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Mechanisms of Human Tumor Metastasis Studied in Patients with Peritoneovenous Shunts[1]

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ABSTRACT

The technique of peritoneovenous shunting for the alleviation of abdominal pain and distension in malignant ascites due to inoperable cancer, returns the fluid to the circulation via a one-way, valved, anastomosis between the peritoneum and the jugular vein. Surprisingly, although the patients treated with this technique receive direct infusions of malignant tumor cells into the blood, this study of 29 patients, 15 of whom came to autopsy, shows that they did not all develop metastases, some being completely free of such lesions despite long survival. Even when metastases do form, they are small and clinically asymptomatic, and the technique is therefore not hazardous. In some patients, inert tumor cells identifiable by natural markers were recognized in the tissues, but no growing metastases were observed. In others, the distribution of secondary deposits was unexpected in that metastases did not form in the organ containing the first capillary bed encountered, although hematogenous metastases had formed in other organs. Despite the fact that various factors such as (*a*) the small numbers of patients treated with the technique; (*b*) the sensitive nature of studies on terminally ill patients; and (*c*) the absence of consistency in the sample population with regard to factors such as length of survival and site of neoplasm, combine to reduce the number of suitable cases for study, the approach has unrivaled power and interest for those seeking to understand mechanisms underlying tumor metastasis in humans.

INTRODUCTION

There have never been acceptable methods for the experimental analysis of mechanisms of metastatic spread in humans. Investigation of this topic has therefore been conducted exclusively in animals (3–5, 14–16, 27), but there is still no proof that the conclusions apply to humans. However, the recent introduction of peritoneovenous shunting for palliation of intractable ascites in patients with incurable abdominal cancer has made ethical opportunities available for investigation of the phenomenon of metastasis in humans. In this procedure, the abdominal effusion is returned to the circulation via an anastomosis, containing a one-way valve, between the peritoneal cavity and the lungs. Since no filter can be interposed without rapidly blocking the flow, large numbers of tumor cells are necessarily infused directly into the circulation. We have studied this unusual group of patients and have reported that palliation is good (24) and complications are minimal (25, 30). Now we present details of laboratory studies on 29 patients treated with this technique and pathological findings in 15 of them who were subsequenty autopsied. Although several reports (1, 7, 8, 13, 18, 19, 23, 26) have been published on the efficacy of this procedure for relief of symptoms, there has been no evaluation of the scientific implications of the observations which can be made. The findings must be interpreted with caution, because the sample of patients that can be studied thoroughly, even by laboratories with adequate resources and appropriate clinical associations, is small, and there are unavoidable differences in length of survival and in diagnosis between individual patients.

However, it is already clear that the findings in humans corroborate and extend those in other species and that patients treated with this technique will, in addition to benefitting from palliation, provide a rich source of information pertinent to mechanisms underlying the metastatic process.

MATERIALS AND METHODS

Clinical Observations. Patient details and diagnoses are presented in Table 1. More extensive clinical details are provided in a previous publication (24). As the shunt is only used for palliation in terminally ill patients with inoperable carcinoma, in whom systemic chemotherapy and other treatment has already failed, no further antineoplastic therapy was given after shunt insertion. Also, anticoagulants were not given for prevention of shunt blockage.

Ascitic Fluid. Ascitic fluid was collected from 22 patients at the time of insertion of the shunts. The samples were collected in sterile plastic tubes and transported in crushed ice. The cells in the fluid were washed by centrifugation and resuspension in minimum essential medium with 10% fetal calf serum and antibiotics. An aliquot of the cell suspension was stained with a mixture of fluorescein diacetate and ethidium bromide (28) and counted in a hemocytometer with an UV microscope to calculate the total cell number and percentage viability. Live cells react with fluorescein diacetate and fluoresce bright green, and dead cells stain red with ethidium bromide in UV, thus making a sensitive test of immediate cell viability.

Capacity for prolonged survival of the ascites cells was tested by culture in plastic tissue culture flasks with minimum essential medium and 10% fetal calf serum, penicillin and streptomycin (50 units/ml), L-glutamine (2 mM), and nonessential amino acids (Grand Island Biological Co. Europe, Ltd., Paisley, Scotland, United Kingdom). The cells were examined regularly for at least 2 weeks by phase-contrast microscopy.

To assess clonogenicity, further cells from samples of ascitic fluid from 12 patients were mixed with 0.33% agarose and plated over a base layer of presolidified 0.5% agarose in 35-mm Petri dishes. Dishes were seeded with either 10^5 or 2.5×10^5 viable cells. After 14 days, the number of colonies of more than 10 cells was counted and the clonogenic efficiency calculated with the following:

$$\frac{\text{No. of colonies}}{\text{No. of cells plated}} \times 100$$

Permanent preparations of the agarose cultures were made following

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Table 1
Autopsied patients: laboratory findings

Patient	Viability of ascites cells (%)	Viable cell no. in 100 ml	Cytological diagnosis in ascites	Ascites cells: Growth on plastic	Ascites cells: Clonogenicity in soft agarose (%)	Blood (growth on plastic)
Group 1						
D. G.	99	40×10^6	M[a]	+	0.012	+
R. H.	95	10×10^6	M	+	0	−
H. M.	98	260×10^6	M	+	NA	NA
J. B.	99	50×10^6	M	+	0.04	NA
B. B.	95	20×10^6	M	+	0.14	NA
Group 2						
F. G.	99	65×10^6	M	+	0.01	++
W. A.	95	20×10^6	M	+	0.02	+
E. R.	99	600×10^6	M	+	0.7	−
D. J. (♀)	95	8×10^6	NA	+	NA	+[b]
E. J. S.	99	20×10^6	M	+	0.003	NA

[a] M, malignant; +, growth; ++, abundant growth; −, no growth; NA, not available.
[b] Few cells in later cultures.

the method described by Salmon and Buick (20). Any remaining cells, or those which could not be cultured immediately, were stored in 1-ml aliquots in liquid nitrogen, with 7.5% dimethyl sulfoxide as cryopreservative (17).

Peripheral Blood. Blood samples (20 ml) were taken at 1 hr before and again 1 hr after inserting the shunt (from 16 patients). Further blood samples were taken at each outpatient visit. The blood, collected in sterile Vacutainers containing EDTA, was mixed carefully and left to stand at 4° for 1 to 2 hr to allow the RBC to sediment. The RBC-free plasma was collected, diluted with minimum essential medium, and centrifuged, and the cell pellet cultured in tissue culture flasks with culture medium (see above). Nonadherent cells were removed after 1 to 2 days by changing the medium. The cultures were maintained and examined regularly for up to 4 weeks.

Autopsy Procedure. Thirty-five patients received shunts at Oxford or High Wycombe, England, in the last 3 years, 15 of whom were autopsied. The remainder either are still alive, died at home, or permission for autopsy was refused.

Full autopsies were performed on 14 of the 15 cases; one (W. H.) had a limited autopsy involving only examination of the thorax at the request of the relatives.

The autopsy procedure consisted of examination of the cranial, thoracic, and abdominal cavities and systematic inspection of all the organs they contained. After surface examination for tumor deposits or other lesions, all solid organs including small ones such as the endocrine glands were cut into thin serial slices for detection of macroscopic metastases. Tubular structures such as the gut and the major vessels were opened longitudinally and the lumen examined. Longitudinal slices of the vertebral column were also studied. The axial (hilar and paraaortic) and peripheral lymph nodes were palpated, and a representative sample was sliced in addition to any that felt abnormal. In the abdominal organs, only deposits deep in the parenchyma were accepted as metastases to exclude confusion with ingrowth of surface tumor nodules.

The lungs were extensively sampled for histology because they contain the first capillary bed encountered by the malignant cells from the shunt. They were fixed by inflation with formalin and sliced 24 hr later. Representative samples of all lesions were taken, together with at least 6 blocks of tissue selected at random from each lung. The extent of sampling from all other organs depended on size and on whether macroscopic lesions were present, but where these were absent, at least one and usually several blocks were taken at random from each organ. In organs that were macroscopically normal, a particularly careful search was made microscopically for micrometastases, tumor cell emboli, or inert tumor cells in the interstitial tissue.

Hematogenous origin of micrometastases could be recognized from knowledge of the vascular and lymphatic anatomy of an organ.

RESULTS

Ascitic Fluid. The viability of cells in the ascitic fluids calculated by the fluorescein diacetate-ethidium bromide staining method was, in all cases, greater than 90% (see Tables 1 and 2). All samples grew well in plastic tissue culture flasks for at least 2 weeks. The clonogenic efficiencies in soft agarose of the ascites cells from 12 patients are also given in Tables 1 and 2. These 3 techniques demonstrate: (*a*) that numerous viable cells were present in the ascitic fluid of these patients; (*b*) that the cells were capable of prolonged survival; and (*c*) that a proportion of them was also capable of growth in semisolid medium, an assay for tumorigenicity (9, 22). At the time of plating in soft agarose, it was not possible to distinguish normal from malignant cells. Therefore, the figure for clonogenic efficiency is calculated from the initial total number of viable cells per Petri dish, which probably underestimates the real value.

Peripheral Blood. Bizarre cells frequently multinucleated were identified in the cultures from peripheral blood of 11 of the patients studied (Tables 1 and 2). The number of cells varied greatly and, in 2 of the patients sampled repeatedly (D. G. and F. G.), reflected the patency of the peritoneovenous shunt since they disappeared, or reduced in numbers, when the shunt was totally or partially blocked. The morphological appearances, although suggestive of neoplastic origin, are not sufficient for establishing this. Further studies with monoclonal antibodies and inoculation of blood cells from these patients into nude mice are in progress.

Autopsy Findings. The main autopsy findings in the 15 patients studied are summarized in Table 3. The primary tumors arose in a variety of sites, although 9 were of the ovary, and the patients survived for periods ranging from 1 to 27 months (median, 4 months) after insertion of the shunt. It is realized that, even with the most thorough technique and systematic search, one cannot be certain that occasional micrometastases have not escaped notice. The separation of patients into 2 groups with and without hematogenous metastasis is therefore a provisional device to aid description and analysis. The magnitude of the difference in collective metastatic performance between the tumors we have placed in each of the 2 compartments certainly suggests the existence of separate populations within the series of cases studied so far rather than that they are a uniform group,

Table 2
Patients without autopsy: laboratory findings

Patient	Diagnosis	Viability of ascites cells (%)	Viable cells/ 100 ml ascites	Ascites cells: Growth on plastic	Ascites cells: Clonogenicity in soft agarose (%)	Blood (growth on plastic)
P. S. Q.	Breast carcinoma	92	6.5×10^6	+[a]	NA	++
E. H.	Non-Hodgkin's lymphoma	100	2.5×10^6	+	NA	−
E. C.	Adenocarcinoma (1° ?)	95	21×10^6	+	NA	+
H. C.	Ovarian carcinoma	98	50×10^6	+	NA	+
C. C.	Pulmonary lymphangiomyomatosis	100	40×10^6	+	0	+
D. B.	Ovarian adenocarcinoma	100	10×10^6	+	0.006	+
J. W.	Large-cell anaplastic carcinoma of lung	99	12×10^6	+	NA	++
A. S.	Adenocarcinoma of colon	99	25×10^6	+	NA	−
L. S.	Breast carcinoma, liver metastases	99	20×10^6	+	NA	−
M. P.	Ovarian carcinoma	96	40×10^6	+	0.007	+
T. M.	Adenocarcinoma of colon	99	5×10^6	+	0.002	NA
I. E.	Adenocarcinoma, probably breast	100	5×10^6	+	0.015	NA

[a] +, growth; ++, abundant growth; −, no growth; NA, not available.

Table 3
Summary of clinical details and pathological findings

Patient	Sex	Age	Site of primary tumor	Survival time after shunting (mo)	Distribution of metastases
Group 1: no hematogenous metastases					
D. G.	F	53	Ovary	27[a]	None
E. H.	F	66	Ovary	2	None
R. H.	F	68	Stomach	1	None
D. J.	M	60	Unknown	2.5	None
A. R.	F	57	Ovary and breast	7	None
H. M.	F	48	Ovary	2	None
J. B.	F	76	Ovary	4	None
B. B.	F	46	Ovary	1	None
Group 2: hematogenous metastases present					
W. A.	F	82	Ovary	4	Several organs[b]
E. R.	F	55	Ovary	3.5	Lungs
F. G.	M	67	Pancreas	9	Lungs, liver
D. J.	F	59	Unknown	5	Liver, vertebrae[c]
W. H.	M	51	Bronchus[d]	1	Other lung
E. J. S.	F	61	Colon	4	Lungs
E. E. S.	F	76	Ovary	5	Several organs[e]

[a] First shunt functioned for 5 months and second for 6 months with an interim period of 16 months with intermittently functioning shunt.
[b] Lungs, liver, spleen, brain, choroid plexus, intestinal wall, adrenals; all tiny deposits.
[c] Large deposits before shunt inserted. Lungs and other organs completely negative.
[d] Pleurovenous shunt.
[e] Adrenals, lungs, and liver.

but observations on a larger sample may later indicate further subdivisions are required.

In some patients (Group 1), there was neither macroscopic nor histological evidence of growth in any organ outside the abdominal cavity. Even within the abdomen, there were no hematogenous metastases in the parenchyma of any organ, although there was often massive local growth of the primary tumor and numerous peritoneal secondary seedlings. (A metastasis was defined as an expanding focus of tumor cells sharing cytological or histological characteristics with the primary tumor either in the interstitial tissue of an organ or completely occluding and distending a blood vessel with associated mural damage.) However, in one patient (D. G.), occasional dispersed single cells or small cell clumps (3 to 5 cells) were seen in the walls of the pulmonary capillaries and in the interstitial tissue of the lungs (Fig. 1). The origin of these from the patient's primary ovarian tumor was confirmed by the presence of psammoma bodies (characteristically produced by ovarian tumors) in and adjacent to the cells (29).

A further patient with no secondary tumor deposits outside the abdominal cavity (A. R.) is of great interest, because she was found to have 2 separate primary carcinomas, one of the breast and one of the ovary. The former was an infiltrating ductal carcinoma and the latter, a papillary cystadenocarcinoma producing psammoma bodies. Numerous seedling tumor deposits of ovarian carcinoma were seen in the peritoneum, but the only

organ in which tumor cells were detected was the liver (Fig. 2). These were occasionally forming psammoma bodies indicating their origin, but the number of cells found was extremely small despite the patient having a clinically functioning shunt for over 7 months. The occasional tumor cells found in these 2 patients, because of the expression of a natural marker, could conceivably have been capable of making deposits at a later time, but no metastases whatsoever were detectable at the time of autopsy although both had survived for many months.

In contrast, the remaining patients studied (Group 2) had either moderate numbers of easily recognized small metastatic deposits in the lungs and other organs or large numbers of viable intravascular tumor cells in numerous organs. Within each patient, the tumor cell aggregates were of a similar order of size, although this varied between patients. F. G. had numerous small macroscopically visible tumor deposits in the lungs (Figs. 3 to 5) and 2 somewhat larger ones in the liver, but none elsewhere. E. R. had many small tumor cell aggregates in the pulmonary capillaries and in the alveolar septae but none in the other organs, and there were no macroscopic deposits anywhere. In contrast, W. A. and E. E. S. had large viable tumor cell aggregates in several organs examined (Table 2; Figs. 6 to 8), but these were still too small to be seen with the naked eye. In F. G., although there was some residual ascites, there were only few abdominal colonies, all in the peritoneum, and the primary tumor in the pancreas was very small. E. R. and W. A. had massive peritoneal colonization, and the ascitic fluid of E. R. was so packed with cells that it resembled a puree.

Four patients (J. B., B. B., E. J. S., and E. E. S.) had firm evidence of lymphogenous dissemination (metastases in hilar lymph nodes and tumor cells in lymphatics in various organs), but only 2 of these (E. J. S. and E. E. S.) had hematogenous metastases. Although B. B. and J. B. only survived 1 and 4 months, respectively, it is interesting that cells clearly capable of survival and growth in the lymphatics and lymph nodes had not exited and formed parenchymal metastases in any organ, although they had been widely disseminated by both the blood and lymphatic pathways.

One female patient (D. J.) was exceptional and deserves special consideration. She had hematogenous spread to the liver and to the thoracic and lumbar spine which had already occurred before insertion of the shunt. Extensive replacement of these organs by tumor was confirmed at autopsy (Figs. 9 and 10) but no deposits, nor even any tumor cells, were found in the lungs (Fig. 11) nor in any other organ, despite survival of the patient for 5 months with a patent shunt.

In no patient was any significant cellular immune response observed to deposits or latent tumor cells. This is of particular interest in those patients with no metastatic tumor deposits outside the abdomen. In one patient (W. A.) occasional small groups of lymphocytes were seen in the vicinity of a few of the tumor cell clumps in the pulmonary vessels, but most of these clumps were not associated with cells of the immune system, nor were those in other organs.

The autopsy findings establish that, even in those patients in whom small deposits had formed, the metastatic sequelae of peritoneovenous shunting were not harmful, because the patients died of their abdominal tumor load before metastases were clinically evident (30).

DISCUSSION

It might be expected that the infusion of large showers of malignant cells into the circulation would produce crops of hematogenous metastases at least in the lungs, and that these lesions would cause respiratory distress and shorten life. This study shows that some such patients indeed probably did form pulmonary and other hematogenous metastases because of the shunt, and the size and uniformity of many of these strongly indicate that they originated since its insertion. Nevertheless, these new metastases were of no clinical importance, because the patients suffered no respiratory distress or other symptoms attributable to metastases and died from the progressive abdominal tumor.

It is remarkable that other patients did not develop detectable hematogenous metastases in spite of massive infusions of malignant cells for up to 27 months. What happened to neoplastic cells shed into the circulation in these patients is not clear, but some did lodge in the vascular tree and even migrate into the interstitial tissue (for example in D. G. and A. R.) but appeared not to grow. It is possible that these cells might in time have produced hematogenous metastases, but in those patients who did not develop secondary deposits, or were slow to do so, there must have been substantially different properties in the malignant cells, the individuals concerned, or both. The special circumstances in patients treated with peritoneovenous shunts make all of these possibilities amenable to investigation.

In the patients with no evidence of metastatic spread, clinical records confirmed the patency of the shunts, and laboratory studies showed that the ascites cells were of high viability (Tables 1 and 2). In the samples tested, the values for clonogenicity in semisolid medium (Tables 1 and 2) were comparable to those reported by others (10, 12) for solid tumors, and so far the ascites cells from 2 (B. B. and J. B.) of 6 patients produced metastasizing tumors when inoculated into nude mice. The presence of large numbers of carcinomatous deposits in the peritoneum is also compelling evidence that the cells in the malignant effusions had tumorigenic potential.

It is difficult to give accurate figures for the numbers of colony-forming tumor cells being released into the blood via the shunt in each patient, but dosage is clearly an important consideration in this context. Some estimates can be made from the known concentrations of cells in the ascitic fluids, and their clonogenic efficiencies (Tables 1 and 2), which could be evaluated accurately, and the rate of production of ascitic fluid. The latter is difficult to measure directly, but the amounts removed at paracentesis before shunt insertion ranged from 6 liters every 3 weeks to 10 liters/week. Assuming similar production rates after shunt insertion, one can calculate the probable malignant cell infusion in particular patients. This in female patient D. J. was of the order of 1.6×10^8 cells/week, which presented the lungs with at least 16×10^3 clonogenic cells/week. In spite of this, she had no pulmonary deposits or intravascular cell clumps even though she had already developed hepatic and spinal metastases before the shunt was inserted. At the other extreme, D. G. is estimated to have infused 4×10^9 cells, with at least 5×10^5 stem cells/week, without forming any visible secondaries.

The length of survival of the patient is clearly a further factor which affects whether metastases are present at autopsy. However, the absence of detectable hematogenous deposits in Group

1 patients is unlikely to be due to insufficient time being available for tumor growth because, in 3 of these patients [D. G., A. R., and D. J. (male)], the abdominal tumor mass increased considerably in the time the patient survived. One of them (D. G.) in fact lived for 27 months after insertion of the shunt without forming a single macroscopic deposit in any organ. Even in the remaining patients in Group 1, the complete absence of tumor cells in the lungs, despite many malignant cells being shed into the circulation for several weeks, is surprising. Collectively, these findings corroborate the observations of earlier workers (2, 21) who found that the presence of malignant cells in blood smears did not reliably predict subsequent metastasis, although at the time there was no way to prove the viability of these cells.

One of the most important issues which the study of shunt-treated patients may illuminate is that of individual variation in behavior among tumors. There are considerable differences in the degree and distribution of metastatic spread in patients even when the tumors are of the same organ and have similar histology, and this makes evaluation of individual prognosis on the basis of histopathology difficult, even when the statistical likelihood of the outcome is well-known. Laboratory investigation of behavioral and biochemical properties of the cells in the ascites and correlation with the degree of metastasis affords opportunities to seek markers which might be of prognostic value. Such information would clearly help to adjust therapy to an individual patient's needs.

The finding that the tumors studied heavily colonized the peritoneum but sometimes could not grow elsewhere directly supports the interpretation that, in humans, as in other species, the microenvironment in the sites of tumor cell arrest influences whether metastases develop [see Tarin and Price (28); Hart and Fidler (6)]. The findings in female patient D. J. are particularly interesting in this regard, because the cells of her tumor had already shown capability to form blood-borne metastases (in the liver and vertebrae) before the shunt was inserted, yet did not form any elsewhere even after the cells were directly infused into the systemic veins. The collective evidence from these patients therefore provides direct experimental support for Paget's (11) "seed and soil" hypothesis. This proposed that tumor cells ("seeds") randomly scattered by vascular routes could only form metastatic deposits if they had appropriate intrinsic properties and also landed in congenial territory ("soil"). The molecular mechanisms by which the microenvironment affects tumor cell growth are presently unknown but, clearly, such evidence of epigenetic suppression of the metastatic phenotype by normal tissues could have profound implications. Opportunities for investigation of such host-related factors are now available in patients with peritoneovenous shunts.

The failure of tumor cells to grow in particular sites in a patient is not an expression of nonspecific hostility of these organs to neoplasms, since the same organs in other patients did support tumor growth. In those patients without systemic spread, the absence of any consistent cellular immune response to isolated, inert tumor cells in extraabdominal organs as well as to the abdominal tumor suggests that control of distant tumor spread is not by mechanisms akin to allograft rejection.

The method of delivering tumor cells into the bloodstream in these patients is the direct homologue of that used in our studies on the colonization capability of cells from spontaneous murine mammary carcinomas [see Price *et al.* (16) and Tarin (27)]. We, therefore, infer that at least some of the mechanisms of metastatic colony formation are common to both, because the findings in the 2 species are so similar.

The practical significance of this method of treating some patients with incurable cancer is that it also provides opportunity for direct analysis of mechanisms of metastasis in humans. The number of patients fully studied so far is small, and the survival of many of them is short, but the information already available indicates that further studies will in time yield considerable information unobtainable by other means and immediately relevant to human disease. The cells in the malignant ascites can be cryopreserved and recovered for subsequent study which increases the possible applications. However, the laboratory findings are of limited value without autopsy or clinical follow-up.

In conclusion, these autopsy findings corroborate the earlier clinical impressions, that patients treated with peritoneovenous shunting for malignant ascites do not become overwhelmed by widespread and massive metastases. In fact, they succumb to their abdominal tumor before metastases generated by the shunt become detectable or clinically significant. The procedure is not free of potential complications such as blockage, thrombosis, pulmonary embolism, and tumor growth in the s.c. tunnel housing the shunt (25), but for suitable patients, the operation gives valuable palliation. The scientific findings, which are incidental byproducts of these measures, give new insights into factors affecting metastasis in humans.

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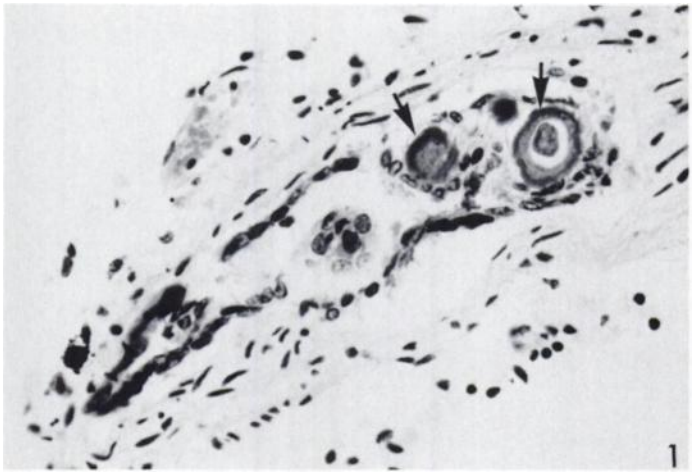
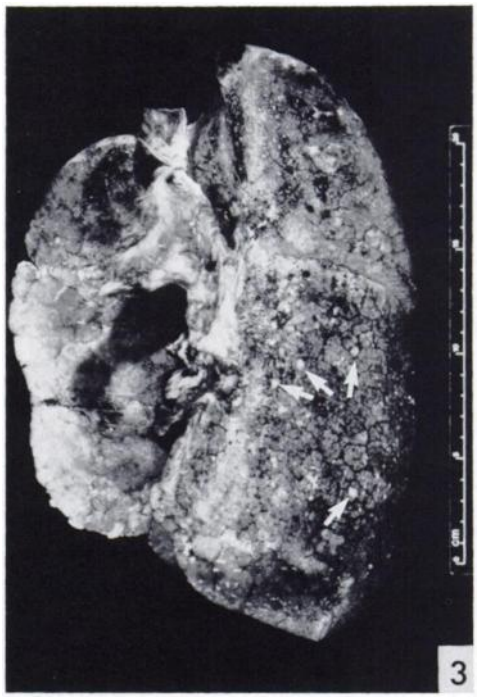
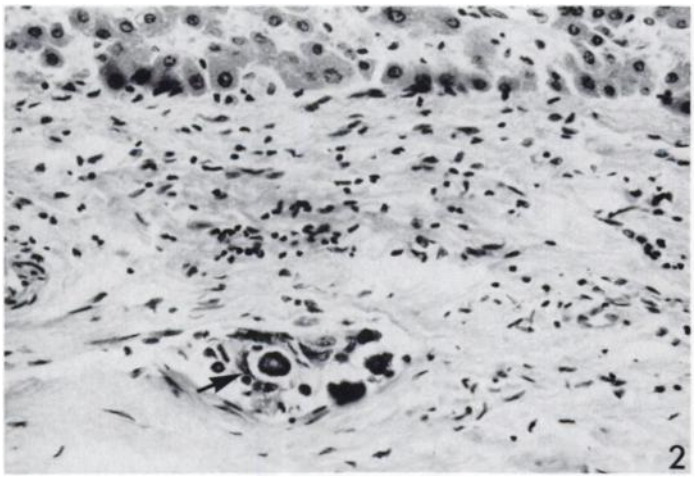






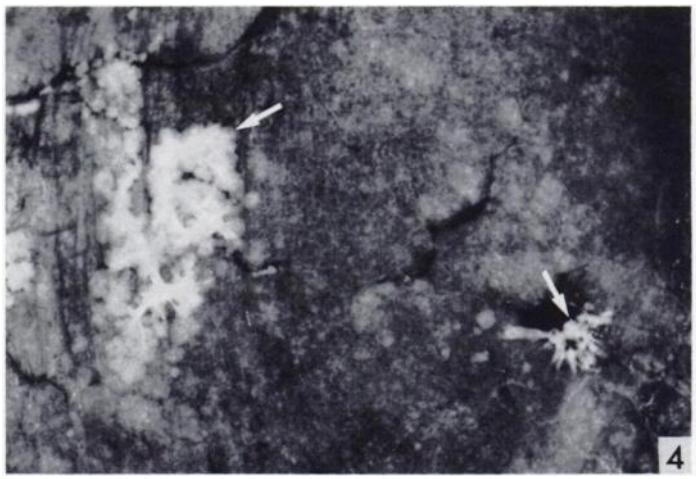
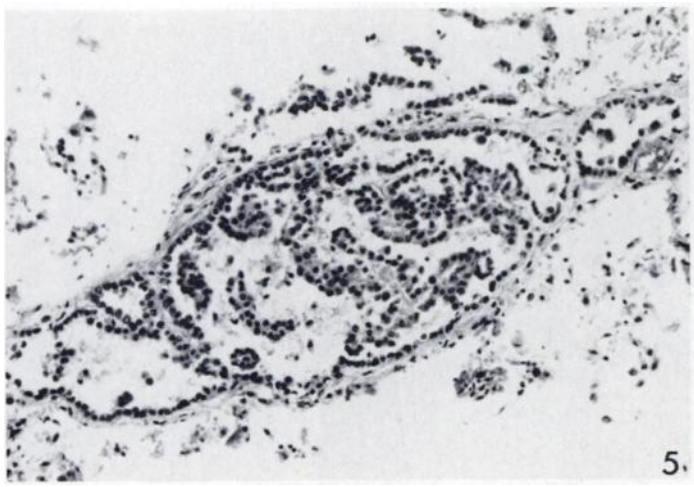


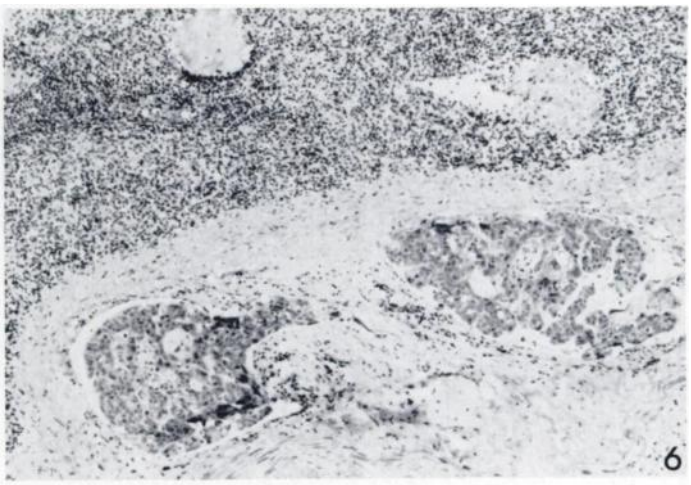
Fig. 1. Patient D. G.: histological sections showing a small group of viable ovarian carcinoma cells, producing psammoma bodies (*arrows*), in a pulmonary capillary. × 300.

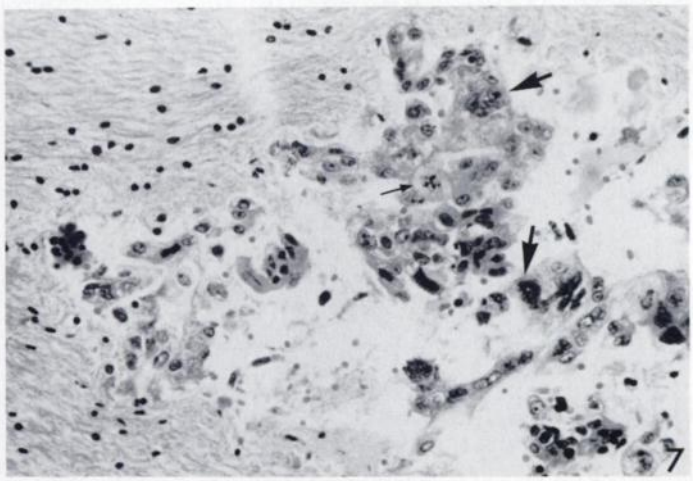
Fig. 2. Patient A. R.: histological section showing a small group of viable ovarian carcinoma cells (*arrow*) producing psammoma bodies in a hepatic portal area. × 190.

Fig. 3. Patient F. G.: survey view of the medial aspect of the right lung showing multiple seedling tumor deposits (*arrows*) of approximately the same size.

Fig. 4. Patient F. G.: detailed view of 2 surface pulmonary deposits (*arrows*) at higher magnification confirming their neoplastic morphology.







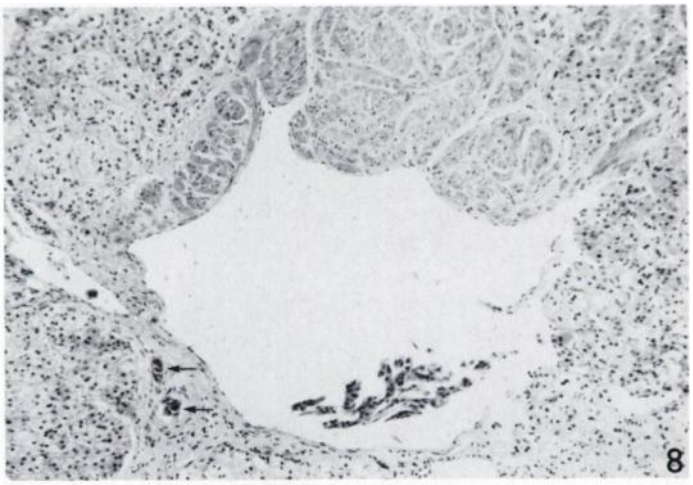
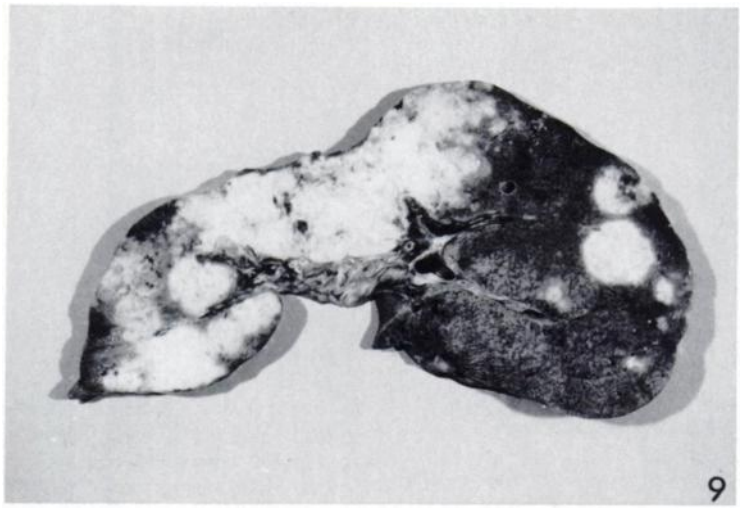


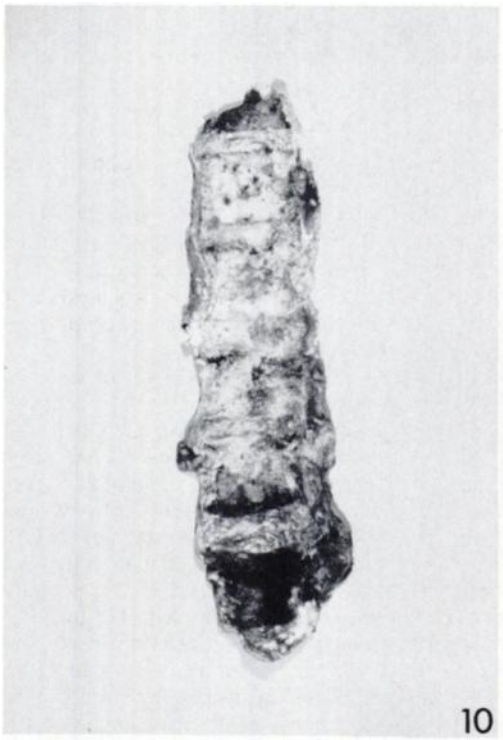
Fig. 5. Patient F. G.: histological section confirming metastatic papillary adenocarcinoma in the lung. × 100.

Fig. 6. Patient W. A.: histological section of the spleen showing large clumps of carcinoma cells in veins in a fibrous trabeculum. × 60.

Fig. 7. Patient W. A.: histological section showing metastatic carcinoma cells in brain parenchyma. Multinucleate cells (*large arrows*) and an abnormal (tripolar) mitotic figure (*small arrow*) are seen. × 190.

Fig. 8. Patient W. A.: histological section showing a clump of carcinoma cells in the central vein of the adrenal and in the substance of the gland (*arrows*). × 75.





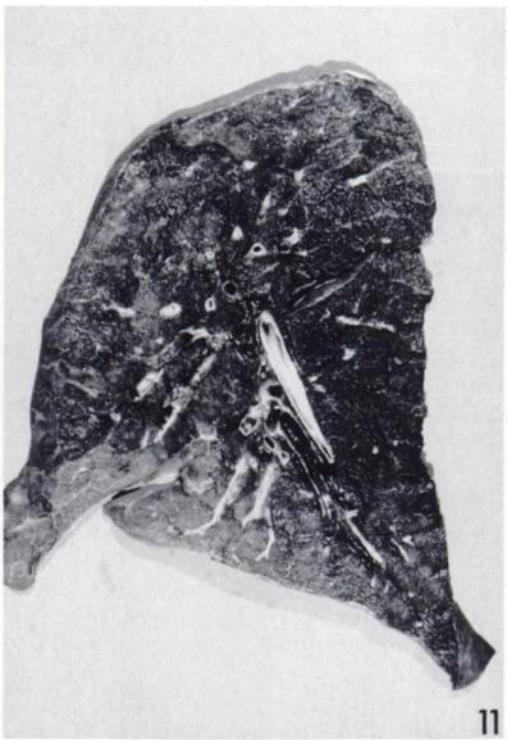


Fig. 9. Patient D. J.: slice of liver showing large metastatic carcinoma deposits.

Fig. 10. Patient D. J.: slice of vertebral column showing subtotal replacement by metastatic carcinoma.

Fig. 11. Patient D. J.: representative slice of lung showing complete absence of macroscopic tumor deposits. No tumor cells were seen in any of the several samples examined histologically.