Loss of Isoantigens A, B, and H in Carcinoma of the Lung

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ISOANTIGENS A, B, AND H, identical with those present in erythrocytes, are also found in some normal tissues. These include endothelial cells lining blood and lymph vessels, squamous epithelium of the tongue and portio vaginalis of the cervix, columnar epithelium of the gastric and bronchial mucosa, transitional cell epithelium of the urinary bladder, and others. They are absent in the basal layer of stratified squamous epithelium, in the cells of connective tissue, and in others to be mentioned later.

Immunofluorescence technique has been used widely for demonstration of these isoantigens. We also used it at first, but we found the mixed cell agglutination reaction (MCAR) to be reproducible, more sensitive, and technically easier. The literature on the use of both techniques in the demonstration of the three isoantigens can be found in our previous publications.[1-4]

The intensity of the MCAR is the same in benign lesions as it is in normal tissues from which they originate. On the other hand, we found the MCAR reduced in intensity or negative in a variety of carcinomas. The gradual decrease of the isoantigens was shown impressively in the cervix where the progression of changes can be followed from benign and reversible lesions (metaplasia and dysplasia) through carcinoma in situ, to infiltrating carcinoma, and eventually to metastatic carcinoma.[4]

In the present report we will relate our studies on the fate of isoantigens A, B, and H in bronchogenic carcinoma of the lung.

Materials and Methods

Biopsy specimens were obtained from 75 patients, and tissues of primary and metastatic carcinomas from 52 necropsies.

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Reagents and methods employed in this study were described in detail in previous publications.[3,4]

The controls employed in this study are listed below:

Reagents

1. Human isologous antisera (anti-A and anti-B)
2. Anti-H reagent: extract of *Ulex Europeus*
3. Isologous indicator erythrocytes (ABO)

Reagent controls

1. Not isologous antiserum and isologous indicator erythrocytes
2. Isologous antiserum and not isologous indicator erythrocytes
3. Antiserum absorbed with isologous erythrocytes
4. Antiserum neutralized with isologous purified blood group substance

Results: *Negative MCAR*

Built-in positive controls

1. Erythrocytes in lumina and endothelial cells lining blood and lymph spaces
2. Tissues normally containing isoantigens A, B, H: squamous epithelium of cervix, pancreas, etc

Built-in negative controls

Tissue normally lacking isoantigens A, B, H—ie, brain, hepatocytes, cells of the adrenal, islets of Langerhans, etc

Results

The diagnoses of the 75 biopsy specimens were: bronchogenic carcinomas in 52, squamous cell metaplasia in 5, chronic bronchitis in 4, and normal bronchial tissue in 14.

The diagnoses in the 52 necropsies included squamous cell and adenocarcinomas with varying degrees of anaplasia, oat cell carcinomas, and bronchiolar carcinomas, in the primary lesions and in metastases. The sites of the metastases are listed in Table 1.

The MCAR in Non-neoplastic Conditions

Normal Tissue. The massive accumulations of indicator erythrocytes on the mucous glands of the normal submucosa of the bronchus and their absence on the serous glands were good examples of a positive and a negative MCAR in normal tissues (Fig 1).

Inflammation. The MCAR is positive in the surface epithelium and on the endothelial cells of the richly vascularized subepithelial connective tissue (Fig 2). The positive reaction in the vascular spaces of the connective tissue is a built-in natural positive control.

Metaplasia. The metaplastic transformation involving the squamous epithelium on the surface and extending into the submucosal connective tissue did not influence the MCAR, indicating preservation of the tissue isoantigens (Fig 3).

Squamous Cell Carcinoma. The section shown in Fig 4 is from the same patient as in Fig 3. Here, in the squamous cell carcinoma, the

Table 1. MCAR in Metastases of Bronchogenic Carcinoma.

Body area		No. of specimens		
		MCAR−	MCAR+	MCAR±
	In Lymph Nodes			
Regional	Thoracic*	40	1	1
Distant	Cervical	5	—	—
	Abdominal	13	—	—
	Retroperitoneal	3	—	—
Total		61	1	1
	In Other Organs			
Regional	Pleura and thoracic wall	4	—	—
Distant	Abd. wall	1	—	—
	Adrenal	20	—	—
	Bones	14	—	—
	Brain	14	—	—
	Colon	2	—	—
	Duodenum	1	—	—
	Gallbladder	1	—	—
	Heart	14	—	—
	Kidneys	15	—	—
	Liver	30	—	—
	Pancreas	7	—	—
	Periaortic tissue	1	—	—
	Peritoneum	2	—	—
	Pituitary	3	—	—
	Scalp	1	—	—
	Skin	1	—	—
	Small Intestine	2	—	—
	Spleen	5	—	—
	Stomach	2	—	—
	Thyroid	3	—	—
	Vena cava	2	—	—
Total		145	0	0

* Includes periaortic, peribronchial, tracheal, and mediastinal.

MCAR is negative. The positive MCAR is limited to a few streaks in the necrotic core in the large semicircular metastatic mass. The positive MCAR in the vascular spaces of the connective tissue is the built-in control permitting the inference that the negative MCAR in the carcinoma is due to the loss of the isoantigen. A comparison with the positive MCAR in Fig 3 allows the further conclusion that the loss of the isoantigen occurred during the transformation from the metaplastic process to the cancer. The same applies to the squamous cell carcinoma invading the pulmonary alveoli in Fig 5. Here, the positive reaction in the framework of the richly vascularized interalveolar septa and in the large distended blood vessel serves again as a positive control and the connective

tissue of the wall of the blood vessel as the negative control of the immunologic isoantigen-antibody reaction.

Anaplastic Carcinoma. In Fig 6 the isoantigen is absent in an oat cell carcinoma that extends into pulmonary alveoli. The negative MCAR in the thick wall of the blood vessel in the right lower corner of Fig 6 is accentuated by the positive MCAR of the delicate string of the indicator erythrocytes along the endothelial lining of the same blood vessel: built-in positive and negative controls in one.

Adenocarcinoma. In this variety of bronchogenic carcinoma, we encounter the same phenomenon of isoantigen loss (Fig 7).

The relation between the histologic structure of the neoplasm and the loss of tissue isoantigens is summarized in Table 2.

Metastatic Carcinoma. The MCAR was negative in all distant metastases as summarized in Table 1. The rare exceptions in metastases in regional lymph nodes will be discussed later.

A metastasis in the liver of a highly anaplastic bronchogenic carcinoma, sometimes referred to as giant cell carcinoma, is shown in Fig 8. The MCAR is negative in the carcinoma as well as in the hepatocytes, in the latter because normally they do not have the isoantigens A, B, and H. The sinusoids of the hepatic parenchyma are positive. The situation is analogous in the adrenal. The isoantigens A, B, and H are not found in the cells of the adrenal parenchyma. Accordingly, the MCAR is negative in them as well as in the metastatic carcinoma. The rich vascular supply of the adrenal is responsible for the positive MCAR in the top half of Fig 9.

The epithelial cells of the exocrine parenchyma of the pancreas contain the isoantigens, whereas the cells of the islets of Langerhans do not. Here again built-in positive and negative controls are present (Fig 10).

A metastasis to the kidney of an anaplastic bronchogenic carcinoma is shown in Fig 11. The renal tubular epithelium lacks the isoantigens as does the metastasis. The positive control in this situation is furnished by the glomeruli, as expected, and by the other blood vessels. A metastasis of a bronchogenic carcinoma to the brain is illustrated in Fig 12.

Table 2. MCAR in Bronchogenic Carcinoma.

	MCAR Results											
	Squ cell		Oat cell		Anaplastic		Bronchiolar		Adeno			
	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(±)	(−)	
Biopsy	0	32	0	9	0	3	0	0	2	0	6	
Autopsy	0	16	0	15	0	11	1	1	1	1	6	
Totals	0	48	0	24	0	14	1	1	3	1	12	
Neg MCAR (%)	100		100		100		50		75			

Cerebral parenchyma lacks tissue isoantigens. This then is the same situation as we saw it in the kidney, liver and adrenal. Again, positively reacting blood vessels are helpful.

Positive MCAR in Bronchogenic Carcinoma

The MCAR was positive in five carcinomas, two in a series of 52 surgical biopsies (4%), and three in 52 necropsies (6%) Table 2). In the biopsies, there was no clinical or radiologic evidence of metastases at the time of the operation. The situation was similar to the previously reported positive MCAR in three carcinomas of the cervix in a series of 35 cases.[4]

The three necropsies, two adenocarcinomas, and one bronchiolar carcinoma provided the opportunity for a more extensive study and for formulation of a hypothesis relating to the pathogenesis of this rare immunopathologic finding.

The MCAR in the anaplastic infiltrating carcinoma was positive in one field (Fig 13B) and negative in another field of the same side (Fig 14B). The same applies to metastases in regional lymph nodes: positive MCAR in Fig 15B and negative in Fig 16B. On the other hand, there were no discernible morphologic differences in the degree of anaplasia between the corresponding hematoxylin and eosin-stained sections in Fig 13A, 14A, 15A, and 16A. This shows that the sensitivity of the immunologic reaction is considerably greater than the morphologic or cytologic changes seen in the hematoxylin and eosin-stained sections would indicate. The MCAR was negative in all distant metastases including the one in the liver as shown in Fig 17.

The 19 illustrations used in this paper came from tissues of 12 patients. The incidence of A, B, and O blood groups in this small sample is not random. The reason for the small number of O group was the difficulty in preparing a good anti-H reagent from *Ulex Europeus*. It was adequate for identification of group O in tissues but the photographs of preparations with the MCAR were not as good as those obtained with anti-A and anti-B reagents. The incidence of A, B, and O blood groups in the total material was the same as in the general population.

Discussion

Transition from Reversible Lesions to Carcinoma

This change in morphology was usually, but not always, accompanied by a change in immunologic reactivity, namely, the loss of demonstrable tissue isoantigens A, B, or H. Both conditions could, in some instances,

be demonstrated simultaneously as shown in Fig 3 with a positive MCAR and in Fig 4 with a negative reaction. Both lesions were present in the same patient at the same time.

In the case of the positive MCAR in adenocarcinoma in which necropsy material was available, positive and negative MCAR were present side by side in the primary carcinoma and in the regional lymph nodes, whereas the MCAR was negative in all distant metastases.

These findings permit the following interpretation: The loss of the isoantigens expressed in the negative MCAR need not be a nothing or all phenomenon. It may be developing gradually in the primary lesion. In case of a positive MCAR in a carcinoma, examination of additional sections may be rewarded by finding a negative reaction, as it happened in our case.

The finding of a positive MCAR in a regional lymph node attached to or near the primary lesion may be explained by continuity of growth from the primary carcinoma of both positively and negatively reacting portions of the cancer. Obviously this hypothetical explanation will have to be checked by the study of larger necropsy material. The fact remains that in our study of 44 biopsies and 42 necropsies of squamous cell, oat cell, and anaplastic carcinomas, the MCAR was 100% negative (Table 2).

These conclusions are supported by the not infrequent finding of metaplasia and infiltrating epidermoid carcinoma side by side as illustrated in Fig 3 and 4. This is an accepted finding in the natural history of the development of carcinoma. It is proper to apply the same reasoning to the presence of positive and negative MCAR in the primary carcinoma and in regional lymph node metastases.

Here we wish to submit a possible explanation of the phenomenon: The findings in the primary carcinoma suggest that the loss of the isoantigens need not necessarily take place in the entire carcinoma at the same time. If the concept is correct that the change from a positive to a negative MCAR is the result of an immunologic dedifferentiation, then it would not be surprising to find different stages of dedifferentiation side by side. The regional lymph node in Fig 15 and 16 was contiguous with the bronchus and the primary carcinoma. Contiguity may explain the presence, simultaneously, of metastatic lesions with positive and negative MCAR.

The other metastases, including those in the liver, kidney, brain, and bone, all of them distant, had negative MCAR. The same applies to the long list of metastatic carcinomas, 145 of them in various organs, and in the 21 lymph nodes other than regional. In all of them, the MCAR was

negative as shown in Table 1. Even in the 42 regional thoracic lymph nodes only one had a positive MCAR, and the other, the case reviewed here, had both a positive and a negative MCAR.

The second instance of a positive MCAR in the primary lesion and in a regional lymph node metastasis was found in a bronchiolar adenocarcinoma. This patient, a 68-year-old nonsmoker, had a lobotomy in which a bronchiolar carcinoma was found. He expired suddenly, 6 months later. The cause of death was pulmonary embolism. No other metastases were found at necropsy.

The Isoantigens A, B, and H and the PAS Reaction

Sections of adenocarcinomas were studied with the PAS stain. There was no relation between the presence and absence of the isoantigens A, B, and H and the PAS reaction.

The Relation Between the Degree of Anaplasia and the Loss of Isoantigens

Simultaneous and side by side positive and negative MCAR may be seen in carcinomas with morphologically different degrees of anaplasia. Such findings are recorded in Fig 18 and 19. In Fig 18, a highly differentiated infiltrating adenocarcinoma gave a positive MCAR. In Fig 19, a highly anaplastic carcinoma with a negative MCAR in the same patient surrounds two isolated glands morphologically identical with those seen in Fig 18. The two morphologically distinct neoplasms give two different immunologic reactions.

Summary

Isoantigens A, B, and H are present in some normal tissues and in benign neoplasms originating in these tissues. We used the mixed cell agglutination reaction (MCAR) for detection of these antigens. The MCAR can be applied to recent and old sections prepared from formalin fixed and paraffin embedded tissues. We were able to demonstrate the described phenomena in tissues stored for at least 10 years.

The normally positively reacting tissues (eg, the endothelial cells lining the vascular spaces and the erythrocytes present in the lumina, the normal stratified squamous epithelium of the cervix, the columnar epithelium of the bronchial mucosa and of the mucous glands of the bronchial submucosa) and the normally negatively reacting tissues (eg, connective tissue stroma, hepatocytes, and islands of Langerhans in the pancreas) served as built-in positive and negative controls.

The MCAR was positive in hyperplastic and metaplastic bronchial epithelium, and negative in over 90% of bronchogenic carcinomas.

In fifty cases of metastatic bronchogenic carcinoma, the isoantigens could not be demonstrated in the primary carcinoma and in the distant metastases. The findings suggest that the loss of demonstrable isoantigens A, B, and H precedes the formation of distal metastases. In 2 cases metastases to peribronchial lymph nodes had demonstrable isoantigens. A hypothesis was suggested to explain the pathogenesis of this phenomenon.

The generally accepted criterion of malignancy is anaplasia, the morphologic evidence of dedifferentiation. We interpret the loss of the A, B, and H isoantigens as the result of immunochemical dedifferentiation in the course of cancerous transformation. The change is analogous to morphologic dedifferentiation.

In case of carcinoma of the bronchus, the results of the MCAR may influence the diagnosis and prognosis. In cases in which the diagnosis of carcinoma in situ is being considered, and the opinions are divided, a negative reaction will favor the less auspicious interpretation. The probability of distant metastases is greater in the presence of a negative reaction and vice versa. The potential clinical implications of these findings, if confirmed by sufficiently large and varied material, are obvious.

The decrease and loss of the isoantigens may be the result of: (1) decrease of ability of epithelial cells to produce them, or to store them, if produced elsewhere; (2) possibly changes in the cellular membranes; (3) a change in their demonstrability by the MCAR; (4) other as yet unknown factors; or (5) of a combination of these.

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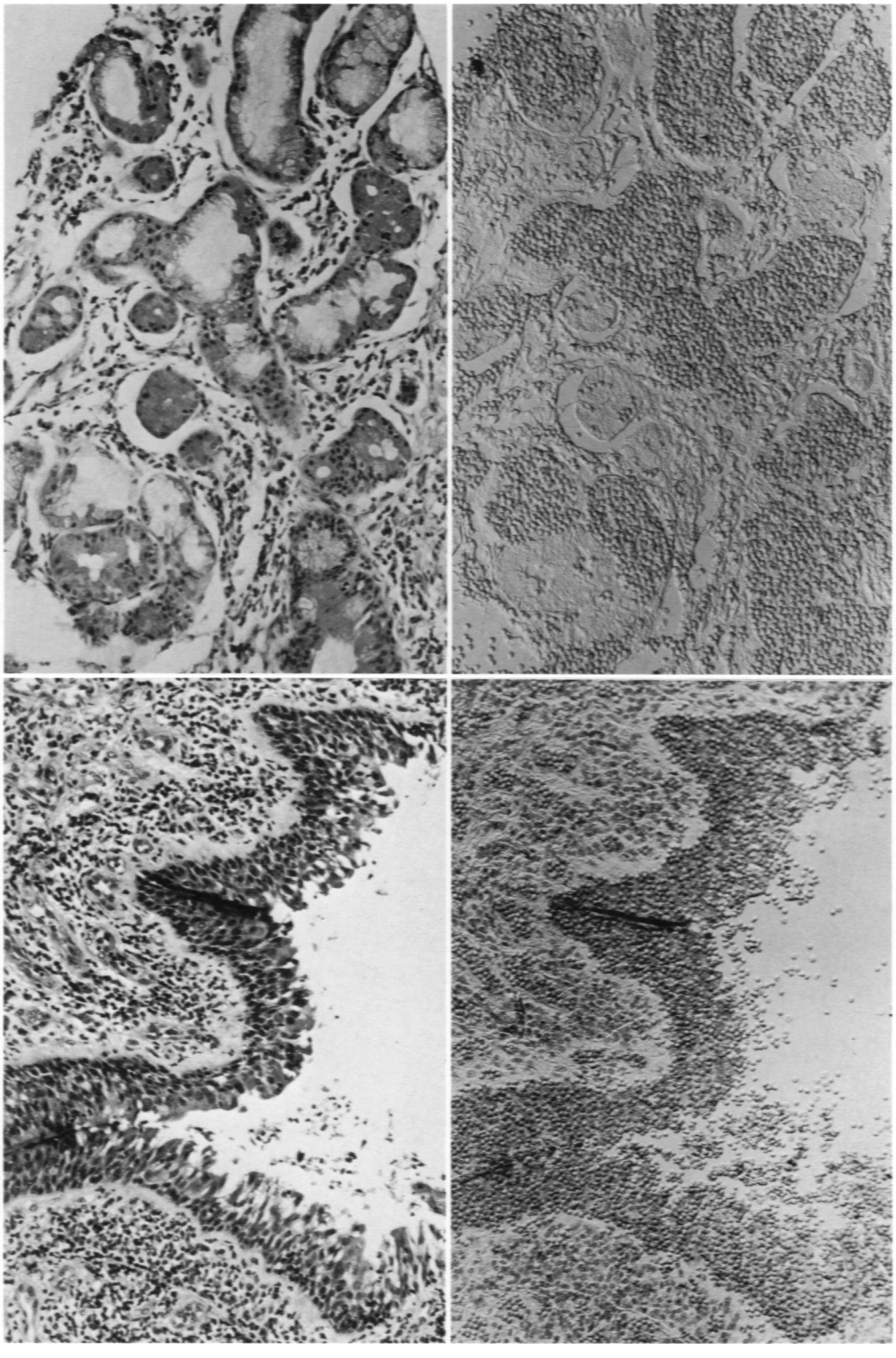
[*Illustrations follow*]

Legends for Figures

Fig 1. Bronchus, submucosa. Female, age 50, blood Group B. **A.** Normal mucous and serous glands. H&E. ×160. **B.** MCAR. Positive reaction over distended mucous glands lined with pale epithelium. Negative reaction over serous glands with pinpoint sized lumina and lined with dark epithelium. ×160.

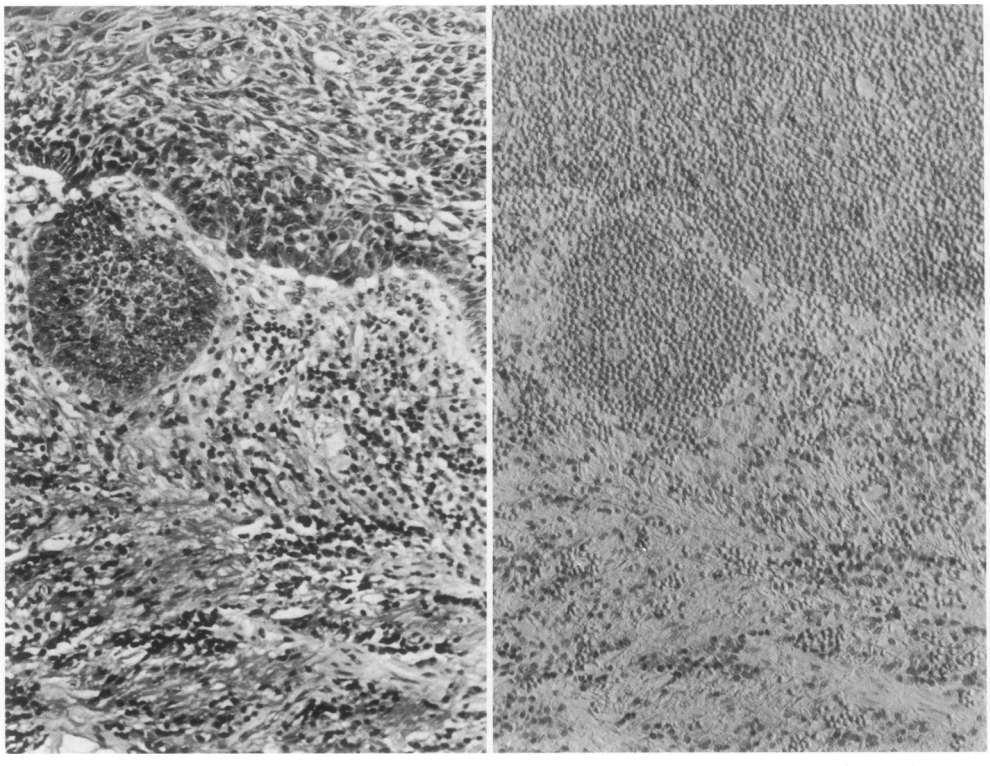
Fig 2. Chronic bronchitis. Same patient as in Fig 1. **A.** High columnar epithelium. Stroma richly vascularized and densely infiltrated with lymphocytes. H&E. ×160. **B.** Strongly positive MCAR. Surface columnal epithelium is covered densely with indicator erythrocytes of Group B. Streaks and groups of erythrocytes are also aggregated in lumina and along endothelial lining of vascular spaces. ×160.



1A 1B

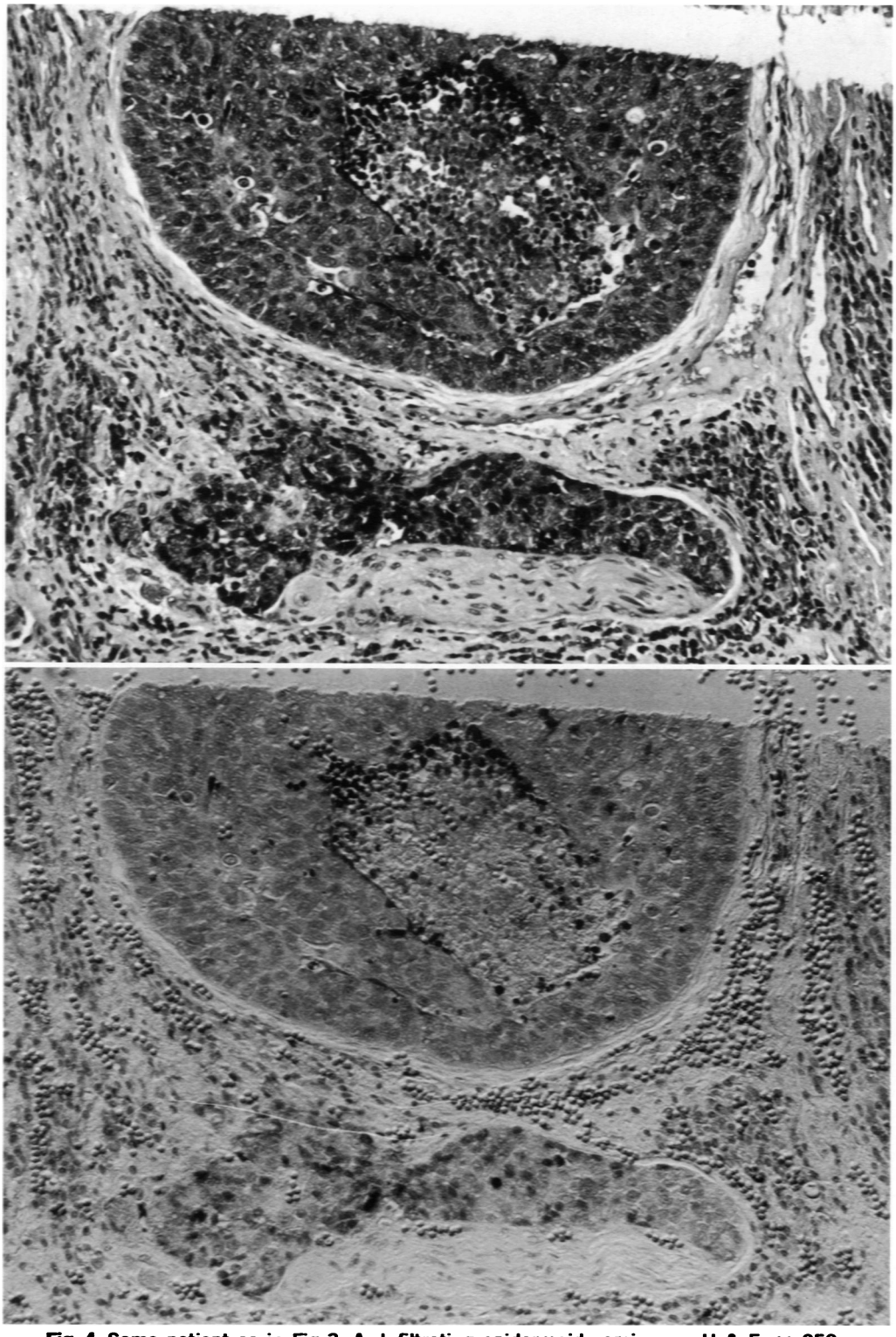
2A 2B

3A



3B

Fig 3. Bronchus. Male, age 46, blood Group B. **A.** Squamous cell metaplasia. H & E. × 160. **B.** Positive MCAR. × 160.



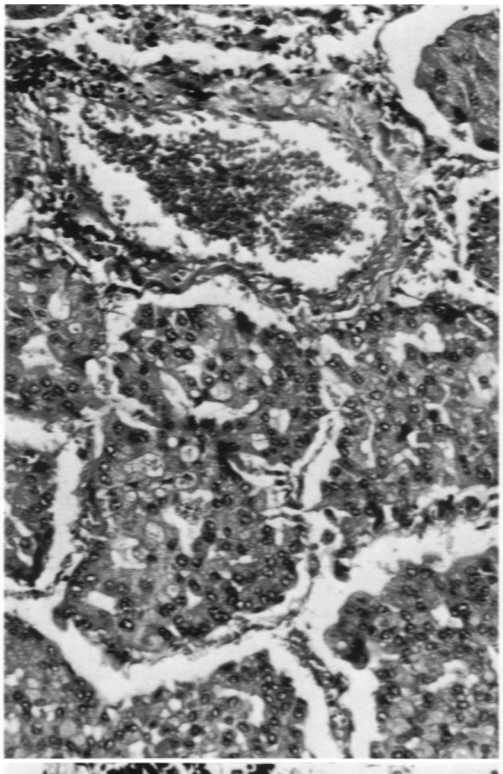
4A

4B

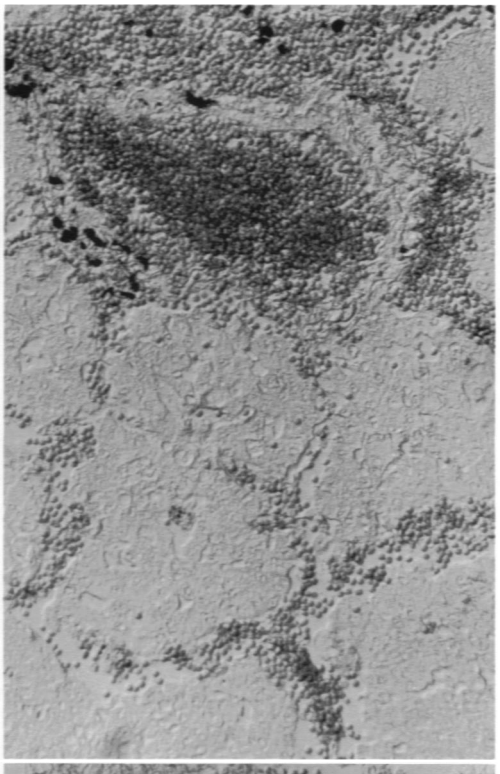
Fig 4 Same patient as in Fig 3. **A.** Infiltrating epidermoid carcinoma. H & E. × 250. **B.** Negative MCAR over carcinoma, positive in vascular spaces and, in spots, over central necrotic area. × 250.

Fig 5. Male, age 50, blood Group A. **A.** Infiltrating squamous cell carcinoma invading alveoli. H & E. × 160. **B.** Positive MCAR on alveolar septa and in distended vein at top contrasts sharply with negative reaction of invading carcinoma × 160.

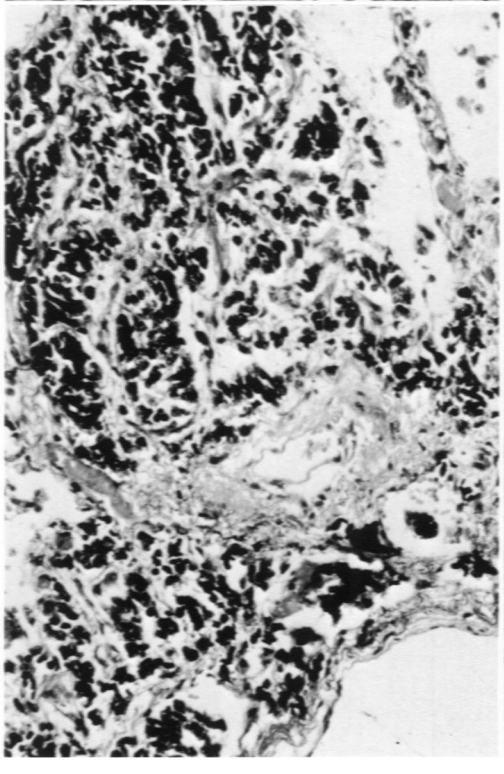
Fig 6. Male, age 63, blood Group A. **A.** Oat cell carcinoma, infiltrating. H & E. × 160. **B.** Negative MCAR. Endothelium lining segment of blood vessel at lower right corner is distinctly outlined by indicator erythrocytes. × 160.



5A



5B



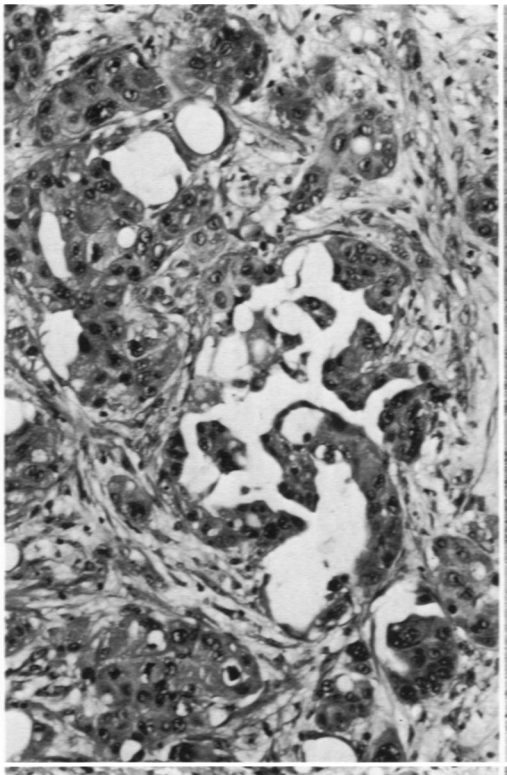
6A

6B

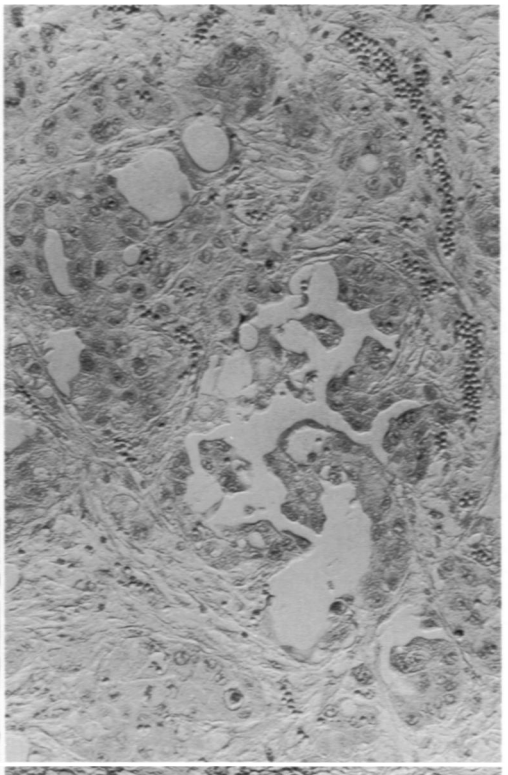
Fig 7. Female, age 49, blood Group B. **A.** Infiltrating anaplastic adenocarcinoma. H & E. × 160. **B.** Negative MCAR. Positive MCAR streaks of indicator erythrocytes in blood vessels. × 160.

Fig 8. Male, age 73, blood Group B. **A.** Bronchogenic anaplastic carcinoma. Liver metastasis. H & E. × 160 **B.** MCAR is negative in metastatic cancer cells and in hepatocytes, but positive in sinusoids. × 160.

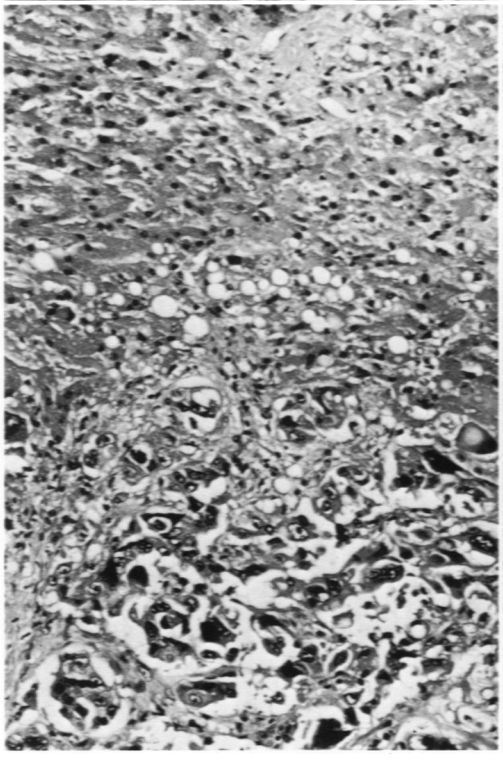
7A



7B



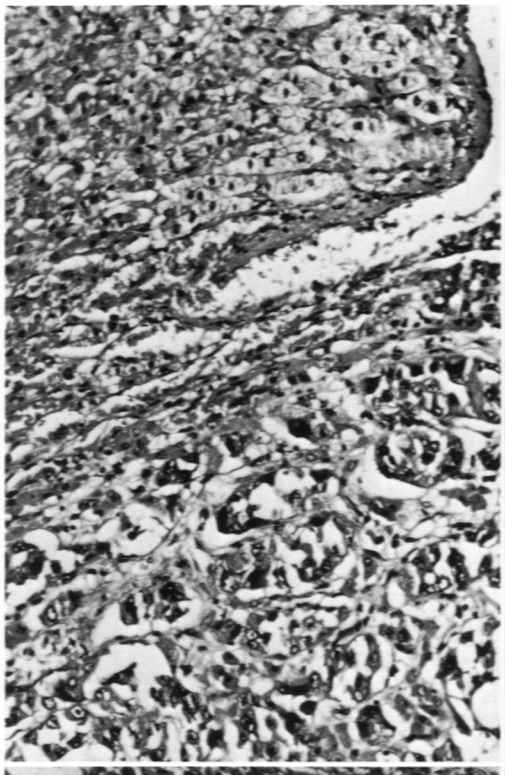
8A



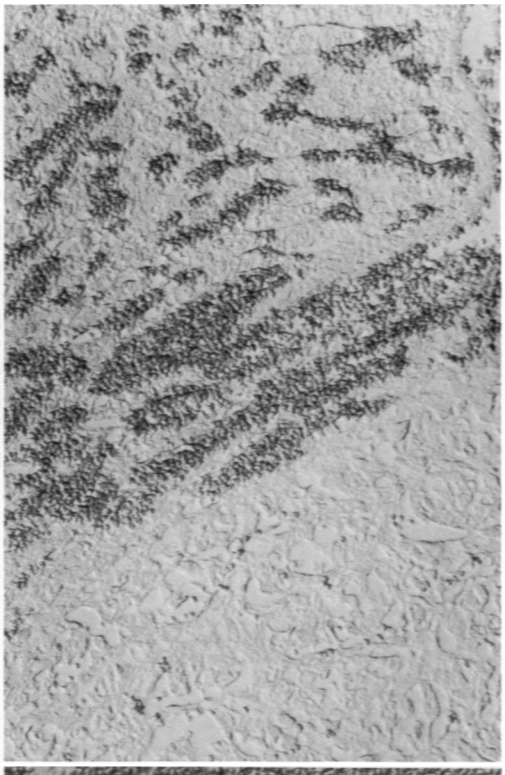
8B

Fig 9. Male, age 39, blood Group A. **A.** Bronchogenic anaplastic carcinoma. Adrenal metastasis. H & E. × 160. **B.** MCAR negative in cells of gland in which isoantigens are not found normally; negative in invading carcinoma in lower part of Figure and positive in capillaries. × 160.

Fig 10. Male, age 39, blood Group O. **A.** Bronchogenic anaplastic carcinoma. Metastasis in pancreas. H & E. × 160. **B.** MCAR positive in exocrine pancreatic parenchyma in upper right corner. A few negatively reacting punched-out spaces correspond to islets of Langerhans, cells of which do not contain A, B, and H isoantigens. Carcinoma in lower part of Figure is MCAR negative. × 160.



9A



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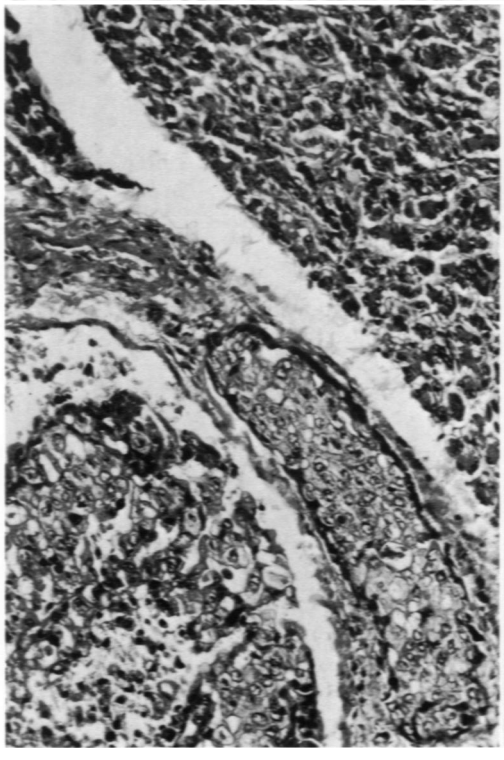
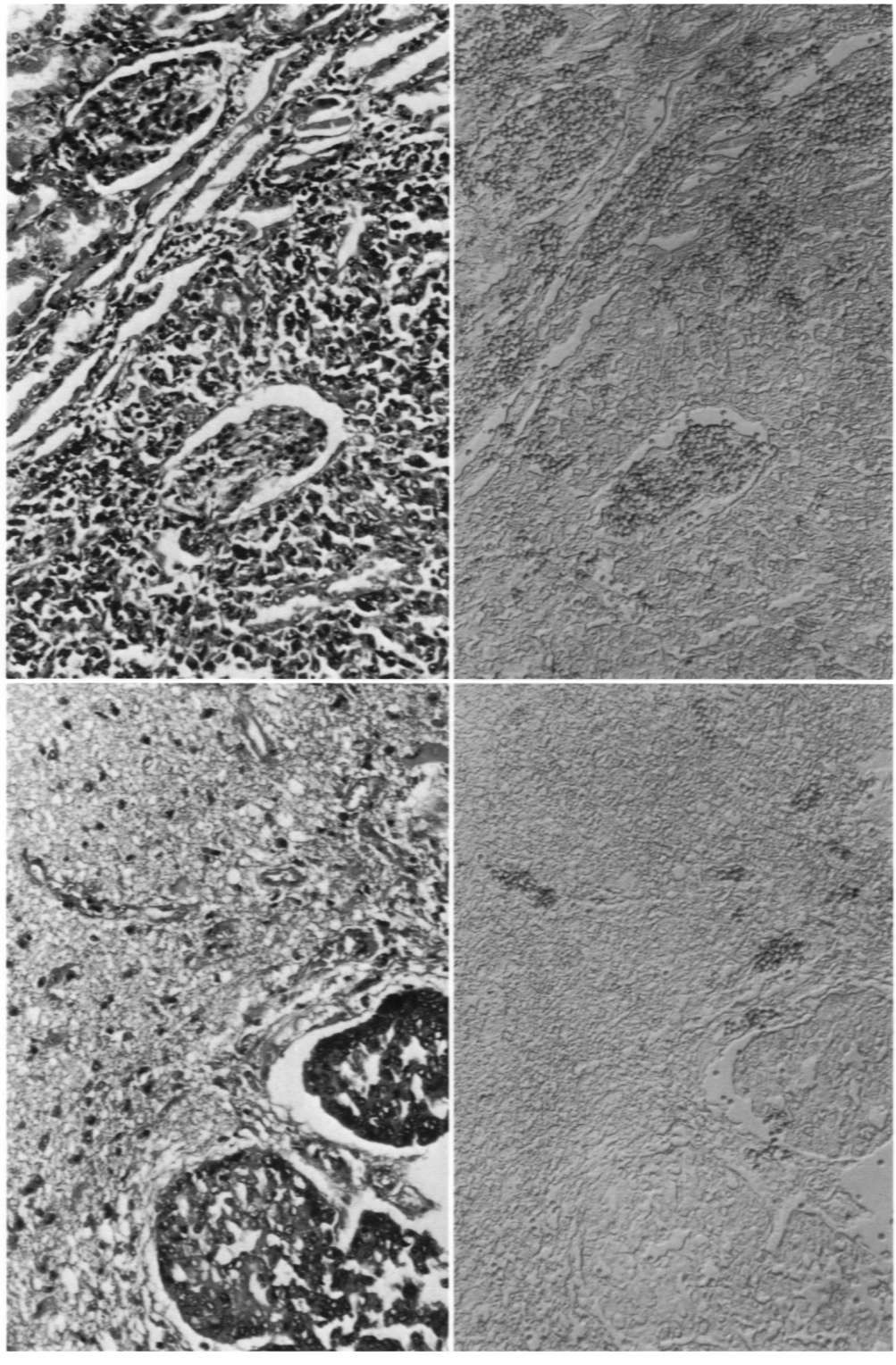


10A

10B

Fig 11. Female, age 44, blood Group A. **A.** Bronchogenic anaplastic carcinoma. Metastasis in kidney. H & E. × 160. **B.** MCAR is negative in renal tubules from which isoantigens A, B, and H are normally absent. It is positive over capillaries between tubules. Glomerulus and its capillaries form an MCAR-positive island in midst of MCAR-negative carcinoma in lower part of figure. × 160.

Fig 12. Male, age 80, blood group A. **A.** Bronchogenic anaplastic adenocarcinoma. Metastasis in brain. H & E. × 160. **B.** Isoantigens A, B, and H are not found in brain tissue. Consequently, MCAR is negative in brain. It is also negative in metastatic carcinoma. At bottom, outlines of adenocarcinoma are clearly discernible. × 160.

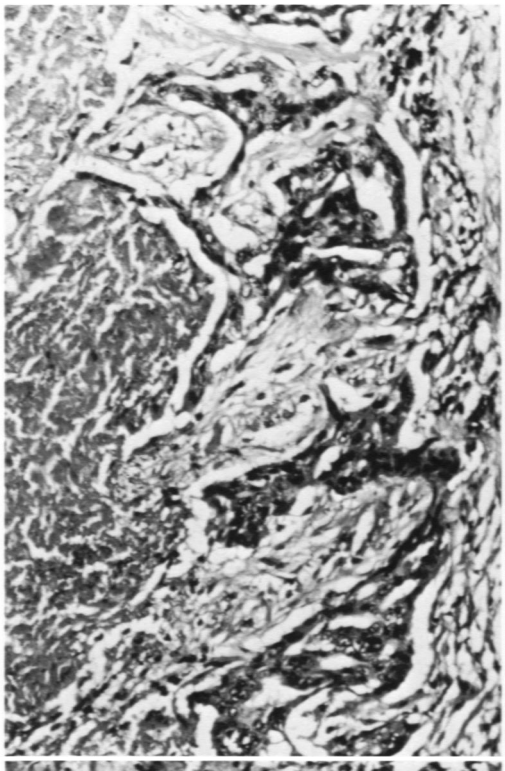


11A 11B

12A 12B

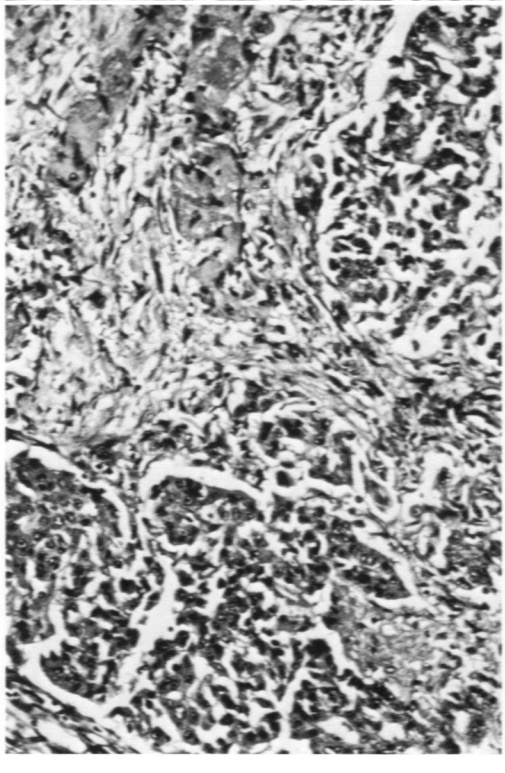
Fig 13. Female, age 68, blood Group B. **A.** Bronchus. Desmoplastic adenocarcinoma, infiltrating. Extensive necrosis and hemorrhage. H & E. × 160. **B.** MCAR is positive in carcinoma (an exceptional finding) and in hemorrhage (as expected). × 160.

Fig 14. Same patient as in Fig 13. **A.** Different field in same section as Fig 13A. Histologic findings are indistinguishable from Fig 13A. H & E. × 160. **B.** MCAR is negative in area of infiltrating carcinoma. Streaks and patches of positive MCAR correspond to blood vessels. × 160.

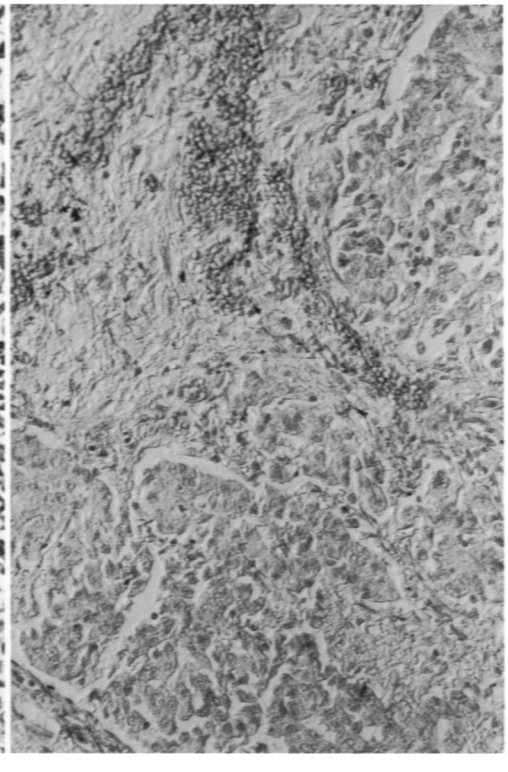


13A

13B



14A

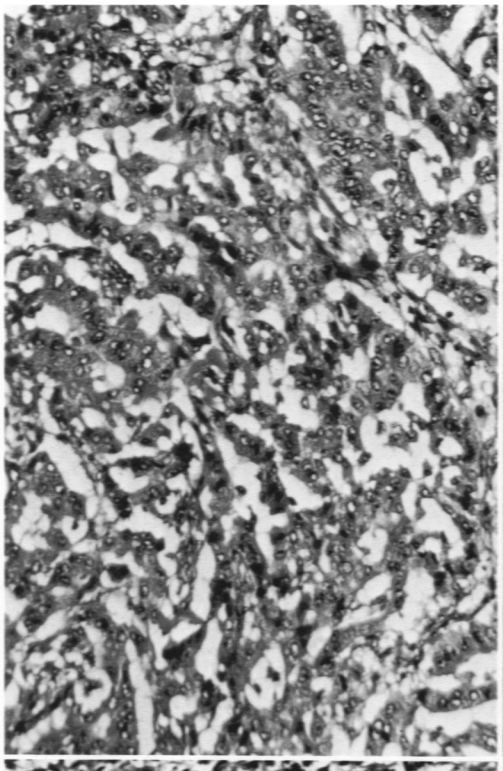


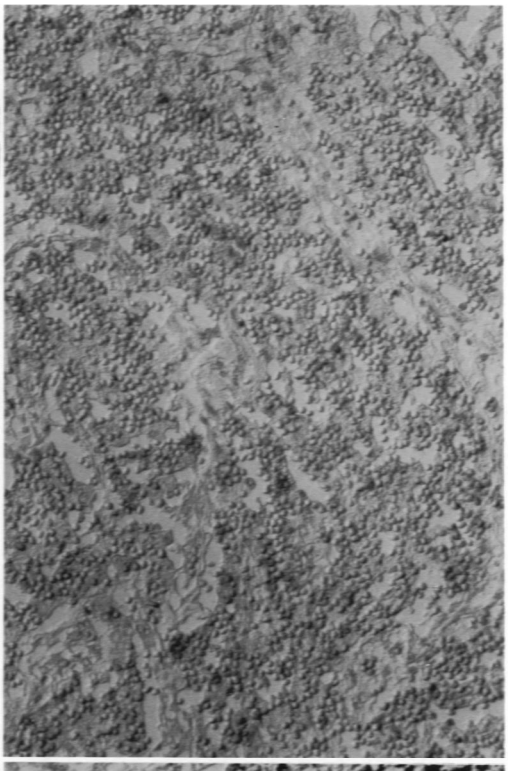
14B

Fig 15. Same patient as in Fig 13. **A.** Regional bronchial lymph node, completely replaced by metastatic adenocarcinoma. H & E. × 160. **B.** MCAR is positive in areas of infiltrating carcinoma. × 160.

Fig 16. Same section as in Fig 15A, but different field. **A.** Histologic appearance identical with Fig 15A, except for some anthracotic pigment near top. H & E. × 160. **B.** Here MCAR is negative. × 160.

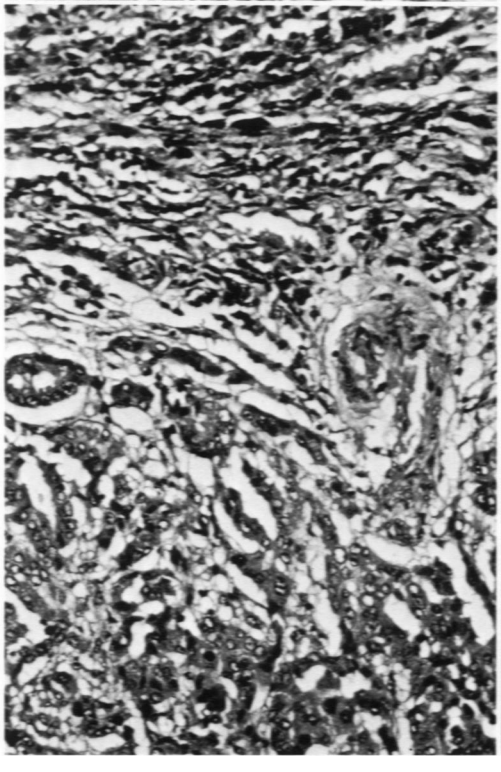
15A

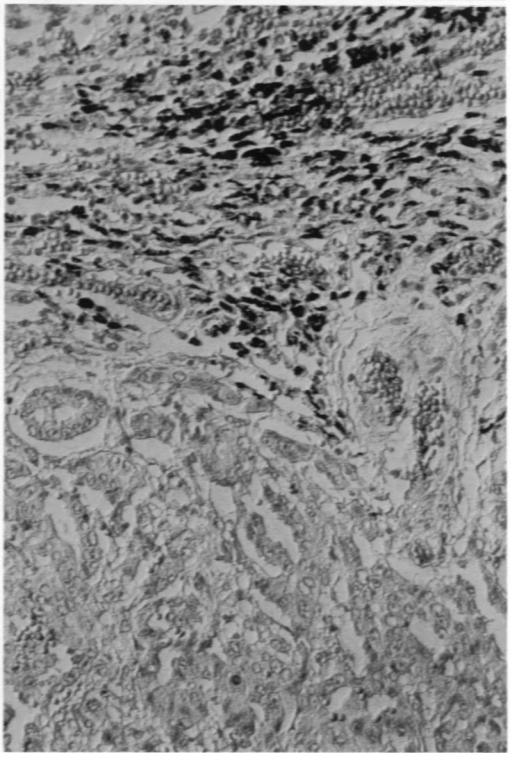




15B

16A

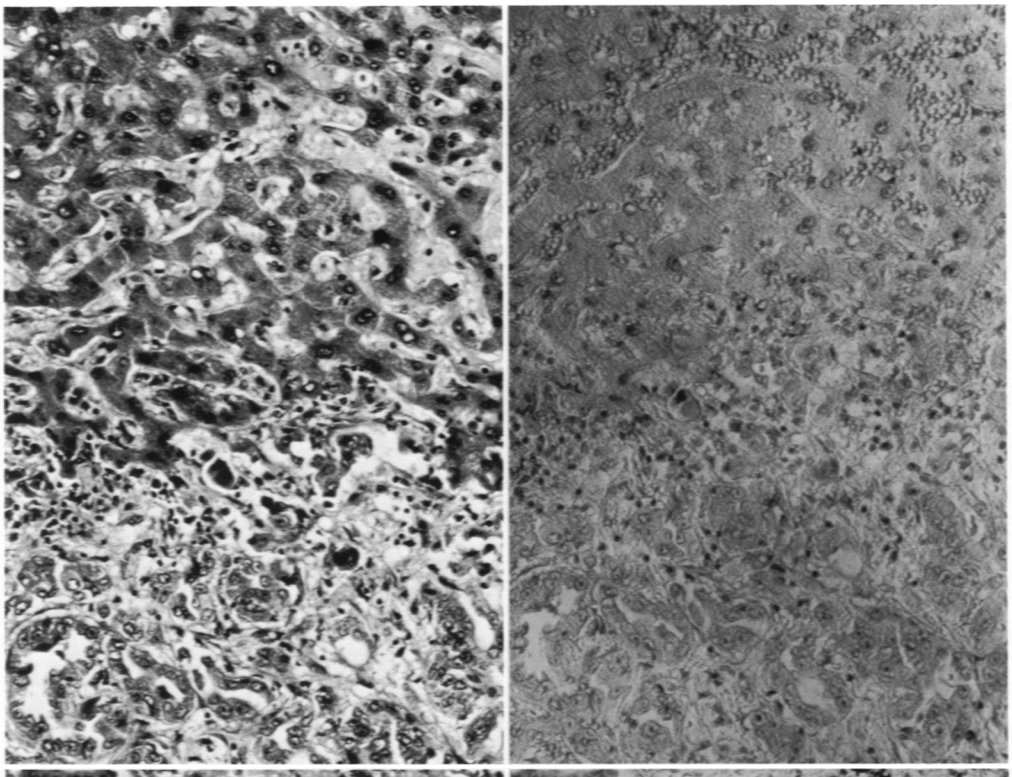




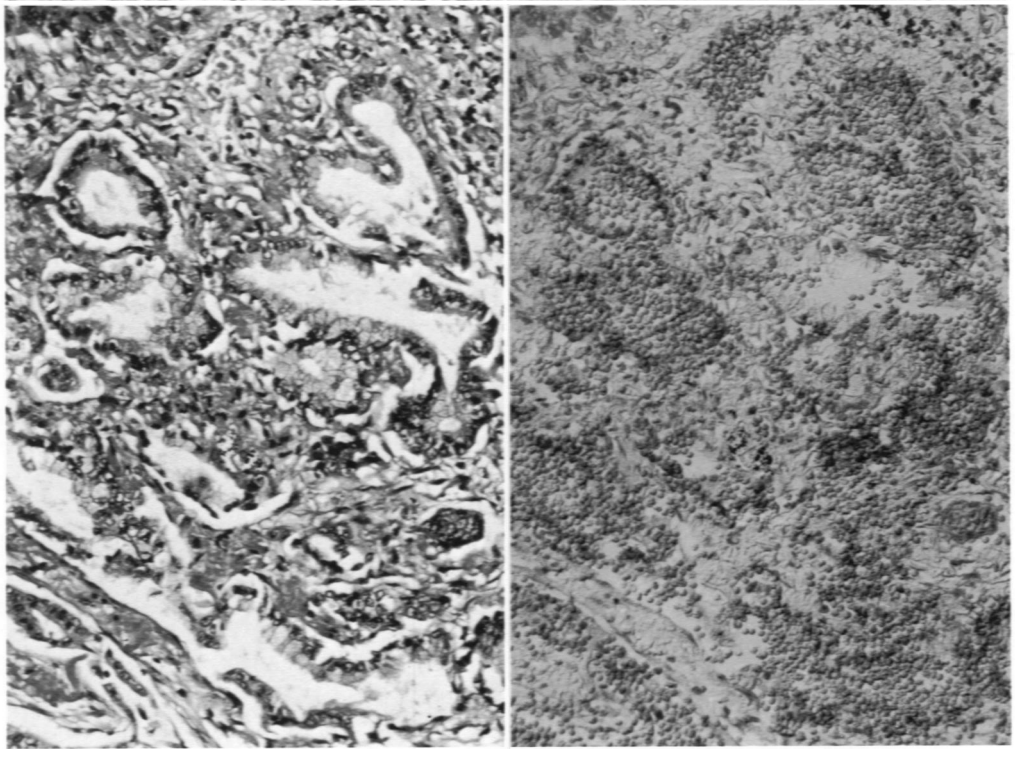
16B

Fig 17. Same patient as in Fig 13. **A.** Liver metastasis. Normal hepatic parenchyma at top. Glandular structures are well preserved in carcinoma. H & E. × 160. **B.** MCAR is negative in metastasis as it is in normal hepatic parenchyma. MCAR positive streaks at top correspond to sinusoids. × 160.

Fig 18. Female, age 78, blood Group A. **A.** Bronchus. Anenocarcinoma, infiltrating, well differentiated. H & E. × 160. **B.** Positive MCAR. × 160.

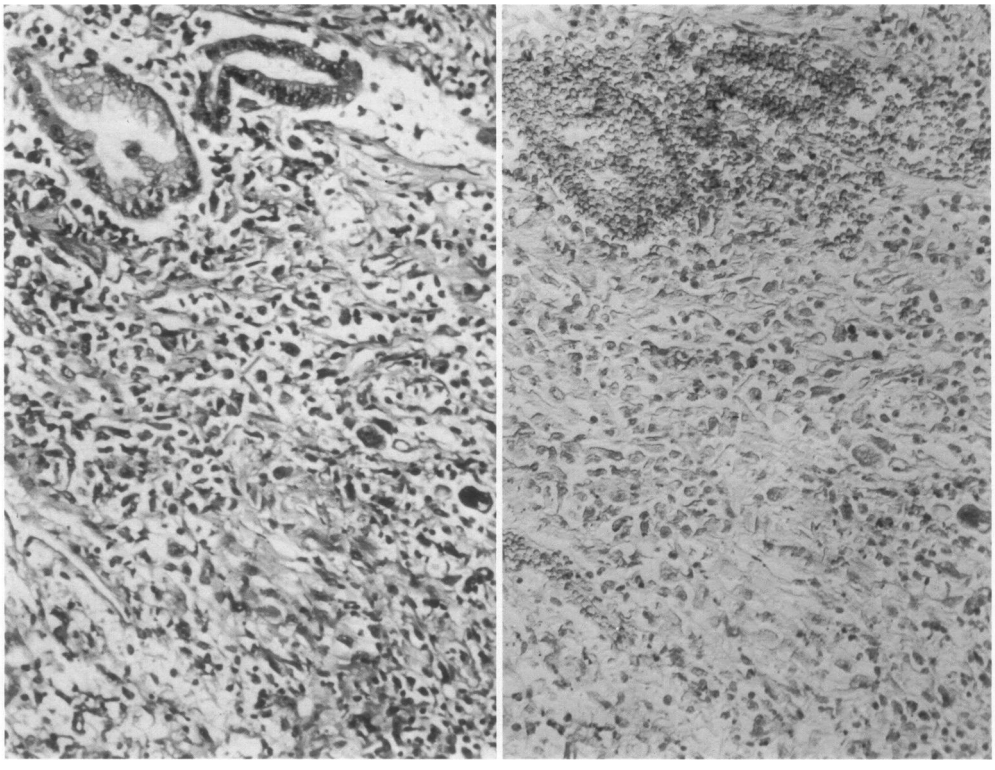


17A 17B



18A 18B

19A



19B

Fig 19. Same patient as in Fig 18; same section, different field. **A.** Highly anaplastic infiltrating carcinoma surrounds island of isolated tubules morphologically identical with well differentiated adenocarcinoma in Fig 18A. H & E. × 160. **B.** MCAR is negative in area of anaplastic carcinoma and positive in island of well differentiated adenocarcinoma. × 160.