III), moderate in 3 cases (2 in type IIc and 1 in type III) and severe in 2 cases (1 in type IIb and 1 in type IIc). Tumour tissue necrosis was observed near the erosive and ulcerative lesions respectively in 5 cases of IIc and in 3 cases of III type; microfoci of tumour tissue necrosis involving single glands were present in the remaining 5 cases (2 in type I and 3 in type IIb).

In one case of type I minute EGC, showing a diameter of 4.5 mm, multiple erosions associated with microfoci of tissue necrosis were observed; slight tissue eosinophilia was associated. There were 12 cases of IIb type minute EGC with diameter ranging from 1.80 to 3.50 mm in which tumour tissue did not show mucosal erosions, extensive tissue necrosis nor single gland necrosis; they were characterized by slight tissue eosinophilia in 7 cases, moderate in 4 cases or severe in 1 case. In particular, the last case presented an erosive lesion in adjacent non-tumoral mucosa showing a slight eosinophilia (Figs. 3a, b, c). There were 8 cases of IIc type minute EGC with diameter ranging from 3.10 to 4.70 mm. Microfoci of tissue necrosis were present near the erosions. Slight tissue eosinophilia was observed in 3 cases, moderate in 2 cases, and severe in 3 cases. There were 4 cases of III type minute EGC. Their diameter varied from 3.30 to 4.80 mm. Necrosis and granulation tissue could be seen at the base of the ulcer. They were associated with slight tissue eosinophilia in 3 cases and moderate in 1 case. There was no correlation between the degree of eosinophilia and necrosis in the tumours (Table 6) (Fig. 4).

At ultrastructural level, eosinophils were readily

Table 4. Correlation between degree of eosinophilia and gastritis activity.

	TISSUE EOSINOPHILIA		
	<10	≥10<20	≥20
ACTIVE	4	0	0
QUIESCENT	18	10	6

χ^2: 3.251. P: N.S.

Table 5. Correlation between tumour and adjacent non-tumoral mucosa with regard to degree of tissue eosinophilia.

	TISSUE EOSINOPHILIA		
	<10	≥10<20	≥20
TUMOUR	22	10	6
NON-TUMORAL MUCOSA	38	0	0

χ^2: 20.267. P<0.001.

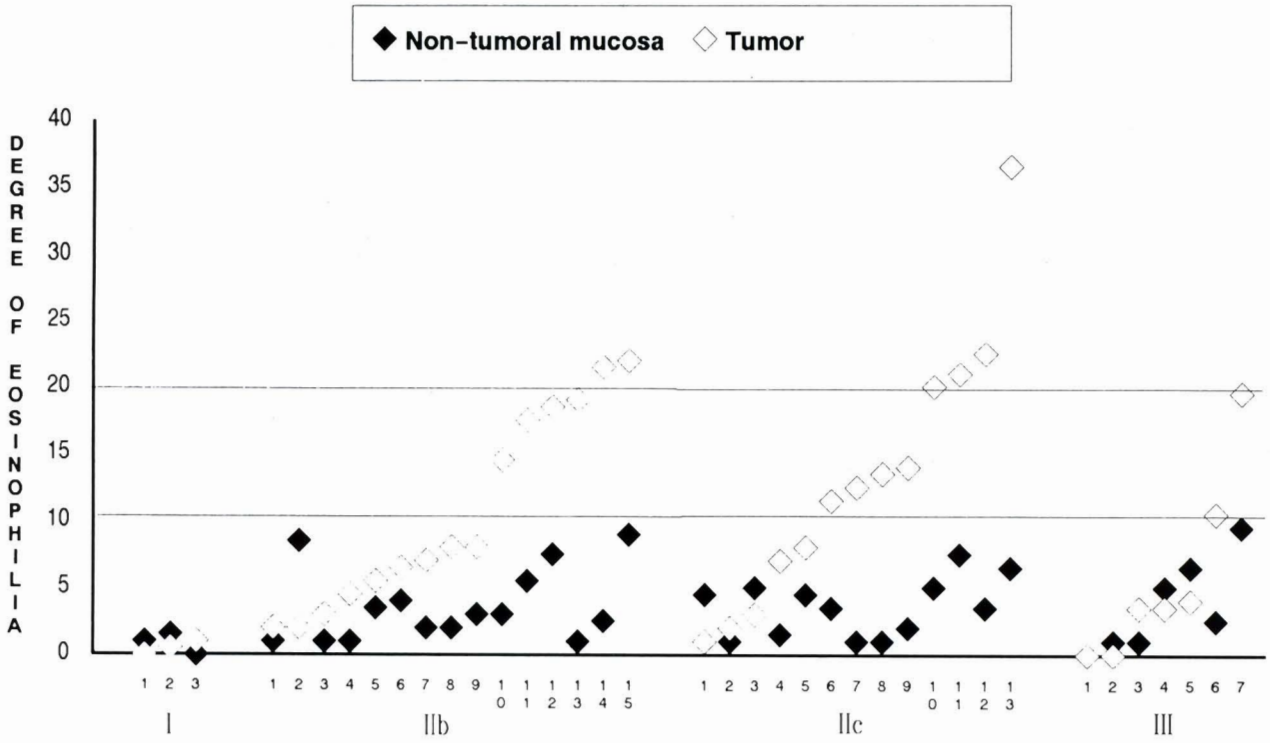
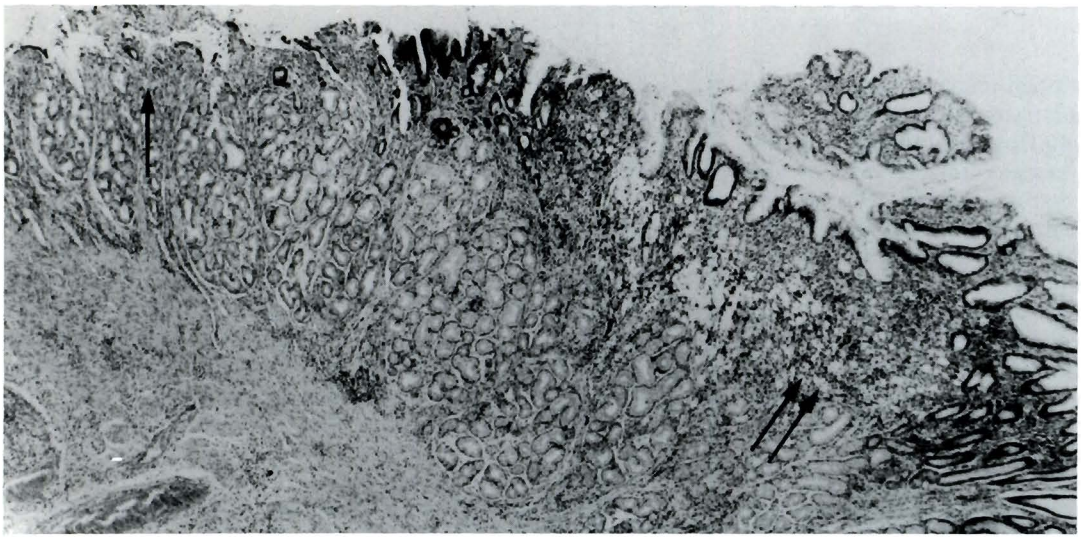
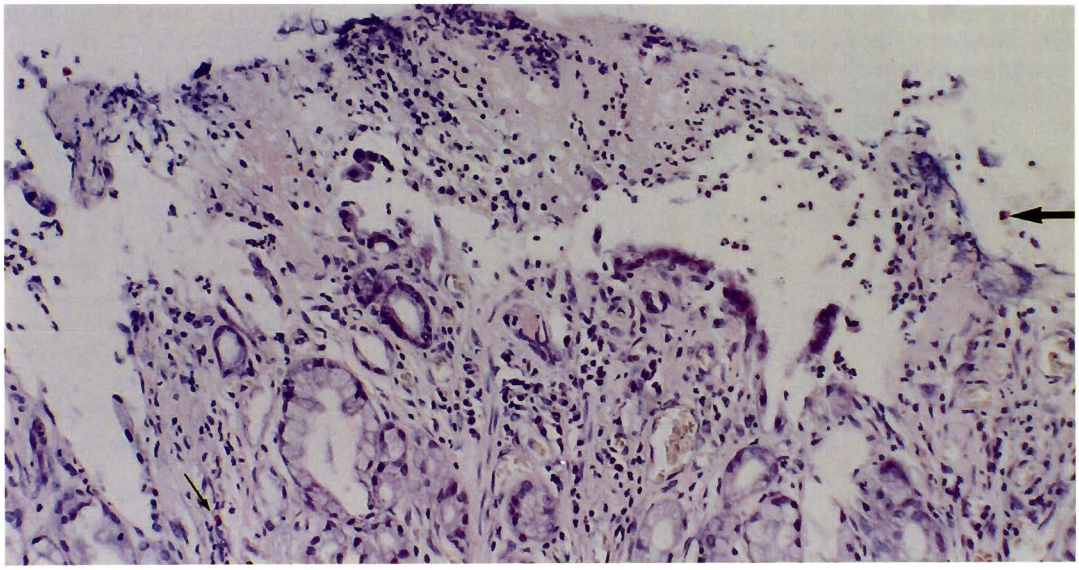


Fig. 2. Scattergram showing the degree of eosinophila in tumour and in adjacent non-tumoral mucosa for each case examined.





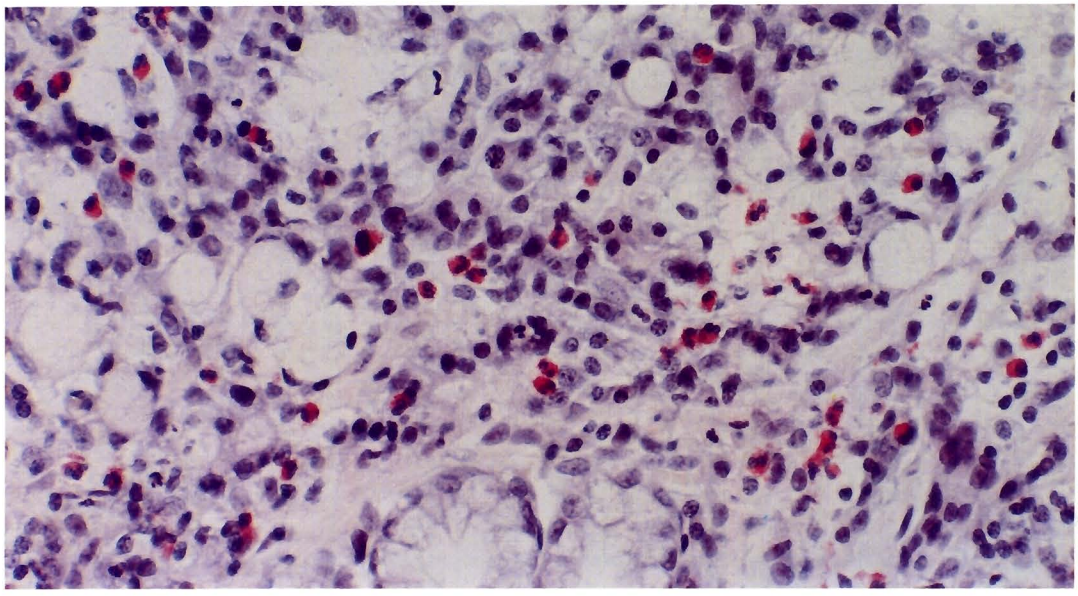


Fig. 3. a. Gastric specimen showing IIb type minute EGC (double arrow). Note the presence of a separate erosive lesion (arrow). H&E. x 35. **b.** Particular view of figure 3a showing erosive lesion with occasional eosinophils (arrow). H&E. x 188. **c**. Particular view of figure 3a showing minute EGC heavily infiltrated by eosinophils. H&E. x 380

recognized by their polylobed nucleus, specific granules and smooth tubulovesicular structures (Fig. 5). Specific granule changes were frequent and ranged from loss of density of the normally dense central crystal to marked loss of density of the entire granule (Fig. 6). Some stromal eosinophils contained reduced numbers of specific granules (Fig. 5). Focal intimate contacts were made between eosinophils and tumour cells; these consisted of sites approximately 1-1.5 μm in length, where the plasma membranes of the respective cells smoothly paralleled each other and were separated by an extracellular gap of uniform width (approximately 25 nm) (Fig. 7). At the region of contact, eosinophils could exhibit micropinocytotic vesicles (Fig. 7) or elongated finger-like surface villi. The subjacent eosinophil cytoplasm occasionally showed classic microtubules (straight, parallel-walled structures, 25 nm across and measuring up to 1.20 μm in length) near the region of membrane contact (Fig. 7). Tumour cells could exhibit focal changes including cytoplasmic vacuoles and blebs as well as markedly dilated perinuclear and endoplasmic cisternae; in some cases, these cytopathic lesions appeared to be localized near the contact region (Fig. 8).

Table 6. Correlation between tumour necrosis and degree of eosinophilic infiltrate.

	TISSUE EOSINOPHILIA		
	<10	≥10<20	≥20
NECROSIS	15	6	5
NO NECROSIS	7	4	1

χ^2: 0.946. P: N.S.

Mitochondrial «high amplitude» swelling was not observed in carcinoma cells (Fig. 8). No evidence of eosinophil phagocytosis of either tumour cells or identifiable tumour cell components was ever observed.

Discussion

Our data showed that the degree of eosinophil infiltration is significantly related to the tumour stroma rather than the adjacent non-tumoral mucosa of minute-small EGC. This TATE was not correlated with the activity of gastritis in the background mucosa. Thus, the possibility that the eosinophil reaction was merely a nonspecific inflammatory phenomenon elicited by surgical tissue manipulation is ruled out.

We discarded the hypothesis that TATE was a necroinflammatory reaction. Solid tumours must induce a new blood supply if they are to grow beyond a diameter of a few millimeters. Furthermore, in most experimental tumours of a size less than 0.5 cm, new vessels are open throughout the tumour, and there is no necrosis (Folkman, 1985, 1990). In the gastric mucosa, cancerous lesions are easily damaged by acid secretion, leading to erosions or peptic ulcerations in the tumour. However, Oohara et al. (1982) showed that type IIc or III minute EGC mostly had a diameter between 3-5 mm, whereas type IIb minute EGC were for the most part less than 3 mm. This is also confirmed in our cases, where IIb type minute EGC are of dimensions less than 3.2 mm, whereas IIc and III type minute EGC ranged, respectively, from 3.10 to 4.70 mm and from 3.30 to 4.80 mm. Moreover, we were unable to observe tissue necrosis in IIb type minute EGC, and no correlation

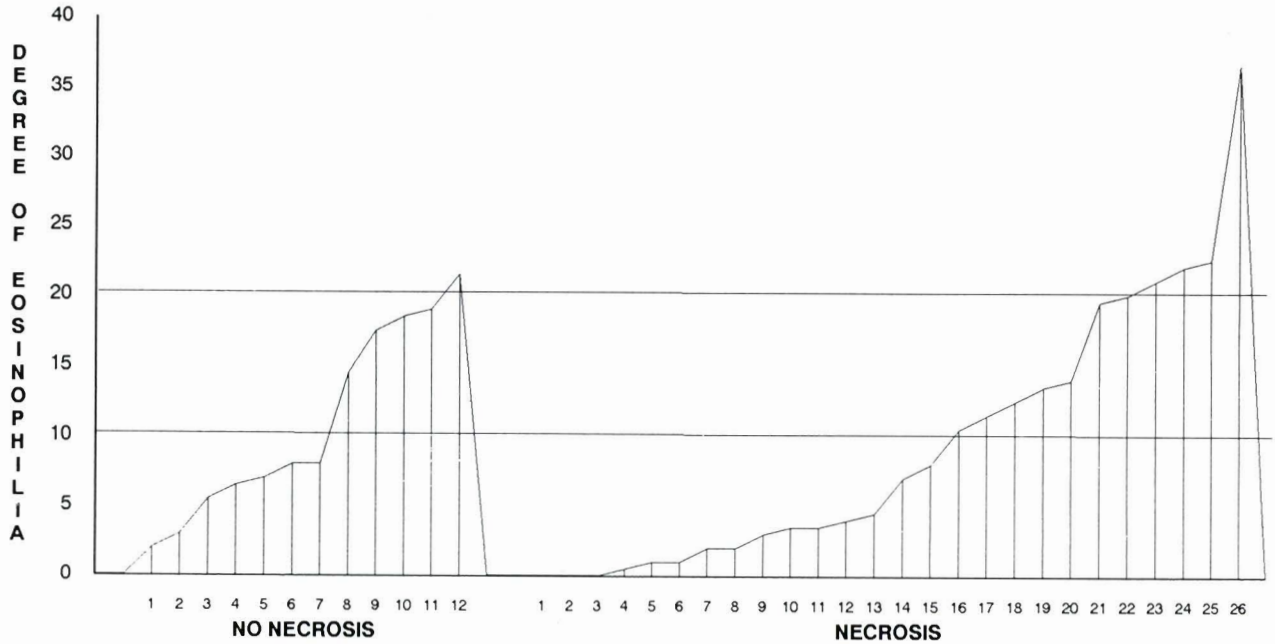


Fig. 4. Histogram showing the degree of eosinophilia in minute EGC without necrosis and in minute-small EGC with necrosis.

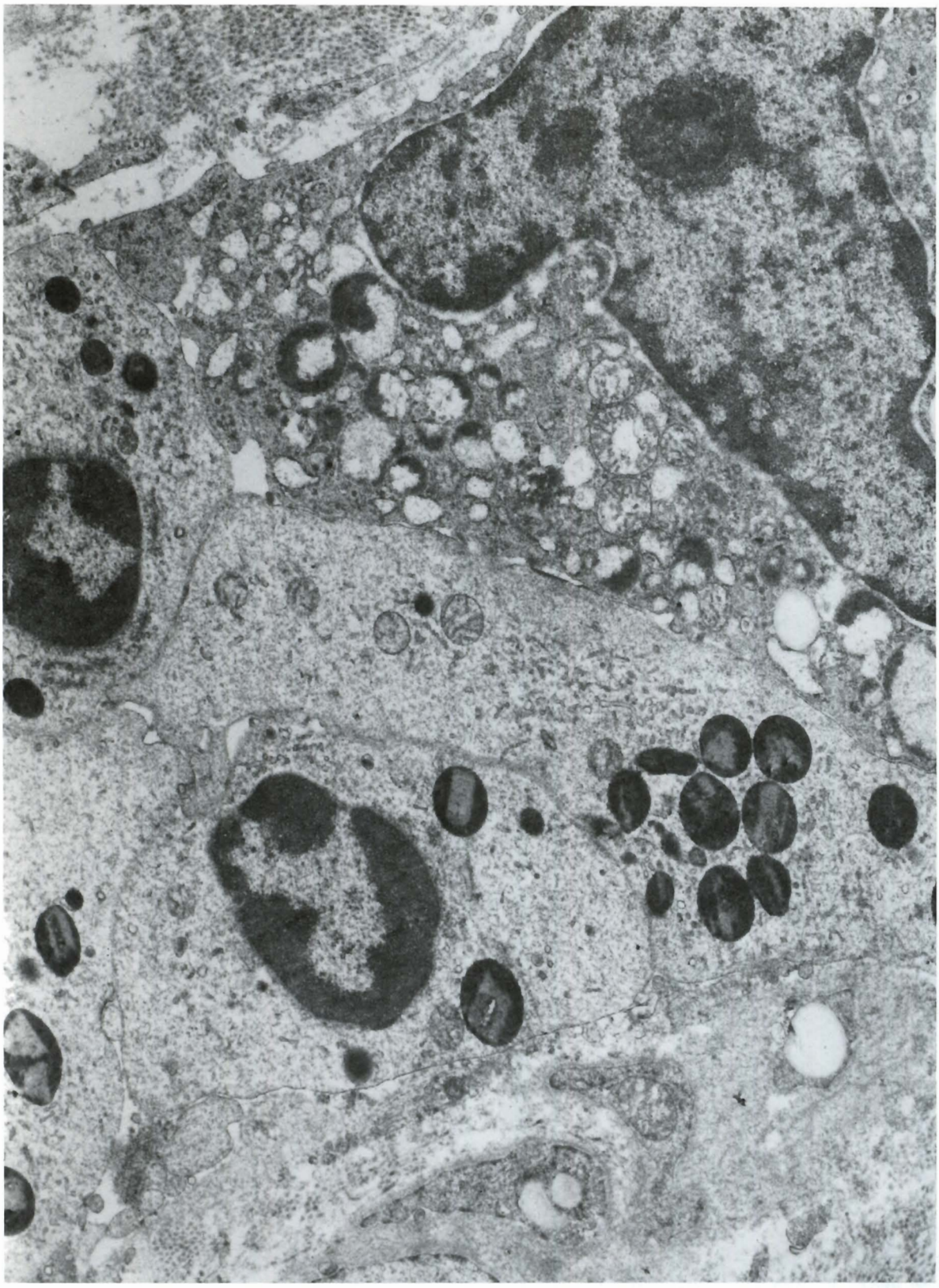


Fig. 5. Eosinophil cluster in proximity of a carcinoma cell. The eosinophil cytoplasm is partially devoid of specific granules. x 12,500

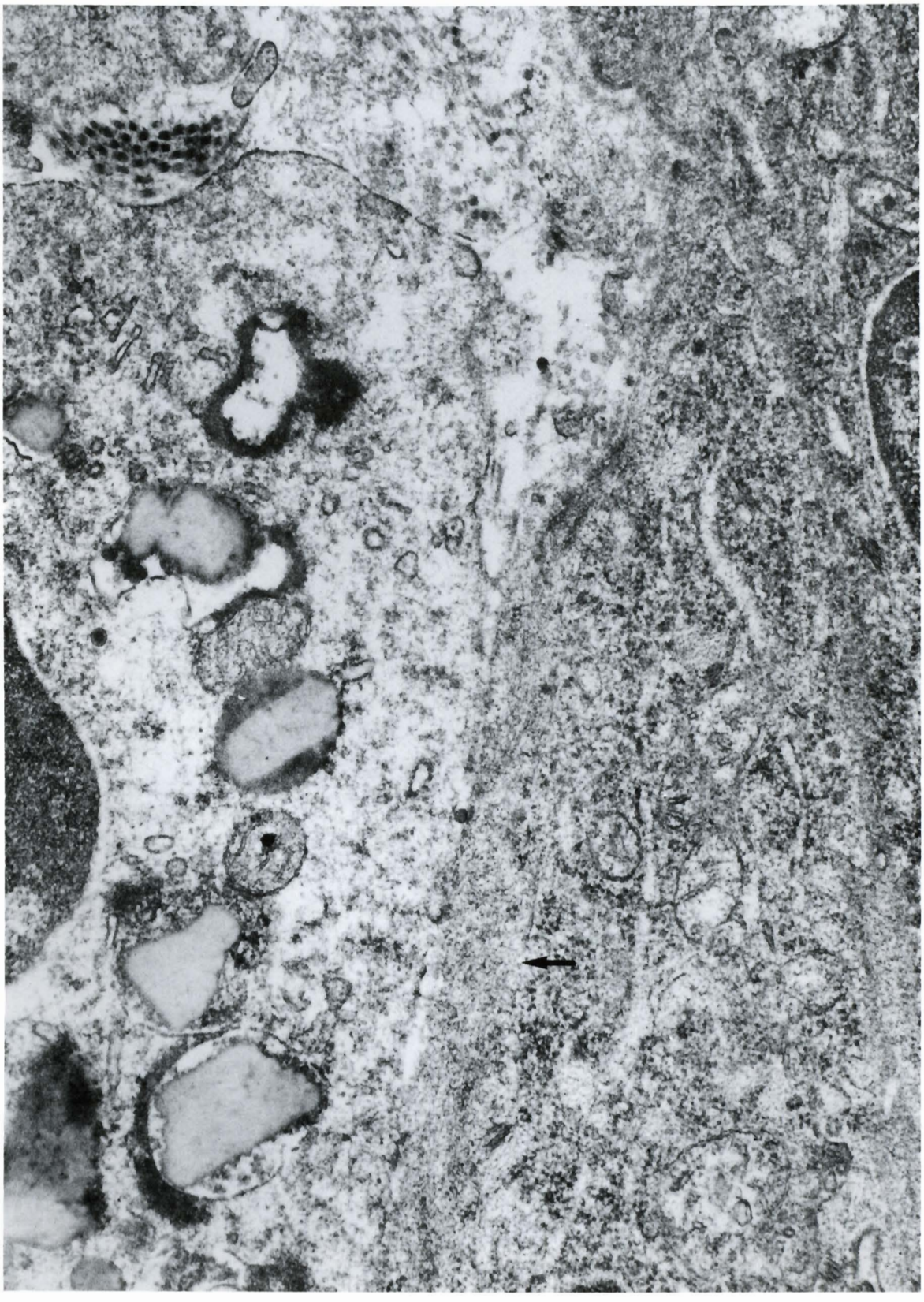


Fig. 6. Eosinophil in contact with tumour cell shows numerous specific granule changes such as loss of their membrane continuity, leakage of the matrix into the cytoplasm or depletion of granule content. Note the aggregates of micro-filaments in the tumour cell cytoplasm at the region of contact (arrow). x 40,000

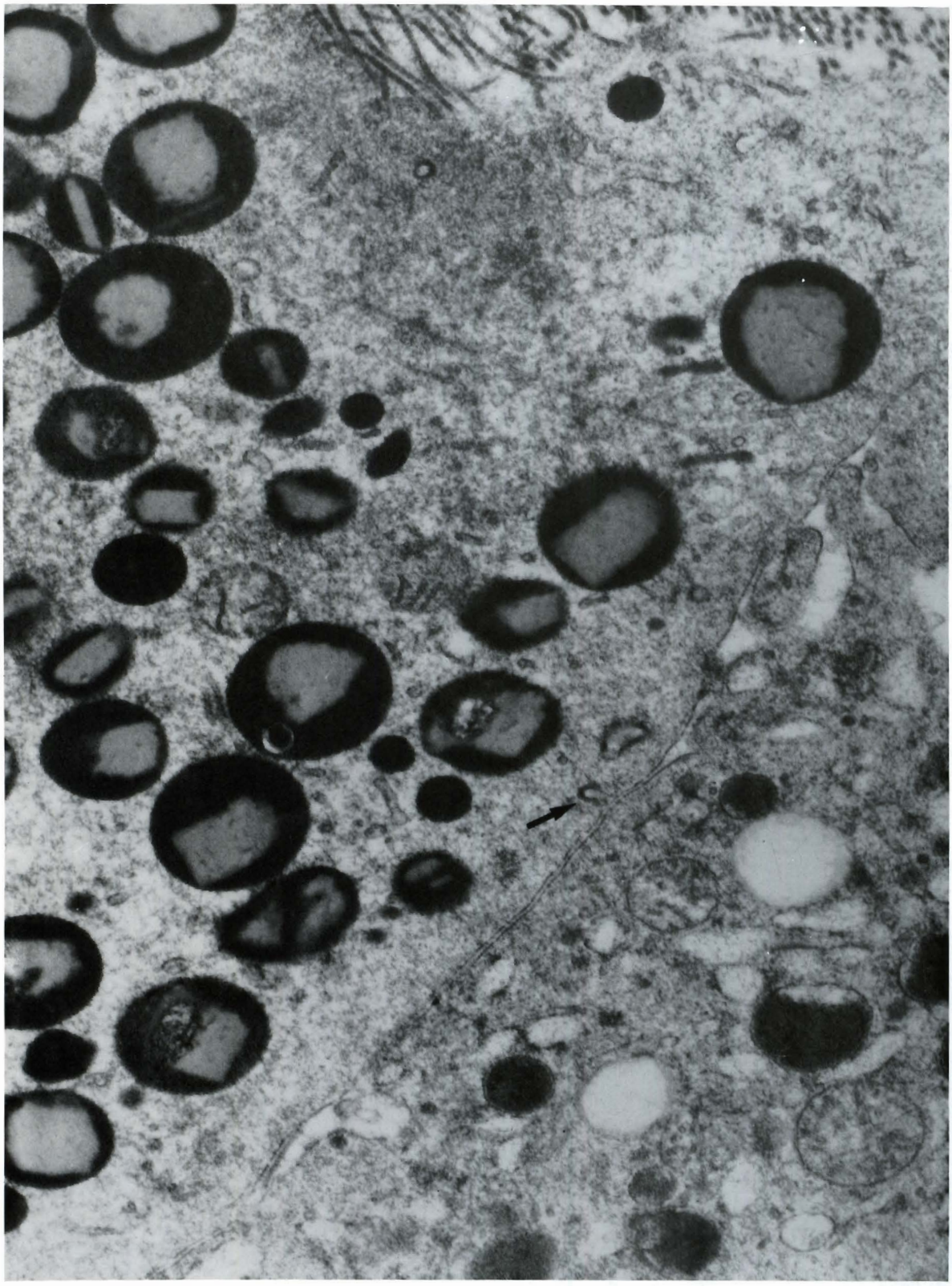


Fig. 7. An eosinophil adhering to a carcinoma cell. Note the plasma membranes of the two cells closely apposed, approximately 25 nm apart. A micropinocytotic vesicle is also observed (arrow). Perpendicular to the region of membrane contact classic microtubules can be observed in the eosinophil cytoplasm. x 40,000

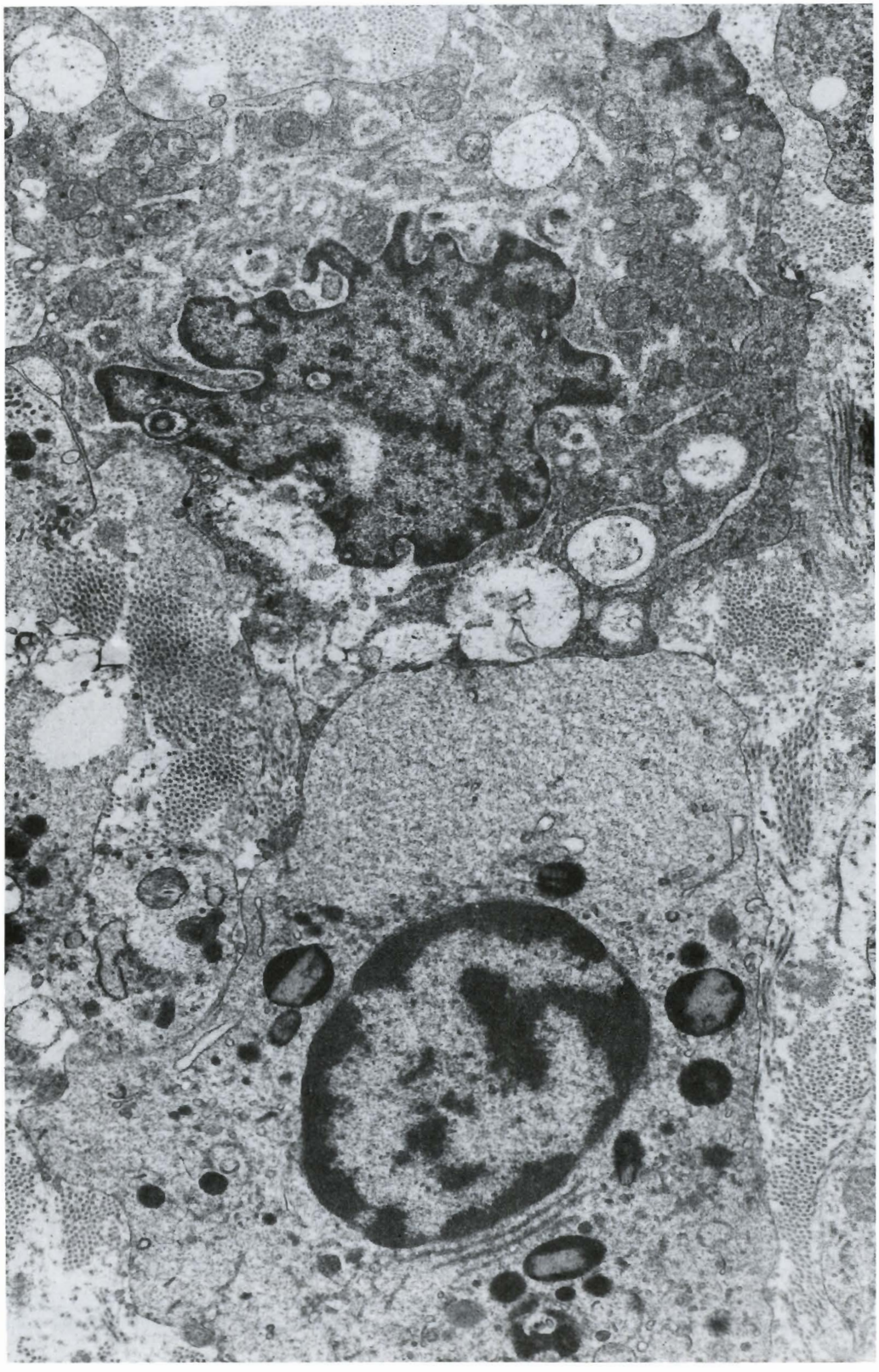


Fig. 8. Eosinophil cytoplasm, devoid of specific granules, is apparently interacting with a carcinoma cell. The latter displays some vacuolization and dilatation of perinuclear cisterna near the contact site. Note the mucin granule and well preserved mitochondria in the remaining cytoplasm of the tumour cell. x 20,000

could be found between the degree of TATE and tumour tissue necrosis. These histopathological observations were confirmed by electron microscopy, which failed to show in the tumour cells the «high amplitude» swelling of mitochondria with loss of cristae, generally considered to be a feature of irreversible injury, related to coagulative necrosis (Trump et al., 1981).

Morphological activation of eosinophils, characterized at light microscopy by eosinophil clusters in the tumour stroma, is confirmed by electron microscopy, which reveals cell-to-cell adhesion of eosinophils as well as diminution of specific granules, increased number of tubulovesicular structures and variable alterations in the matrix and crystalloid compartments of specific granules (Dvorak et al., 1989). Similar eosinophil granule changes have been described in ultrastructural analyses of human eosinophils, studied both *in vitro* and *in vivo* in tissues (Komiyama and Spicer, 1975; Dvorak et al., 1989, 1990, 1991a,b), and may represent release of some granule material (Gleich and Adolphson, 1986; Dvorak et al., 1991b). In our cases the presence of classical microtubules and large nude areas in the eosinophil cytoplasm further support this concept.

There are several potential cytotoxins that reside within the specific granules which lyse tumour cells *in vitro* such as eosinophil cationic protein (Young et al., 1986), peroxidase (Jong and Klebanoff, 1980), and major basic protein (Butterworth et al., 1979). Furthermore, the killing mechanism in some instances requires eosinophil-tumour target cell contact (Young et al., 1986). According to these experimental data, it is tempting to speculate that an eosinophil-mediated colloid osmotic cytolysis occurs in some gastric carcinoma cells showing focal cytopathic changes (i.e. dilatation of the perinuclear cisternae as well as cytoplasmic vacuoles and blebs) in the cytoplasmic areas of intimate contact with partially degranulated eosinophils. It is unlikely that these changes were fixation and embedding artifacts, because they are concentrated at the region of contact with eosinophil, the rest of the tumour cell being morphologically well preserved.

In conclusion, we think that TATE observed in small and minute EGC represents a local host response, not correlated to tissue necrosis. Further studies of the eosinophil-tumour cell interactions are warranted.

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Distribution of cortico-visual neurons projecting to the pons in the cat. A retrograde labelling study with rhodamine latex microspheres

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Summary. Injections of the low diffusion retrograde tracer rhodamine latex microspheres were made in the pontine grey matter of the cat in order to study the cortical convergence to the pons. We found a different distribution of cells in the convex surface of the brain hemisphere making ventral or dorsolateral injections. In the first case, cells were grouped in the top of the gyri. In the second case, cells were more frequent in the bottom of the sulci. Our results show a possible retinotopic organization of this projection.

Key words: Visual cortico-pontine grey matter projection. Rhodamine latex microspheres, Retrograde labelling

Introduction

The cortico-visual pontine grey matter pathway is involved in many important processes such as control of ocular position, the following of objects, etc. This is because it is integrated in the cortico-ponto-cerebellar circuit. Numerous studies have been carried out using degeneration or peroxidase transport techniques.

The first description of the advantages of the rhodamine latex microspheres (RLM) was provided by Katz et al. in 1984. It involves small acrylic spheres (0.02-0.2 μm in diameter) which, when injected into the nervous tissue, diffuse very little, even over long periods of time, giving a very well delimited injection site. The transport time is very short, and generally speaking, after twelve hours it is already possible to detect a clear labelling. This marker is neither phototoxic nor cytotoxic. Once retrogradely tranported, the spheres are confined to the cytoplasm and adjacent parts of dendrites and axon (Katz, 1987). It is thought that they are trapped by the damaged fibres as a result of the injection, although it is also possible that they are uptaken by synaptic clefts through an endocytosis process (Gonatas et al., 1972). The microspheres are probably transported inside vesicles, as their negative charge would cause them to be transported anterogradely rather than retrogradely (Adams and Bray, 1983). It appears that the uptake process requires the spheres to undergo a carboxylation process (Cornwal and Phillipson, 1988). The transport capacity of the RLM depends on their size; those measuring 100 nm or more in diameter remain at the injection site or along the axon; however, those that reach the cellular soma, included in lysosomes, are always the smaller spheres (Holländer et al., 1989). This is because of the relatively small size of the extracellular space (in the case of broken ends) or of the synaptic clefts (in the case of synaptic capture), both of which act as «filters» for diffusion of the larger globules (Cornwald and Phillipson, 1988). The RLM may also be used as a tracer visible under an electron microscope, employing potassium permanganate for negative contrast (Egensperger and Holländer, 1988). One particular interesting application is the simultaneous implementation with Golgi's technique, which may constitute an alternative to the intracellular injection in the entire animal (Cactsicas et al., 1986). Katz and Iarovici (1990) have recently introduced to neurobiological research a new tracer in which the acrylic spheres emit a green light when illuminated with the appropiate filter set. The use of fluorescent dyes may be of special interest in double labelling experiments.

Our aim was to study the visual corticopontine projection by means of the transport of RLM, presenting the advantages and disadvantages of its utilization.

Materials and methods

We used 5 adult cats of both sexes, weighing between 1,700 and 2,500 grs. The animals were anaesthetized with a mixture of Ketamine chlorhydrate (Ketolar®) (0.2 ml/kg of a solution 50 mg/ml) and tiazine chlorhydrate (Rompun®) (0.06 ml/kg of a solution 23.32 mg/ml) and then placed in a stereotaxic frame. 0.5 μl of RLM (Luma Fluor, Inc.) were injected into the ventral and

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