REVIEW ARTICLE

HEPATOMA—NATURE'S MODEL TUMOR

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A Review

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PRIMARY HEPATOMA is a tumor of special interest in the field of human oncology. The study of primary hepatocellular carcinoma, hereafter referred to as hepatoma, has opened exploration of the role of naturally occurring carcinogens, the fetal manifestations of malignancy and the use of an organ's physiology for diagnosis and therapy. It is no surprise, therefore, that this tumor has been the focus of a wide variety of experimental programs, nor that many of the findings which resulted from these investigations appear directly applicable to man.[1-8] This review will attempt to survey recent developments with respect to hepatoma and to focus upon areas as yet unclear. The experience at the New York University-Bellevue Medical Center from 1950 to 1970, a total of 210 hepatomas, will be used in addition to a review of the literature.

Etiology

The greatest contribution of human hepatoma as a model tumor has has come from the study of its etiology.

In 1934, Yoshida reported the induction of hepatomas in animals following their ingestion of azo dyes.[9] Since those publications, an impressive number of chemical substances have been demonstrated to induce these malignancies after chronic ingestion. These range from plant and microbiologic products to synthetic chemicals. Indeed, attempts to induce animal hepatomas by their ingestion is now a standard means of examining food additives, drugs and other chemical materials for carcinogenic potential, and many of these have since been removed from human use.[10] The study of chemical hepatocarcinogenesis in animals has disclosed several findings which may be related to the human situation. First, all of these agents are relatively toxic and alter the normal metabolic capabilities of hepatocytes. Second, almost every carcinogen induces excessive hepatocytic division (above basal rates)

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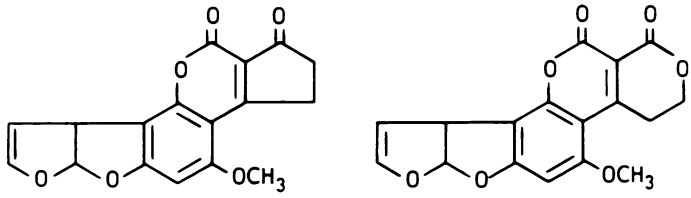
whether or not necrosis is evident.[11] Third, foci of altered cells, termed hepatic nodules, result from this hyperplasia and from other cytologic changes. After sufficient exposure to the carcinogen, these foci persist for long periods and appear to be at high risk for malignant alteration. Within the nodules, subpopulation of cells appear with metabolic or histologic atypia (Figure 1), but it is uncertain whether these aggregates or cells elsewhere within the nodules give rise to the final evolutionary alteration, malignancy.[11-13] Fourth, the evolution of malignancy appears to be a multiphasic sequence. Thus, should the exposure to carcinogen be halted prior to that point when the altered hepatocytes achieve "persistence" the sequence is reversed, the histology returns to normal and malignancy is averted.[14] In addition, further alterations may occur during the life history of the nodules' cells even after cessation of the carcinogen, which suggests a progression of cellular events.[15]

Examination of differences in geographic tumor incidence has contributed as much as animal experiments to our understanding of the etiology of this tumor.

In African sub-Saharan countries and in areas of Asia, the incidence of hepatoma exceeds that in the United States and European countries by 5- to 25-fold and upwards of 100-fold if only younger age groups are compared.[16,17] In these areas, hepatoma is a major component of all deaths and is often the most frequent form of malignancy. For years, special conditions indigenous to these high-risk areas were proposed as obligatory in carcinogenesis. These included malnutrition, parasitic infestation, excess iron intake and amebiasis. The failure of an exact correlation between the areas of maximum hepatoma incidence and those in which these phenomena existed and the discovery of aflatoxin carcinogenesis have greatly diminished the probability of a major role for these factors; although one or more might contribute to a degree.[18] In 1945, Ninard and Hinterman first suggested the possibility that a specific ingested "substance" might be responsible for the high incidence of tumors in animal livers in Morocco.[19] This report and subsequent analyses of the geographic distribution of hepatoma in addition to the demonstration of the induction of animal hepatomas by purified mycotoxins culminated in the suggestion that contamination of food by the fungal product, aflatoxin, was responsible for endemic areas of hepatoma. (Text-figure 1).[20] Aflatoxin, a composite of crystalline fungal toxins (*Aspergillus flavus*), has now been demonstrated to induce hepatoma in a broad spectrum of animal species at doses lower than all other hepatocarcinogens.[21] Although final proof that aflatoxin is the agent of human hepatocarcinogenesis in these areas is lacking, the circumstantial



TEXT-FIG 1—The chemical structure of two aflatoxin crystalline derivatives.

evidence is strong. Aflatoxin contamination of food is evident in the highest risk areas, and the level of contamination is roughly proportional to the incidence of tumor.[22-24] The estimates of the levels ingested as measured from average contamination of staple foods are well within the estimated carcinogenic range as determined by animal experiments. The pervasiveness of this contamination is startling. It occurs almost everywhere that nut meal is stored under warm and humid conditions. The link to focal endemic areas of hepatoma prevalence, therefore, might well be aflatoxin. It has also been suggested that other food contaminants, plant products or, in the industrialized countries, the synthetic chemicals which exist locally as a result of a given industrial process[25-27] might be responsible for other foci.

The rate of hepatoma diagnosed at the New York City's Bellevue Hospital was five times that of neighboring University Hospital, a distance of one-fifth of a mile, when compared per adult admission or per autopsy during the period from 1950 to 1970. These "local" differences, and similar findings elsewhere in the western hemisphere, are found to be roughly proportional to the incidence of cirrhosis and to the intake of alcohol. All authors agree that the incidence of hepatoma and cirrhosis are parallel.[8,28] An overall estimate is that roughly 60% of all hepatomas in these "nonendemic" areas of the world are associated with cirrhosis, and this figure may reach 90% in hospitals with high alcoholism admission rates.

If cirrhosis, regardless of etiology, is a premalignant lesion, what are the determining factors in this pathogenetic sequence? Several possibilities can be summarized as follows: a) A pathogenic factor common to all forms of cirrhosis is chronic cell damage and resulting cell division. There is speculation that repetitive mitosis in non-stem cell populations, such as hepatocytes, may induce genetic aberrations and malignant transformation. b) It is more likely, however, that the repeated demand for the residual cells to divide, while they themselves are "abnormal"

may be basic to this process. Thus, liver damage due to chronic alcoholism reflects the direct toxicity of alcohol itself in the presence of nutritional deficiency, with a resultant diminished capacity for normal cell functions. Abnormal "repair" is likely to occur. c) The liver as the major filter of the body has a prime responsibility to metabolically alter and eliminate the natural or synthetic carcinogens which are ingested daily. In the face of a diminished metabolic capacity, the liver itself may become even more vulnerable to the action of these agents. To this possibility must be added the well-demonstrated increase in susceptibility to carcinogens which occurs when hepatocytes are in division. It has been reported that single doses of several agents, which are noncarcinogenic for the resting liver, induce hepatoma when administered during hepatocyte cell division.

Another form of chronic liver injury leading to cirrhosis is that reputed to result from persistent viral infection. In an increasing number of studies, an association has been reported between chronic liver disease, cirrhosis, hepatoma and the purported viral antigen HB Ag. A number of these have demonstrated a significantly higher incidence of HB Ag positivity in hepatoma patients than in the general population of the area. In addition, an increased rate of HB Ag prevalence in patients from those areas of tumor prevalence is also used by some authors as evidence for a relationship between the two entities.[29-33] Although the link between this antigen and its virus and the further necessity of linking this virus to tumor production is tenuous at present, the possibility cannot be ignored. Further, it is interesting to speculate that a synergism could exist between a low-grade viral infection in the presence of a carcinogen, *ie*, aflatoxin ingestion. However, it is possible that a tumor or tissues under its influence may be highly susceptible to secondary viral infection, thus explaining the apparent relationship to viral antigen without implicating the "virus" as an oncogen.

In every series reported from nonendemic areas thus far, hepatomas have also been noted in the absence of cirrhosis. These tumors therefore, represent spontaneous malignancies. Heston,[34] Wolff [35] and others have demonstrated that genetic determinants are involved in the evolution of hepatomas in some species. It is possible that some individuals are much more susceptible than others to carcinogenic stimuli and do not require the intervening phase of cirrhosis. This susceptibility could take the form of an altered metabolic pattern for chemical carcinogens or a defect in some enzyme(s) obligatory for the maintenance of normal macromolecular homeostasis, *ie*, DNA repair.

Pathology

The gross and histologic appearance of hepatoma have been available for the past 70 years.[28,36–38] Although Eggel's gross classification of massive, multinodular and diffuse forms is still used, the existence of a diffuse form is conceded to be rare.[39] In the 210 cases of hepatoma, studied at New York University, which were examined as background for this review, not a single case of the diffuse form was found. The most common form was that of a major tumor mass (65%), usually of the right lobe and frequently having satellite nodules (Figures 2–4). The penetration of these satellites into adjoining parenchyma was very varied, and occasionally the left lobe was fully involved, leading to a picture simulating that of "multinodular" form. The purely multinodular form was suggested by the presence of tumor nodules which were of roughly equal size throughout a large portion of the parenchyma and occasionally throughout the entire liver (Figure 5). This presentation suggested a "field-effect" in which malignant transformation had occurred simultaneously in many foci. The possibility of multiple primary origins was supported by the histologic finding of malignant foci entirely confined within cirrhotic nodules, at widely separated distances, and without obvious connections.[36] However, in other instances, a remarkable penetration of cirrhotic septa by tumor suggested a transhepatic lymphatic penetration which could have evoked the multinodular picture. This then was the picture of tumor permeation (Figure 6).[40]

The most common gross finding associated with either tumor type was the penetration of the tumor into the venous system. Tumor thrombi were often demonstrated in the hepatic veins and in two cases, the tumor demonstrated transcaval growth (Figure 7). One of these tumors grew in contiguity from liver to the right artium. More frequently, the tumor invaded and grew in retrograde fashion into the portal system. In several cases, total or near total occlusion of the portal vein might well have contributed to the ruptured eosphageal varices which were also noted.

Aside from the frequent finding of spread to the lungs, local nodes and a significant number of vertebral and adrenal (often right side only) metastases, two less frequently described areas of spread occurred (Figure 8). In approximately 5% of these cases, tumoremia was demonstrated. Tumoremia is defined as the presence of tumor thromboemboli in vessels of many of the organs (Figure 9). This may result from an explosive growth due to total loss of host resistance terminally or acquisition of an inordinate "loosening" of tumor cells. In three cases, the tumor had grown down into the major bile duct, causing obstruction.

The histology of the primary tumors in our study was identical to that described in detail by Edmundson [28] and others. They varied from those which bore a remarkable resemblance to normal liver to others which were totally anaplastic. None of the 210 cases fitted the criteria for a mixed hepatoma-cholangiocarcinoma, although several demonstrated tubuloform variation. (As this review was completed, a surgical specimen of supraclavicular node was processed which demonstrated a mucin- and bile-producing "hepatoma"). One interesting finding which was suggested by the analysis of the New York University case material was a tendency for tumors in noncirrhotic livers to be more frequently of the major mass variety, and these often demonstrated a well-differentiated picture, Figures 10–16).[41]

A number of human hepatomas have been examined by electron microscopy.[42–45] Although their component cells consistently demonstrated loss of cytologic structure, they often revealed a striking resemblance to normal hepatocytes. In our own experience with 4 cases, there has been a strong tendency to internal variation of the cytology of cells within tumor areas. Some demonstrated a remarkable preservation of granular endoplasmic reticulum, bile canaliculi and other markers of hepatocyte function; others were very poorly differentiated. The finding of "capillarization of sinusoids" which has been reported previously, was seen in all of these tumors. It is possible however, that these vessels actually represent new capillaries which develop in response to elaboration of a tumor angiogenic factor and form the blood supply of growing tumors, rather than tumor sinusoids (Figures 17 and 18).[46]

In accord with the findings of others, patients with hepatoma in this series died frequently with ruptured esophageal varices or in hepatic failure. The former might have been related in part to tumor thrombi in the portal circulation, but it should be pointed out that roughly 95% of those patients who demonstrated ruptured varices were also cirrhotic. All patients who demonstrated severe hepatic decompensation were cirrhotic. Two interesting and less discussed causes of death were also detected in this study. Seven patients died with hepatorenal syndrome, while 18 demonstrated severe gastrointestinal hemorrhages from acute gastric ulceration. In addition, a large number of patients, 93/210, died of various causes including pneumonia, cardiovascular disease, thromboembolism or other conditions which are related only indirectly to the tumor. The percentage of patients dying from causes directly related to the tumor is much higher in endemic areas. There, clinical presentation often results from the tumor's growth, hemorrhages and rupture, and the patients are usually younger.

Most of the analyses of the patterns of cirrhosis which were asso-

ciated with hepatoma were aimed at delineating the etiology and pathogenesis of malignant evolution.[47] For the moment, this goal has not been achieved.

For subsequent discussion in this review, a specific entity, small nodular cirrhosis (SNC) will be defined as that in which more than 90% of the liver mass is composed of nodules measuring 0.5 cm or less, with fibrous septae, scars or areas of confluent parenchymal loss of any size. More than 90% of these nodules do not possess normal architectural landmarks such as portal areas and central veins. All other forms of cirrhosis will be termed non-small nodular cirrhosis (NSNC). It should be pointed out that in reality, the vast majority of cirrhotic livers demonstrate a mixture of these patterns. Unfortunately, it is not possible to determine an etiologic or pathogenetic sequence from the pattern of cirrhosis alone, since "both" types have been found to result from diverse causes, and the duration of disease may determine the variation in pattern.[49]

However, in many previous reports, a strong relationship existed between NSNC and the presence of hepatoma. This association held true in high and low risk areas. In the cases studied at NYU however, 44% of hepatomas were associated with SNC; 43% with NSNC and the remaining 13% with livers which could not be classified as cirrhotic. This somewhat unusual distribution may reflect the very high incidence of alcoholism at Bellevue Hospital. Indeed, one-quarter of the patients without definable cirrhosis also had an alcoholic history. These findings however, appear to strengthen rather than weaken the association between hepatoma and NSNC in view of the relative infrequency of this latter diagnosis in Bellevue Hospital autopsy material.

It is of interest that one group of patients at NYU with an incidence of hepatoma far in excess of that expected from admission or autopsy rates were Chinese who had immigrated to this country. This finding has held true at Bellevue Hospital for more than 50 years.[51] Although exact rates of hepatoma for Chinese emigrees cannot be determined, in other countries it has been reported to be directly related to the period spent in China prior to emigration

Another approach to establishing an etiologic association based upon pathologic analysis might be the histology of the tumors themselves. However, in all series reported, from high and low risk areas, as well as in the alcoholic and nonalcoholic, no significant histologic differences have been found. Therefore, one cannot use the same technic which has been relatively successful in the analysis of lung cancer, wherein specific histologic appearances correlate with exposure to chemical carcinogens. This is not surprising since, in this instance, the cell respond-

ing to challenges, the hepatocyte, is not of the multipotential stem cell type and gives rise to all forms of hepatoma.

No means of characterizing hepatomas now available permits differentiating these tumors in terms of prognosis. Although some tumors bear a close histologic resemblance to liver and others are anaplastic, the range of patient survival is limited. The vast majority of patients were dead within 1 year of presentation. Some hepatomas, histologically well differentiated, have been termed minimal deviation tumors. This designation was based partially upon their morphology and, in the experimental situation, upon metabolic similarity to liver. However, the more closely the minimally deviated experimental tumors were examined, the more significant differences were found between them and normal liver and the use of the term minimal deviation therefore, has no applicability at present. Although some experimental hepatomas have sufficient phenotypic variation to divide them roughly into groups, all of these were induced by a single carcinogen.[52] Perhaps some form of functional analysis of human hepatoma, such as alpha fetoglobulin production, will allow a differentiation in terms of prognostication.

Biology

One of the most fascinating aspects of tumor biology is the relationship between the tumor and host. Invasion of vasculature and destruction of vital tissues are common pathways of interrelationship, often the ultimate cause of demise. However, we understand much less about the severe wasting seen in so many patients in the face of maximal efforts to supply nutrients. In some experimental hepatomas, an intense avidity for selected amino acids has been demonstrated. In these instances, the tumor may act as a nutrient trap, selectively depleting the patient of a single essential amino acid and thus thwarting any efforts to maintain normal protein synthesis.[53] Other workers have persistently claimed that a "toxo-hormone" is produced by several tumors, a material with vague detrimental effects on the host. The role of such factors remains to be defined.[54]

Several reports indicate that human hepatomas interact with their host by other means. Prominent among these have been the production of hypoglycemia and erythropoiesis, but in neither is the hepatoma unique. It has been postulated that many large rapidly growing tumors may utilize sufficient glucose to overcome the host's capacity for production; while in other cases, insulin-like substances have been identified. Although some studies have suggested the presence of insulin-like substances in hepatomas, a consensus at present would not accept this single hypothesis.[55–57] The hypoglycemia associated with hepatomas may

represent a combination of the following factors: a) high glucose utilization by the tumor; b) depressed gluconeogenesis by the liver, possibly resulting from the decreased availability of substrates and c) glycogen storage in tumors and liver simulating an acquired glycogenesis, possibly due to decreased phosphorylase activity (? decreased phosphorylase kinase).[58]

Clearly, in the instance of hypoglycemia, as in that of amino acid "trapping," administration of exogenous substrates in amounts sufficient to overcome the tumor's capacity to utilize them might stimulate tumor growth as well as sustain the patient. Examination of such phenomenon in experimental situations may produce information with value beyond that which can be anticipated now. One example of this is the unexpected finding that host hypoglycemia "sensitized" experimental hepatomas to the antitumor effect of the enzyme L-asparaginase.[59]

Erythrocytosis reported in 1 to 14% of hepatoma patients seems of less importance to their well-being but illustrates once again the complexity of interaction between host and tumor.[60-62] Although tumors of other types have demonstrable erythropoietin production, as reflected in elevated circulating levels, this has not been clearly demonstrated with hepatoma. Neither, however, have other causes of reactive erythropoiesis such as altered arterial oxygen saturation and splenomegaly.

In addition to these more common presentations, published reports exist which also incriminate hepatoma in the production of porphyria,[63] carcinoid syndrome,[64] severe alterations in calcium metabolism [65,66] and in the production of dysfibrinogenemia.[67]

This last phenomenon, although reported in a single patient, may contribute a great deal to our understanding of the relationship between tumors and their host. These authors suggested that dysfibrinogenemia resulted from the production by the tumor of an abnormal fibrinogen which interfered with normal clotting mechanisms. It has been demonstrated that some experimental hepatomas are capable of vigorous production of normal plasma proteins.[68] This is remarkable in itself, since many of these tumors were cytologically poorly differentiated. These findings suggest that the production of abnormal molecular forms of plasma proteins by hepatomas is possible and that these might interfere in the normal relationships of liver-products and the host.

Alpha Fetoglobulin

In 1963, Abelev identified an alpha-1-globulin (AF) in the serum of fetal animals and pregnant females.[69] Since that time, AF has been demonstrated to be produced by the fetal liver and high levels are available in amniotic fluid. Later, Tatarinov demonstrated AF in the serum

of hepatoma patients, thus beginning an era of diagnostic and biologic investigation.[70] Alpha fetoglobulin has been found in trace amounts in the sera of normal adults, with transient elevations during liver regeneration or toxic liver injury.[69] Rarely, elevations are noted with metastases of other tumors to the liver or in association with embryonal tumors, but these are so infrequent that a sustained elevation of AF in an adult is almost invariably associated with hepatoma.[71]

A large number of immunologic technics have been developed to test for AF. These range from double diffusion gels to radioimmunoassay inhibition (Figure 19).[69,71] Utilizing these methods, a rate of positive reaction of approximately 70% has been demonstrated in Africans bearing hepatoma. The mechanism for the significantly higher rates of positive reactions in these patients when compared to those of other areas has not been explained.[72] Experimental tumors evoked by differing carcinogens demonstrate no significant differences in production of AF.[71] It is also interesting that well-differentiated tumors of humans as well as those of rats generally produce low levels of AF or none at all, while the more anaplastic tumors produce AF in quantity.[71] Although there is an assumption that the synthesis of this protein by hepatomas is evidence for "fetal regression" as an integral part of carcinogenic evolution, the difference in production from one tumor to another suggests that the mechanism for this association is as yet unknown.

A number of recent experimental studies have demonstrated that AF may appear in animals during their exposure to carcinogens long before malignant tumors are detectable.[73,74] Again, this association may give us more important information concerning carcinogenic evolution than has been gained by an examination of AF production by tumors themselves.

The circulating levels of AF can be used to follow therapeutic results, since reversion to negative sera has often been demonstrated after surgical excision.[71] Resumption of a positive reaction usually signals recurrence. Other interesting relationships are also appearing, for example, the high frequency of AF in Indian childhood cirrhosis.[75] This association raises questions concerning the etiology of this syndrome, indicating the possibility of the intervention of an endemic toxic or carcinogenic agent, and raises the question of eventual hepatoma production should these children survive.

Diagnosis

Methods of tumor detection usually aim at two goals: a) early detection and b) definitive diagnosis. It is difficult at present to foresee fulfillment of the former in the near future. With the low incidence of

hepatoma in this country, routine serologic examination for AF may not be warranted, save for patients with known cirrhosis, chronic liver disease or a history of alcoholism. Thus, the complex of vague clinical signs and symptoms, well described in many texts, remains the only warning of its presence. Right upper quadrant discomfort, a sudden increase in portal pressure, or the accumulation of ascites (bloody less often than not) or jaundice must remain the skimpy clinical clues to this entity.[5,6]

From the standpoint of laboratory examination, determination of AF levels is a satisfactory tool. Innumerable reports of other specific laboratory "patterns," abnormal enzymes or protein electrophoresis have not proved to be helpful.[76]

A great deal of effort has been devoted to a variety of technics which visualize the hepatoma for identification and localization once it is suspected. Recently, studies have reported direct visualization and tumor biopsy by laporoscopy.[77] This valuable instrument may represent a highly critical method for the diagnosis of hepatoma and background liver disease wherein the tumor presents at the liver's surfaces.

Other workers have utilized the unique blood supply of the hepatoma to visualize this tumor.[78,79] From its earliest growth, the hepatoma incorporates an hepatic arterial vasculature. Selective angiography of hepatic arterial branches often demonstrates the rush of dye through the capsule of the tumor, when it is of the major mass type, and then flow into its mass via complex vascular patterns. The definition of hepatoma by this means permits excellent localization, differentiation from metastatic disease and a moderate degree of diagnostic certainty.

Visualization by inference has also been developed, utilizing the dye concentrating capacity of the hepatocyte and the phagocytic activity of the reticuloendothelial system (RES).[78,80] Both approaches are based upon the assumption that malignant tissues are not capable of these activities, an assumption which may not be invariably valid. A number of hepatomas produce bile which is histochemically "normal," and the cells of these tumors may be capable of uptake and concentration of dye, while the prominent endothelium of these tumors (Figure 11) might represent endothelial derivatives with phagocytic potential. However, a radioactive nucleotide such as ^{131}I-rose bengal should only appear, albeit transiently, in normal hepatocyte parenchyma, while colloid materials, especially technetium 99m sulfur colloid, would be extracted rapidly from the blood by the RES, concentrated, and then rapidly degraded. Both technics produce only a low liver radiation dose, but are readily detected by scintiscan (Figure 20).

The final definition of the nature of a liver tumor is always at a histo-

logic level.[28,81] Whether tissue is obtained after localization by palpation or radiology or direct visualization, the tumor itself should be biopsied. There is no evidence, at present, that tumor tissue is more prone to postbiopsy bleeding than the liver itself, provided coagulation studies are normal. Our ability to make the histologic diagnosis of hepatoma is based upon the odd rule that "the tumor should resemble hepatocytes but not too closely." This rule elucidates the two major problems in analyzing such tissue: distinguishing it from other malignancies, usually metastatic and distinguishing the tumor from abnormal but nonmalignant liver, usually cirrhotic. To distinguish hepatoma from other tumors histologically, the following points can be sought in order of descending differential importance: a) resemblance to hepatocytes and their trabecular structure b) bile production by the malignant cells, for often hepatomas and their metastases produce bile, even when the tumor is anaplastic; c) the presence of glycogen within tumor cells and d) the presence of papillary-like fronds, with endothelial sheathes (Figure 10).

To distinguish a well-differentiated hepatoma from a cirrhotic liver is often more difficult. The former frequently forms masses without the normal liver landmarks, has no septa (desmoplasia is usually diffuse and helpful when present), demonstrates more basophilia, shows odd papillary endings to trabeculae (Figure 11), occasional mitoses or abnormal nuclei and demonstrates bile formation in aberrant rosette-like structures. Venous "lakes" may be seen.

Therapy

The reported life expectancy of hepatoma patients averages roughly 6 months to 1 year from onset of symptoms and 3 to 6 months from diagnosis. Noncirrhotic patients with well-differentiated tumors may die just as rapidly as the cirrhotic patient with multifocal, poorly differentiated tumors. Despite attempts at grading hepatomas, frequently based on biopsy alone, these tumors demonstrate so narrow a range of life expectancy as to suggest that they are biologically similar, and that this factor may dominate over others such as size, location, local spread and histology. Except for deaths from liver failure or portal venous complications, these patients usually die from tumor spread, or the usual malignancy-associated spectrum, including thromboembolism, pneumonia and "no demonstrable cause." A significant tendency for early nodal and vascular spread is noteworthy. This is nowhere better illustrated than in patients demonstrating widespread metastases and/or tumoremia a short time after total surgical removal of the primary hepatoma by partial hepatectomy or total hepatectomy with transplantation.[82]

Perhaps the most logical form of therapy, albeit generally unsuccessful, is that which is based upon the unique vascular supply of this tumor.[83] As indicated above, these tumors derive all of their blood from the hepatic artery, and the exact source or branch can often be demonstrated radiologically. An initial attempt to utilize this phenomenon was the selective ligation of the appropriate artery. In most cases, the liver, especially if noncirrhotic, survived this focal deprivation while the tumor underwent severe central necrosis. The failure of this method has resulted in large part from the ease with which the tumor subsequently "captures" collateral supply.

More impressive have been the results of selective perfusion of the tumor via its arterial source.[84,85] Chemotherapeutic and radioactive materials (such as microspherules) can be infused at high levels, and in some instances systematic circulation avoided by venous catheterization of the draining blood. The results of this approach are variable, but it may represent the procedure of choice in multifocal tumor where perfusion via the main hepatic artery would affect all nodules.

The most successful approach at present has been the removal of a major tumor mass by partial hepatectomy, in noncirrhotic livers. In many instances, this procedure has resulted in a considerable increase in life expectancy with some survivals exceeding 5 years.[86–88] The impressive capacity of residual hepatocytes to proliferate following surgical amputation of a major portion of the liver has been documented in many mammalian species, including man.[89,90] Within 7 to 10 days, the functional mass is restored, and relatively few postoperative complications are noted (Figure 21). It has been suggested that the cirrhotic liver may also be suitable for this approach, although the residual hepatocytes of such livers (especially in the cirrhosis of chronic alcoholism) are often functioning at a borderline rate. The postoperative course can then be much more threatening.

Perspective and Prospects

An attempt has been made to present an overview of the model human tumor–hepatoma. Many excellent reports have offered detailed studies of selected areas of this topic, and for this reason, the major emphasis of this review was upon newer developments or points not previously stressed. I will conclude by summarizing some of these promising and perplexing areas.

Pathology and Etiology

Clearly, when exposed to endemic dietary carcinogens, many of the inhabitants of select areas develop hepatoma, some at a relatively young

age, Thus, a continuing examination of geographic distribution of this tumor may alert us to new endemic foci and to other carcinogenic agents newly introduced or indigenous, synthetic or natural. The major unanswered question regarding such endemic foci is the possibility of the presence of other factors which might act synergistically with aflatoxin or with other carcinogens. In the long run, it may be easier to eliminate these secondary factors, *eg*, protein or vitamin deficiency, parasitic infestation or excessive iron intake, than to eliminate the endemic *Aspergillus* and its product aflatoxin. In the same way, it has been proposed that elimination of asbestos exposure might greatly reduce carcinogenesis from smoking.

Further, local epidemiologic observations, whether they reveal differences between alcoholics and nonalcoholics or the frequent presentation of hepatomas in patients originating from other countries, such as the Chinese population at Bellevue, should again alert us to etiologic possibilities. For example, in endemic areas, the incidence of hepatoma is far greater in men than in women. Is this another clue to etiologic associations or to a sex-related metabolic difference, as has been shown in animal responses to chemical carcinogens?.[91]

What is the premalignant lesion in the alcoholic patient or in human aflatoxin hepatocarcinogenesis? Relatively little cellular atypia is noted in examinations of most cirrhotic livers prior to the appearance of carcinoma. This may be explained by experimental studies which suggest that premalignant cells may not be readily differentiated from normal hepatocytes. The majority of cells of the hepatic nodule, which is induced by chemical agents, are diploid and are relatively normal histologically.[15] Indeed, "atypical" cells within these nodules are not found to participate in cell division. The cirrhotic nodule may then bear the potential for malignant evolution without clear morphologic stigmata. Indeed, the work of Lee has demonstrated that malignancy may develop in cirrhotic livers years after alcoholic exposure has ceased and that this malignant transformation often occurs in livers with the least diffuse damage to the parenchyma.[49] This finding and the strong association of NSNC with the development of hepatoma reported generally is quite surprising. Based on explanations previously offered to explain the cirrhosis-tumor relationship, cell division, metabolic imbalance, etc, one would have expected that those livers demonstrating SNC would have demonstrated the highest tumor incidence. There is no explanation for this paradox at present.

Our ability to distinguish hepatoma histologically might improve by the application of laboratory findings to biopsied tissue. Perhaps enzyme

histochemical analysis of biopsy tissue is the answer, possibly utilizing glucose-6-phosphatase alterations or other crucial hepatocyte-associated enzymes. Another approach would be the use of anti-AF immunofluorescence. Some tumors which produce too little AF to be detected by serum analysis are "positive" by immunofluorescence technics using antibody to AF.[92,93] In these instances, the production of AF by the tumor may be so low that clearance mechanisms can maintain circulating levels below the sensitivity of commonly used tests. The former might aid in differentiating hepatic tumor from others, the latter to differentiate it from liver and from other tumors.

Therapy

It is probable that the use of selective chemotherapeutic agents will increase in the future. When combined with surgical removal of hepatoma, these could reduce recurrence at metastatic sites. The experimental study of normal liver metabolism might permit a more rational prediction of suitable agents. If there is persistence of specific hepatocyte-associated metabolism in hepatomas, it might enable us to synthesize agents that would be activated only in the vulnerable hepatoma and in the less sensitive, nondividing hepatocytes. The basal hepatocyte is relatively resistant to the effects of most cytotoxic agents. The dividing hepatocyte, after partial hepatectomy however, is vulnerable to the cytotoxic effects of many antitumor agents. Thus, during the regeneration of the liver subsequent to the amputation of an hepatoma, there should be a delay on administering chemotherapeutic agents.[94]

Finally, the obvious goal of the study of this tumor is to reduce its incidence in the world. Whether the attack be upon one factor of a synergistic carcinogenic combination or the reduction of known carcinogens, the elimination of but a few etiologic agents would have a drastic effect upon the aggregate incidence of malignancy.

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Legends for Figures

Fig 1—A photomicrograph of a section through a hepatic nodule induced in a rat liver by ingestion of the chemical carcinogen *N*-2-fluorenylacetamide. Although the majority of cells possess normal diploid nuclei (*arrow*) hepatocytes with severely "atypical" nuclei are seen (2 arrows) (H & E, × 450).

Fig 2—A human hepatoma of the major-mass type is demonstrated in an otherwise normal liver. No satellite nodules, vascular invasion nor intrahepatic spread were noted, and the patient died of causes unrelated to the tumor.

Fig 3—A human hepatoma in a cirrhotic liver of the NSNC type. Striking demarcation of the tumor is noted occasionally in the major-mass type, and this appearance was heightened in this case by intrahepatic hemorrhage and necrosis. Only one satellite nodule was found. This patient died of pulmonary emboli.

Fig 4—A human hepatoma of the major-mass type with extensive satellite nodules spread throughout all areas of the liver. Although the tumor apparently arose in the median portion of the right lobe, the tumor had spread to all margins. Additional tumor was found in the lung, kidneys and adrenal gland.

Fig 5—A hepatoma of the multicentric or multinodular type which was found In a cirrhotic liver of the SNC type. Tumor nodules were found throughout both hepatic lobes and the majority of tumor nodules were approximately 2 to 3 cm in diameter. Many tumor nodules ranging from 0.5 cm to this common size were also noted. Additional tumor was found in the lymph nodes of the porta, lungs, portal vein and vertebral bone.

Fig 6—A photomicrograph of a section through a liver which demonstrated multicentric hepatoma and NSNC cirrhosis. The lymphatic and venules of the liver showed extensive invasion and growth by the tumor. Particularly striking were the lymphatics within cirrhotic septae in all areas of the liver; these were filled with tumor. A longitudinal cut of a lymphatic demonstrates such tumor growth (*arrow*) and suggests a means by which the hepatoma might permeate to all areas of the liver creating a multicentric appearance (H & E, × 150).

Fig 7—A tumor thrombus which arose from a hepatoma of the major-mass type can be seen within a major hepatic vein. It had grown into the inferior vena cava (*arrow*). The liver demonstrated a cirrhosis of the SNC type.

Fig 8—A section taken through the lung from a patient dying of extensive metastatic hepatoma. Although the tumor was considered histologically well-differentiated, it had spread to many organs. Tumor thrombi can be seen filling vessels within the section and completely occluded these vessels (*arrow*). Of particular interest is a tumor thrombus in the subendothelial area of a major pulmonary vein (*2 arrows*) (H & E, × 12).

Fig 9—A section of lung obtained at the autopsy of a patient dying of hepatoma and liver decompensation. Although no gross metastases were detected, extensive tumor thrombi were found in small vessels in many organs. The vessels of the lungs were massively involved although the patient demonstrated no respiratory difficulty. Typical tumor growth in a small vessel is noted (H & E, original magnification × 300).

Figures 10 to 16 are photomicrographs of histologic sections of human hepatomas which demonstrate differing degrees of histologic similarity to normal liver, *ie*, histologic differentiation.

Fig 10—A well-differentiated hepatoma demonstrating a trabecular pattern with cell nuclei which appear to be similar to those of hepatocytes. A number of cells demonstrate a reticular pattern which proved to be glycogen by special staining (H & E, original magnification ×150).

Fig 11—A high power view of an area of the tumor depicted in Figure 10. The "diploid" nature of the nuclei is clearly demonstrated. The sinusoidal structure and endothelial lining cells (*arrow*) simulate that seen in normal liver. Hyalin areas were noted (*2 arrows*) in some of the tumor cells. In many hepatomas, trabeculae end in a blunt tip as seen in this section (*t*), a conformation which is unusual in nontumor livers (H & E, original magnification × 300).

Fig 12—A striking demonstration of bile production by a well-differentiated but somewhat less trabecular hepatoma. Accumulations of bile were seen throughout the tumor, filling spaces in a manner simulating extra hepatic obstruction. The bile was histochemically identical to that produced by normal liver. A metastasis of this tumor to the lung also demonstrated bile in these dilated accumulations and in single cells as well (H & E, original magnification × 300).

Fig 13—A section of a less well-differentiated hepatoma which demonstrates only residual "sinusoidal" structure (*arrow*). The entire tumor was composed of sheets or solid accumulations of tumor cells which demonstrated relatively normal nuclear and cytologic structure. In occasional areas, representing approximately 10% of the tumor, nuclear pleomorphism was noted. This tumor also grew into and occluded a major portal vein branch (H & E, original magnification × 150).

Fig 14—This photomicrograph of an area of tumor demonstrates a spectrum of histology ranging from extremely well-differentiated to poorly differentiated. The residual trabecular structure is still evident, as is the similarity of many cells to normal hepatocytes. However, nuclear pleomorphism is noted throughout the section as is a tendency toward loss of trabecular structure and the formation of solid areas of tumor (H & E, original magnification, × 300).

Fig 15—The typical histology of a poorly differentiated hepatoma is demonstrated. No trabecular structure can be recognized, although one area suggests that such may have existed earlier in the tumor's evolution. The entire tumor consisted of sheets and masses of hepatoma cells alternating with areas of hemorrhage and necrosis. Although this tumor can be construed as poorly differentiated, some cells still bear a resemblance to those of normal liver (H & E, original magnification × 150).

Fig 16—A higher magnification of an area of the tumor seen in Figure 15. Nuclear pleomorphism and a lack of cellular polarity is evident. Although this represents a

poorly differentiated hepatoma, as indicated by a lack of similarity to normal liver, other tumors show an even greater degree of nuclear pleomorphism with almost no cells demonstrating nuclei and cytologic structure resembling that of hepatocytes (H & E, original magnification × 400).

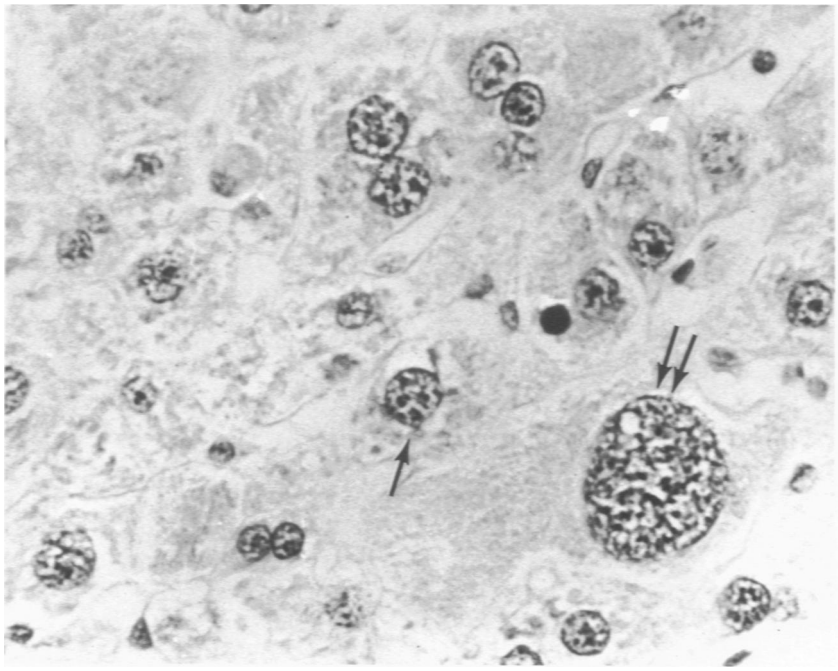
Fig 17—An electron micrograph of an area taken from an histologically well-differentiated human hepatoma. The cells bear a remarkable resemblance to normal hepatocytes with a well formed, although poorly granulated, granular endoplasmic reticulum (GER) (*arrow*). The cytoplasm of these cells was simplified with diminished numbers of mitochondria, most of which are small and round, and lack a prominent Golgi apparatus or any glycogen. Some areas resembling bile canaliculi were detected. The central cell appears to be a "dark-variant," but its internal structure simulated that of the surrounding tumor cells (Uranyl acetate and lead citrate, × 3000).

Fig 18—An electron micrograph of another human hepatoma. This area demonstrates cells which resemble normal hepatocytes in that they possess a GER (*arrow*) and in some areas small aggregates of glycogen. The cells are arranged about a "sinusoidal" structure (s). Within this vessel, an endothelial cell is evident (ec) and in some areas, basement membrane can be detected between the endothelium and the tumor cells (*2 arrows*) (Uranyl acetate and lead citrate, × 3000).

Fig 19—Immunoelectrophoretic patterns using different antisera: *CARS* = serum from an adult rat injected with croton oil (contains the "inflammatory protein" αM-F); *AMF* = amniotic fluid from 15-day pregnant rats; *NARS* = normal adult rat serum; *anti-α1-F* = specific goat anti-α1 fetoprotein; *anti-NARS* = goat anti-normal adult rat serum; *anti-AMF abs with NARS* = goat anti-amniotic fluid absorbed with NARS. Precipition bands specifically identified are: *αM-F* = α-macrofetoprotein, a fetoprotein associated in adults with inflammation; *α1F* = α1-fetoprotein. NARS demonstrates a large number of normal plasma proteins. The α1-F precipitation bands are identical to those seen in human or rat hepatoma sera.

Fig 20—The scintiscan produced by radioactive gold-colloid administration to a patient bearing a large major-mass hepatoma in the upper-lateral area of the right lobe (*arrow*). The "cold area" indicates a lack of phagocytic activity in the area of destroyed parenchyma.

Fig 21—A scintiscan of the above patient demonstrated 1 month after operation. The liver mass was calculated to represent at least 90% of normal having however a globular outline. The spleen is also demonstrated in this scintiscan.



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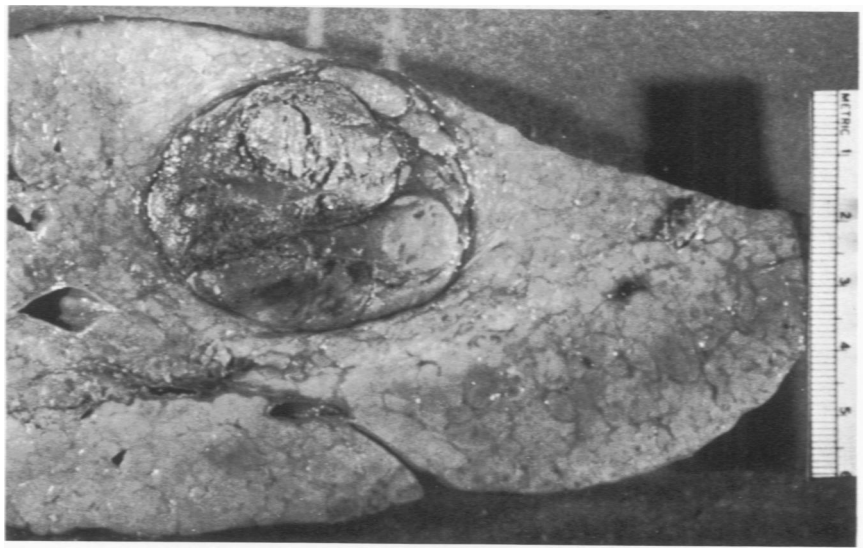

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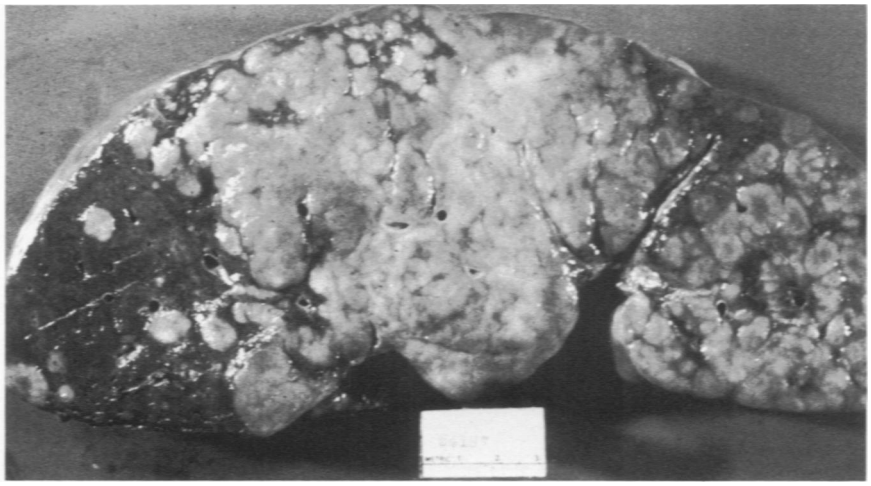

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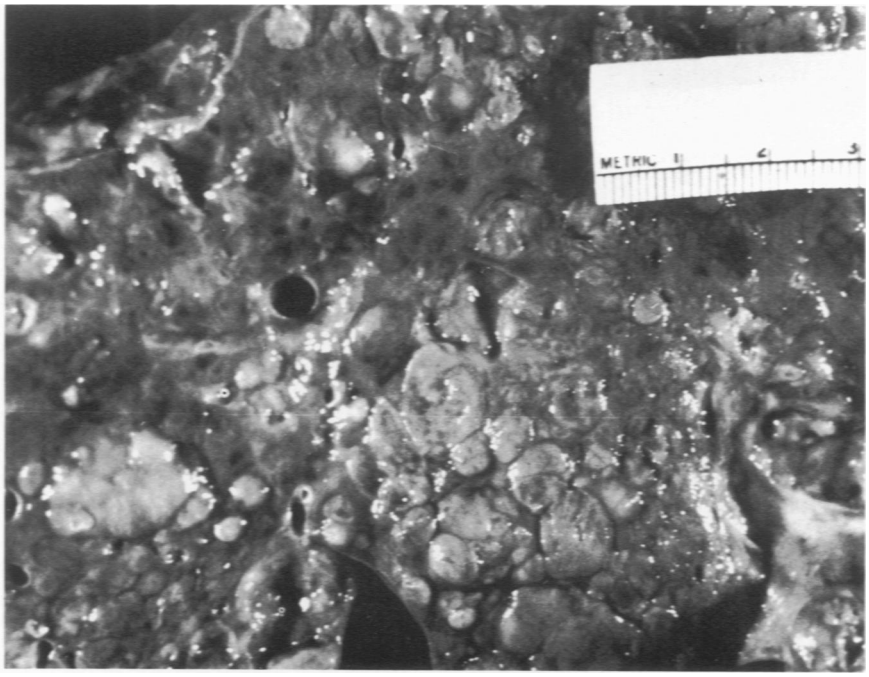

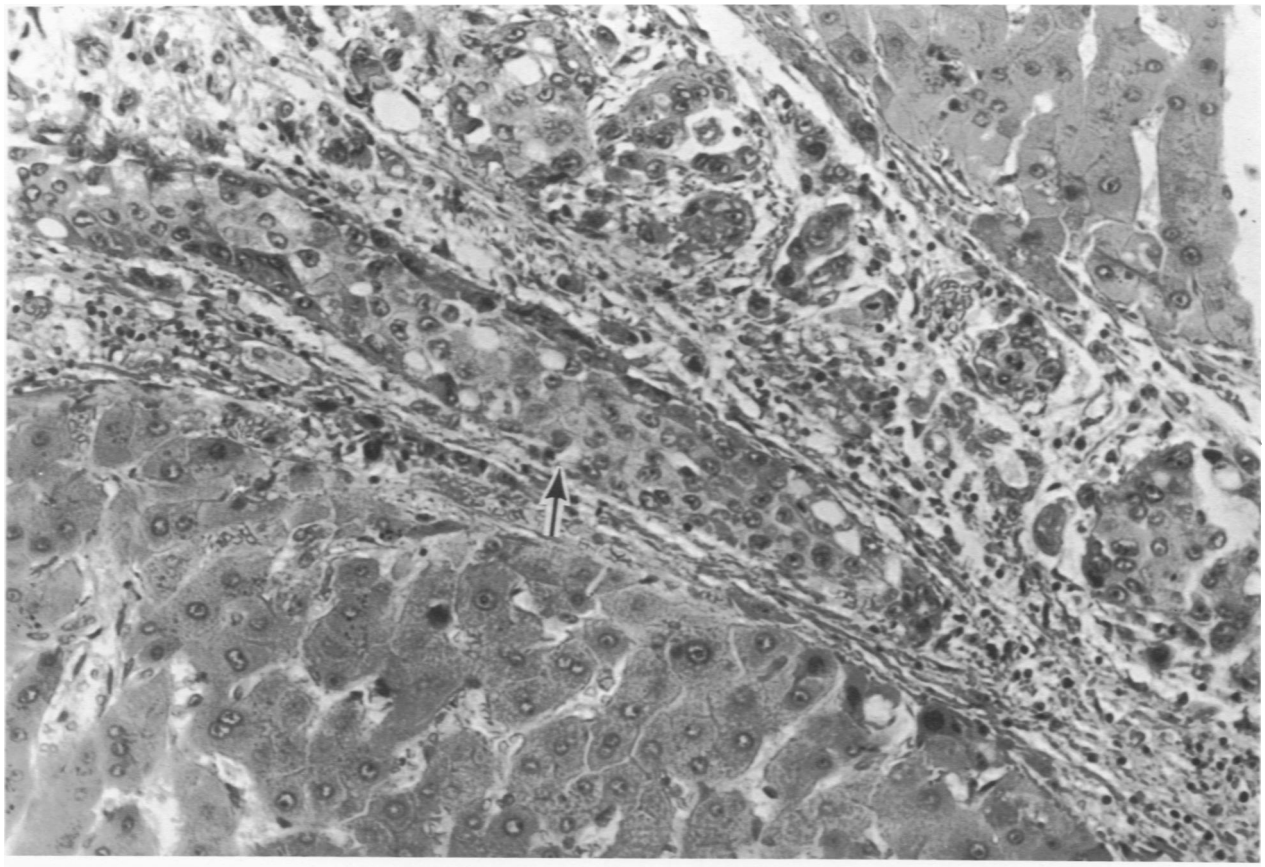

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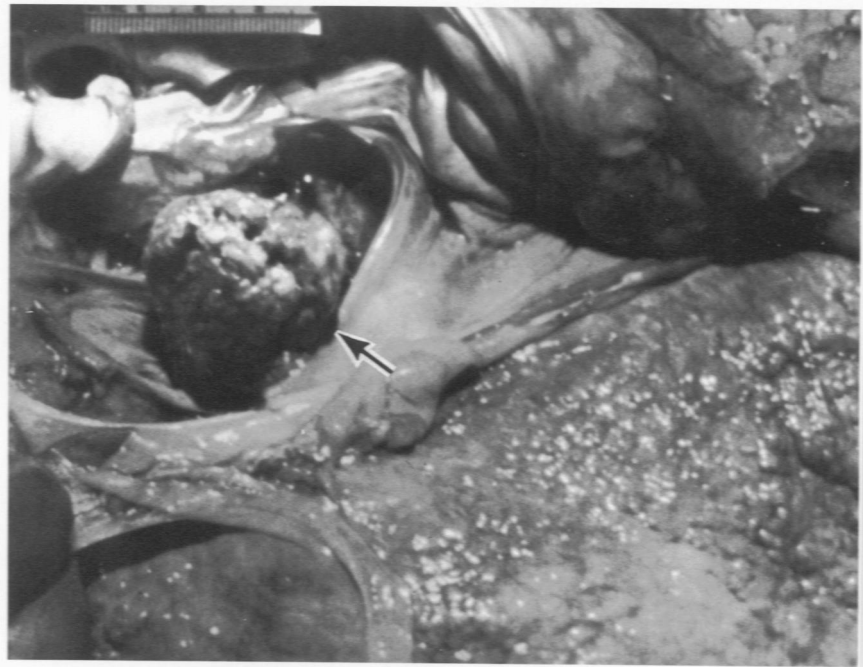

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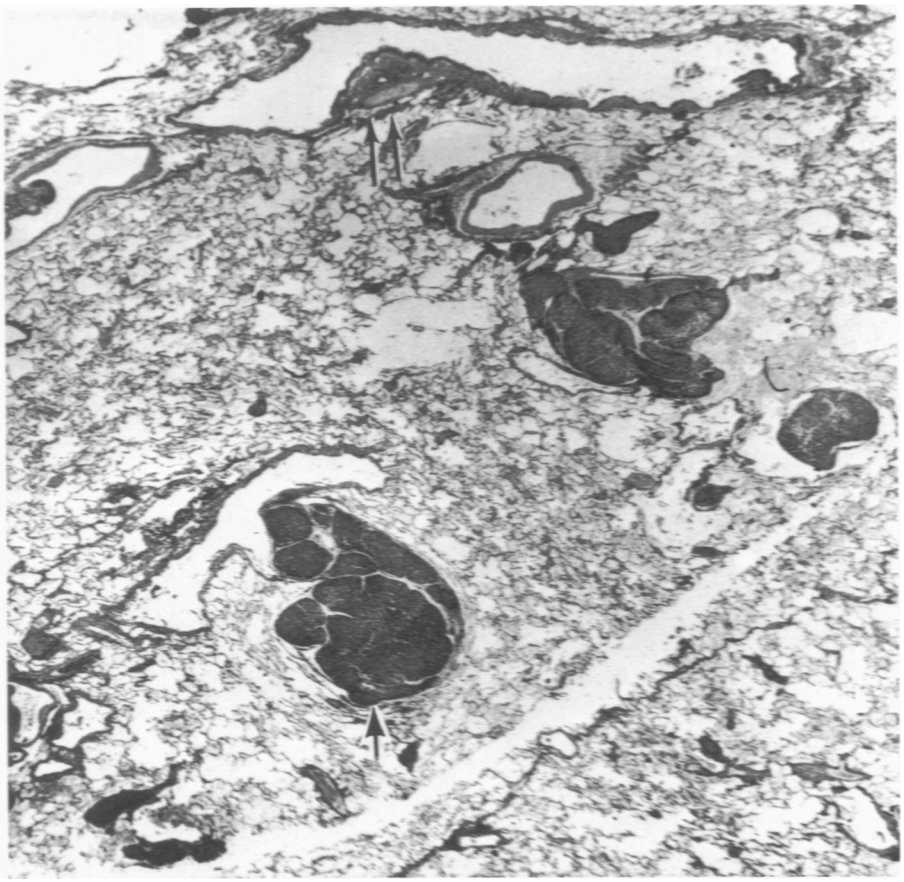

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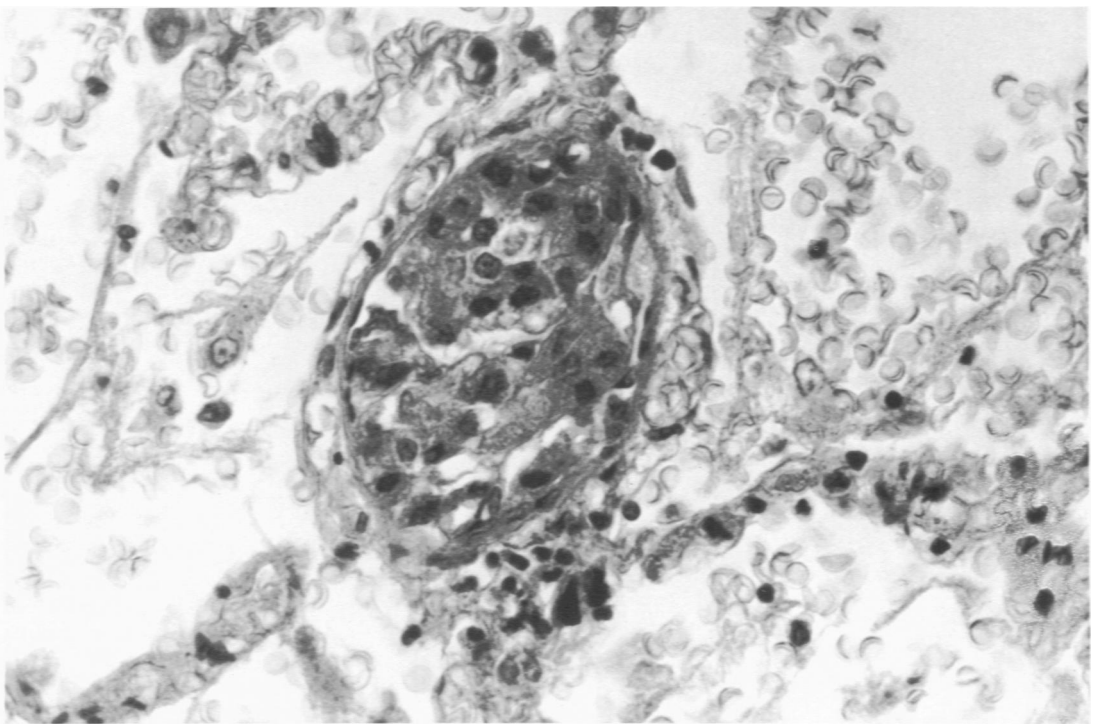

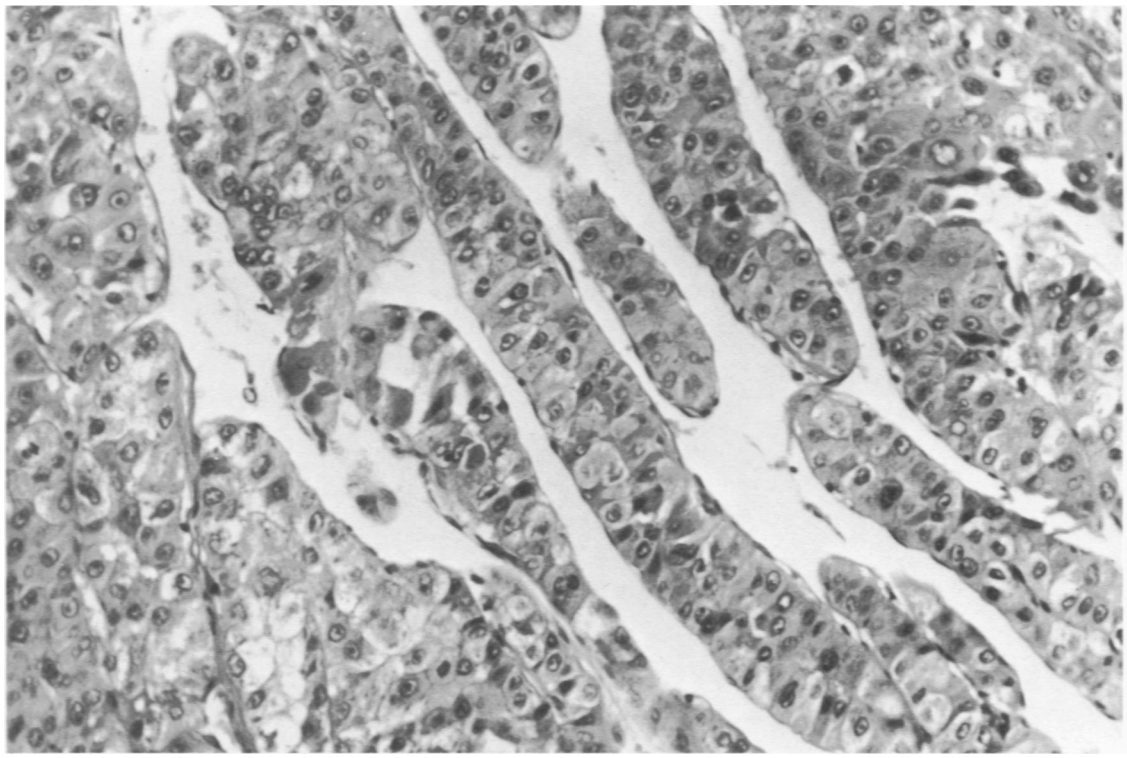

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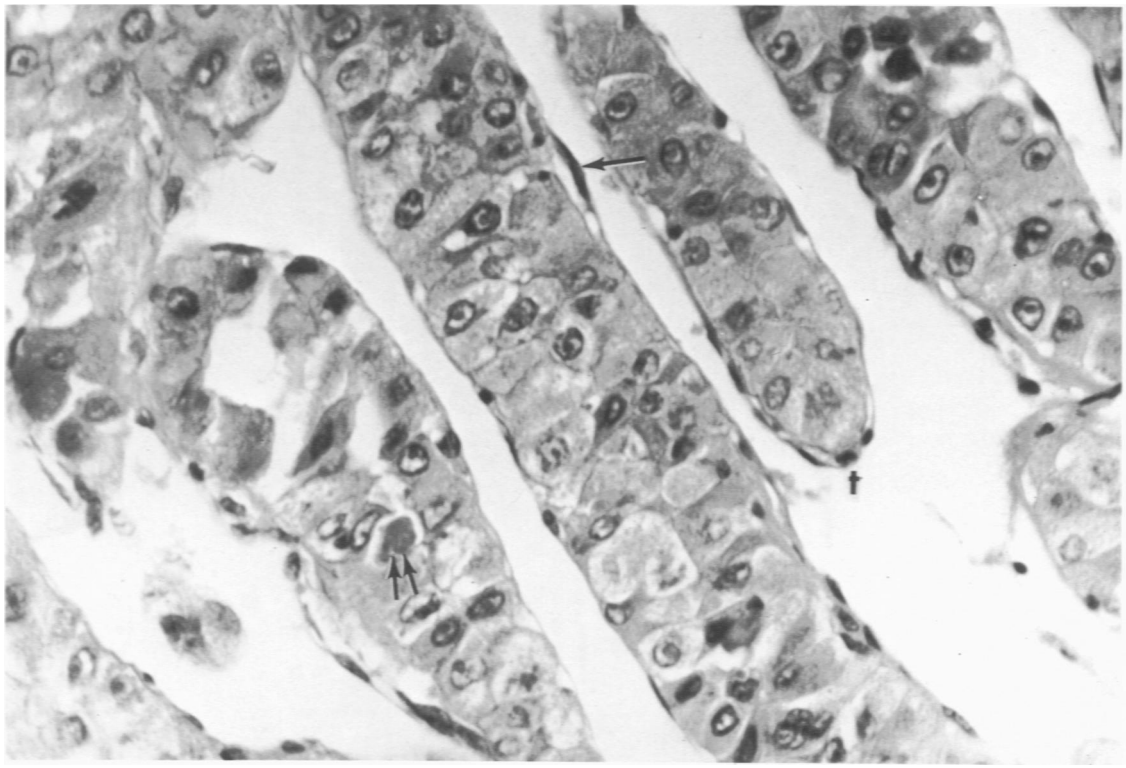

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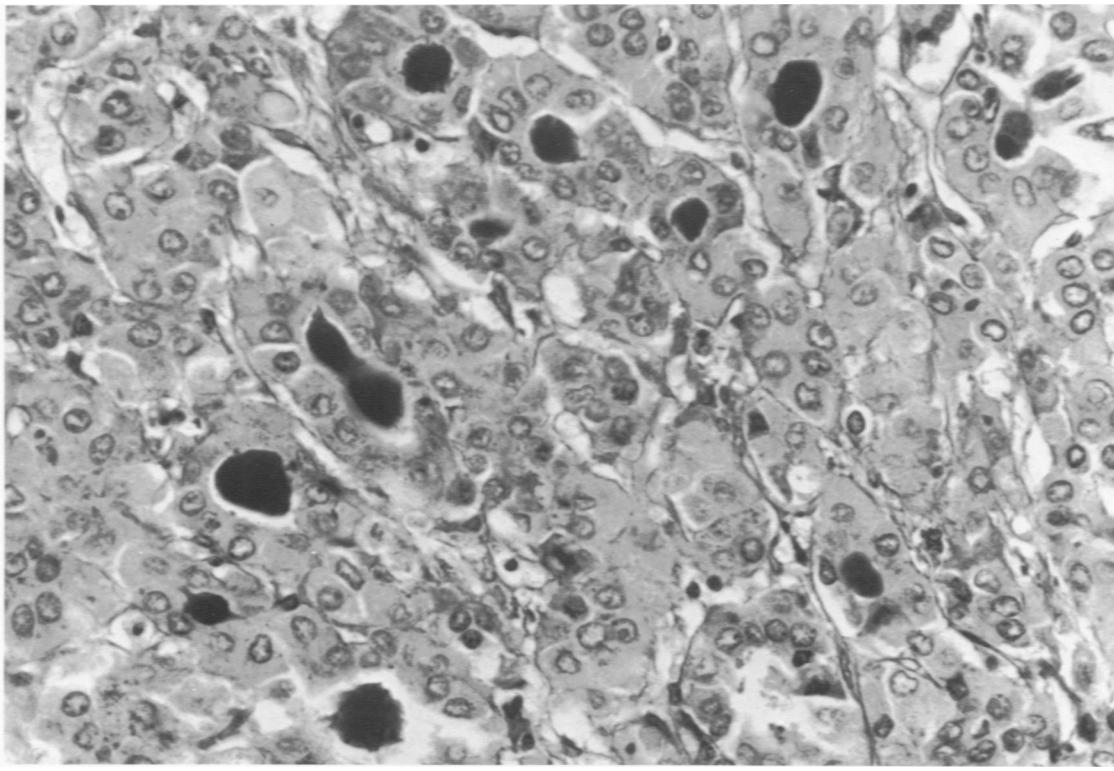

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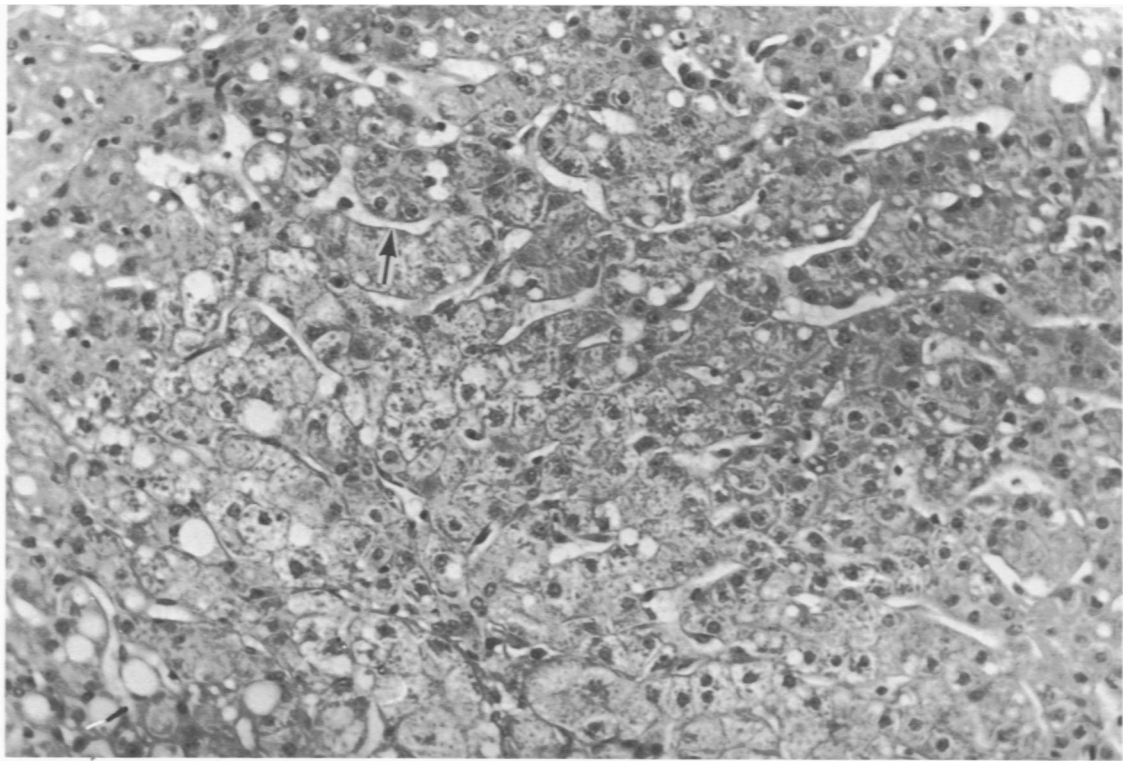

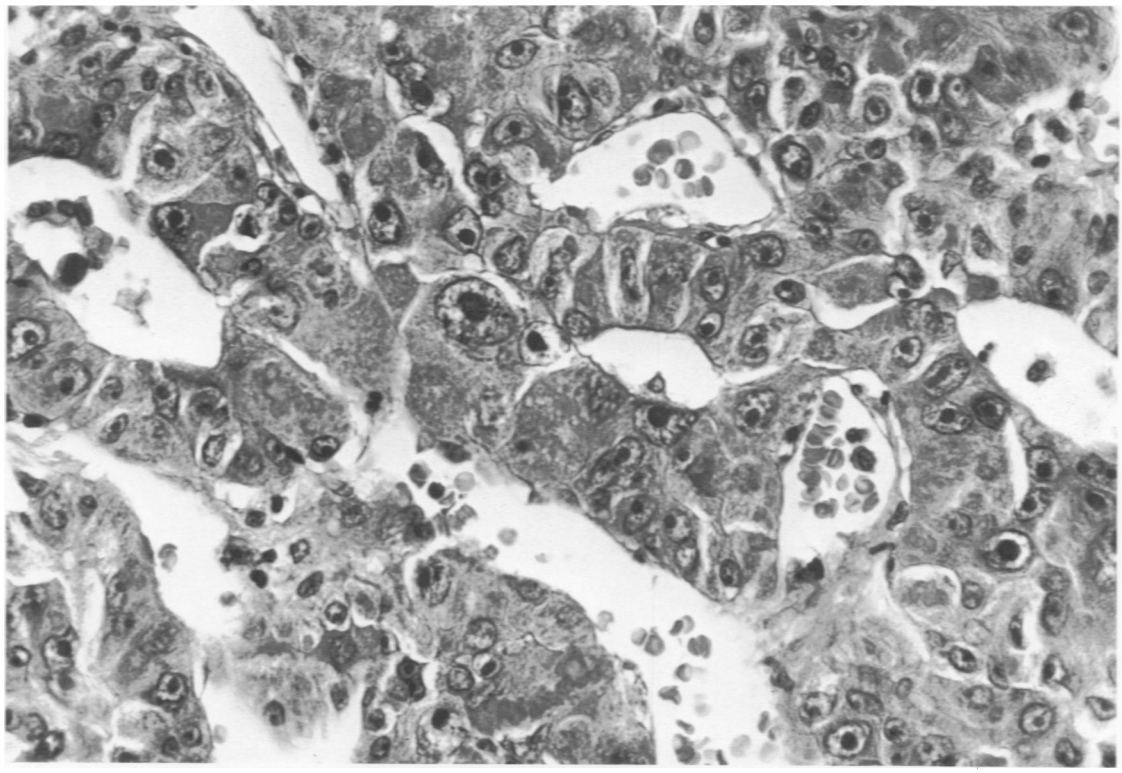

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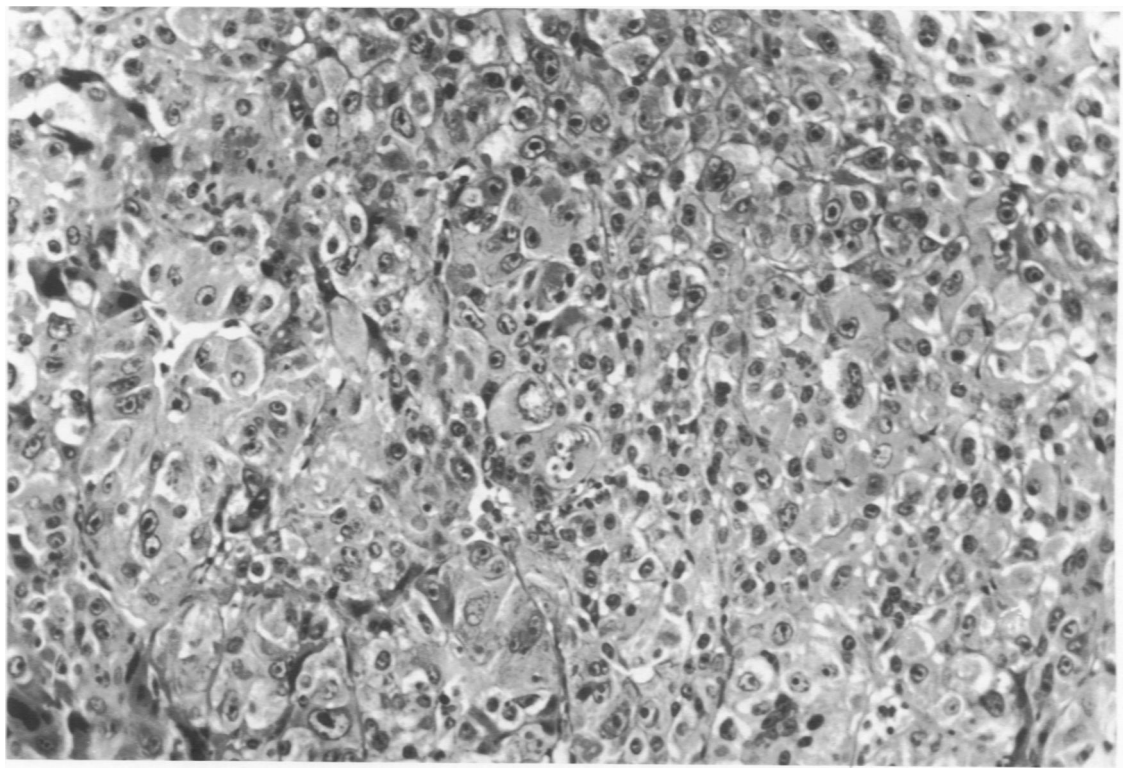

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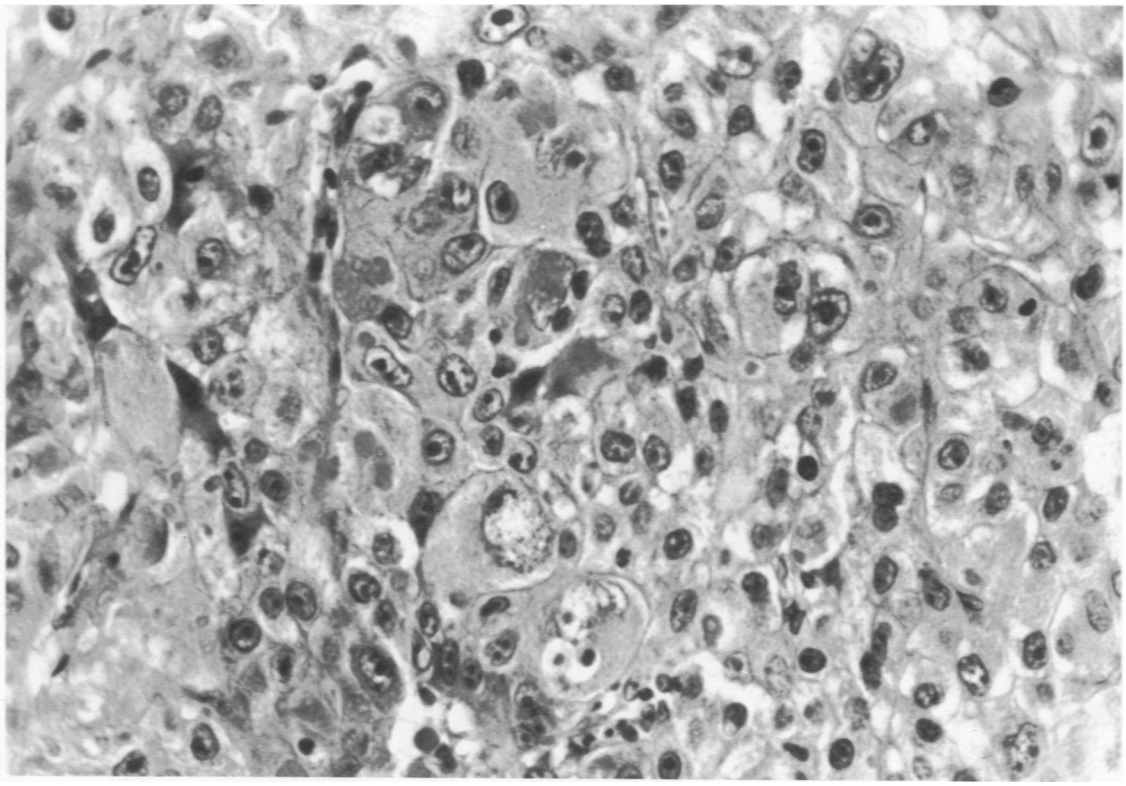

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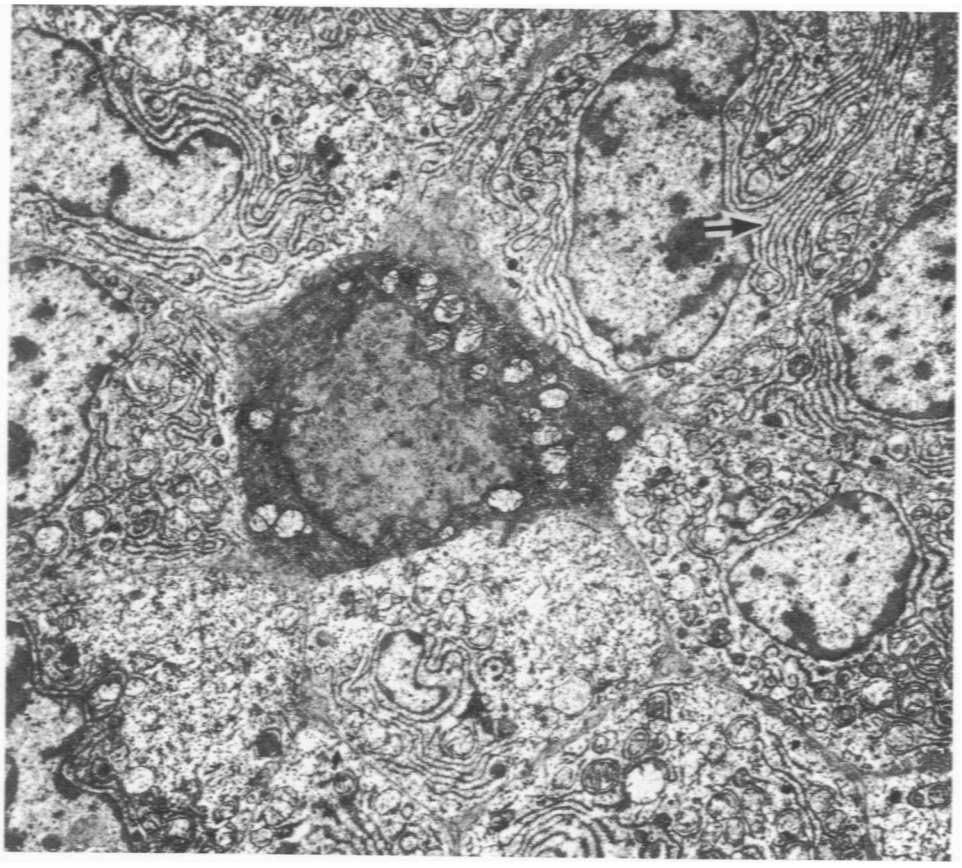

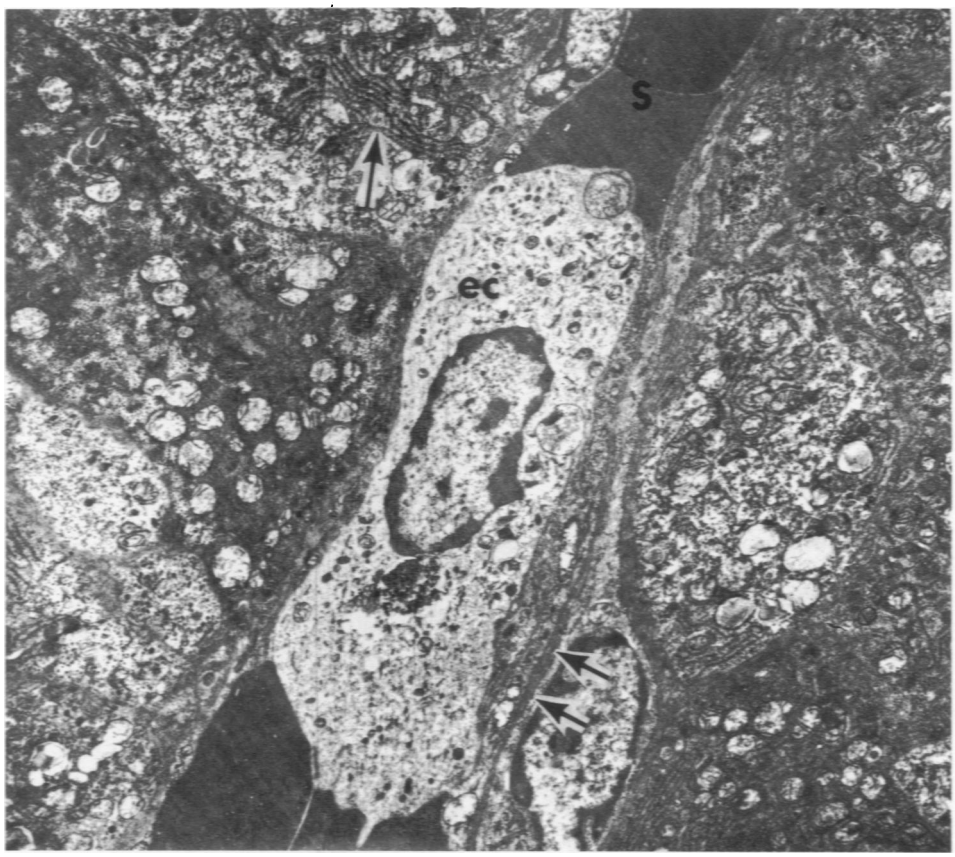
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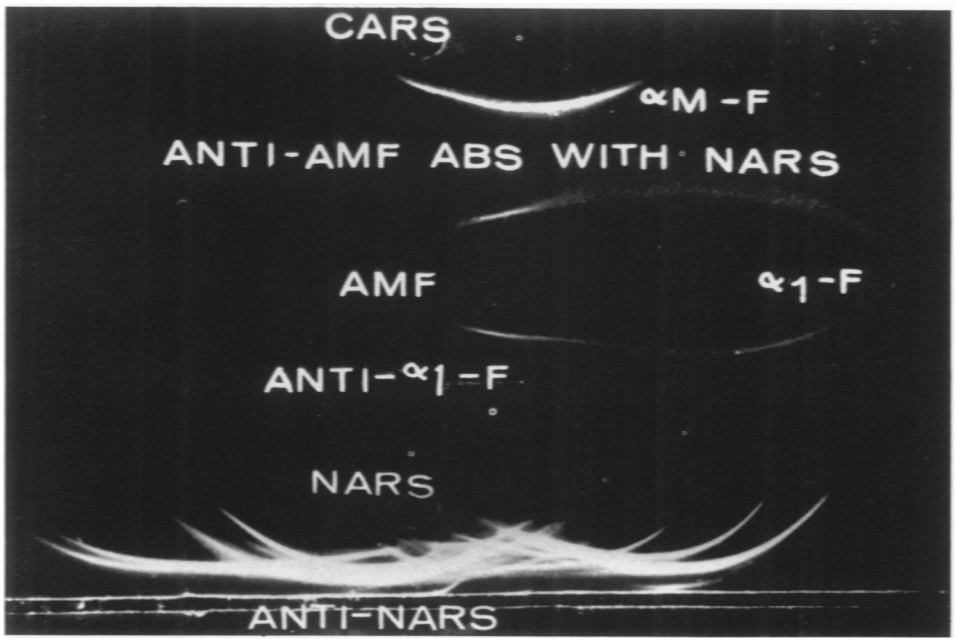
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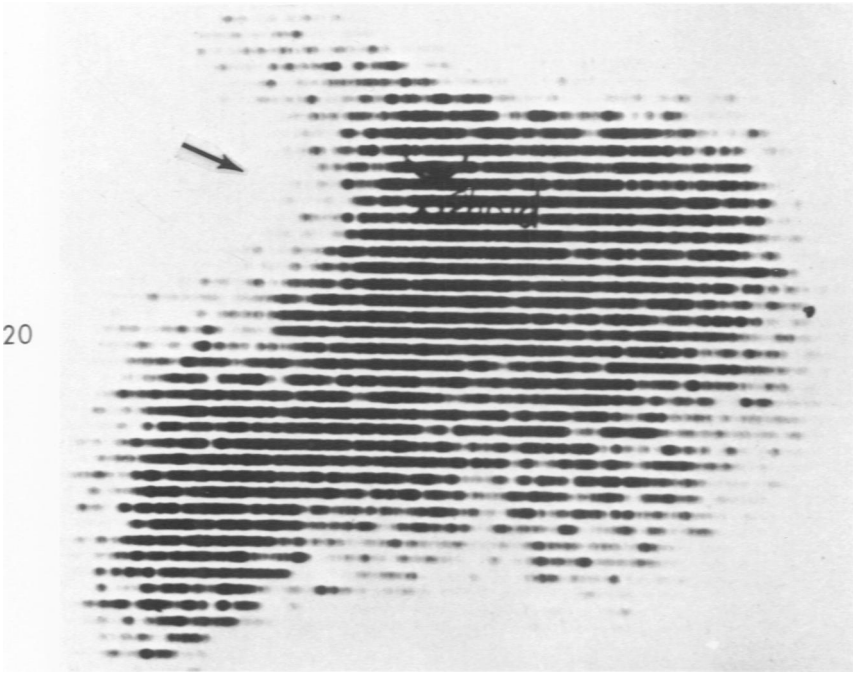

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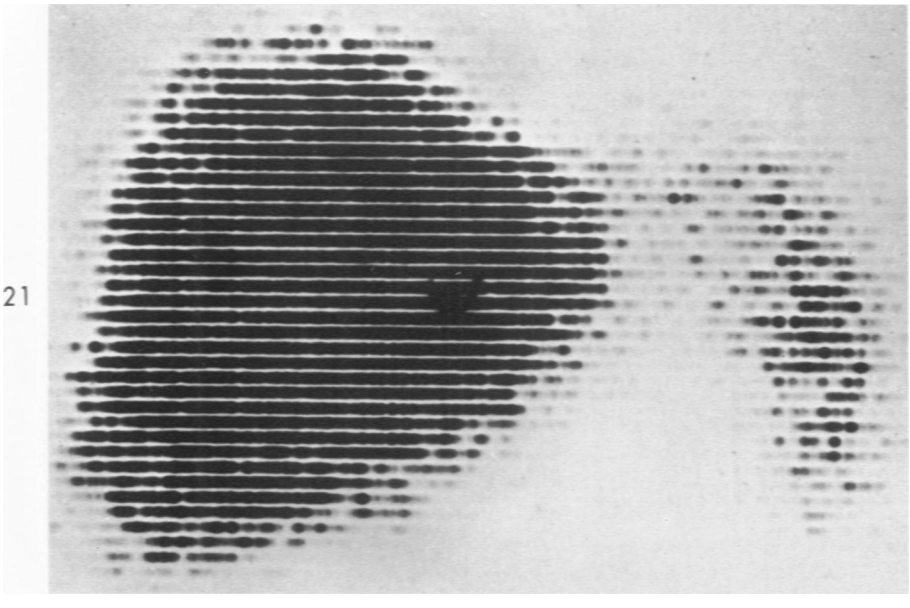

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