Development of Hepatic Angiosarcoma in Man Induced by Vinyl Chloride, Thorotrast, and Arsenic

Comparison With Cases of Unknown Etiology

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Examples of human angiosarcoma following exposure to vinyl chloride, Thorotrast, or arsenic (medicinal and industrial) and cases, including children, of unknown etiology were studied to establish diagnostic criteria and to study their evolution. The uniform evolution suggests an environmental factor also in the cases of unknown etiology, which may be established by epidemiologic studies. A precursor stage is characterized by areas of combined hyperplasia of hepatocytes and a variety of sinusoidal and perisinusoidal cells associated with excess of reticulin and with sinusoidal dilatation. The diagnostically useful picture in silver impregnations indicates reticulum formation by the perisinusoidal cells, presumably the lipocytes. The hepatocytic proliferation suggests a hepatocarcinogenic but usually not fully expressed potential. The mixed hyperplasia of the various sinusoidal cells proceeds to an overgrowth of angiosarcoma cells, presumably derived from endothelial cells. In early stages they are usually in contact with hepatocytes (intralobular growth). A trabecular arrangement results from loosening of the lobular plate arrangement by dilatation of sinusoids, leading to primary peliosis. With disappearance of the hepatocytes, various growth patterns develop, terminating in nodular, solid angiosarcoma composed of either spindle-shaped or polyhedral cells which undergo necrosis or hemorrhage (secondary peliosis). The interaction between hepatocytes and sinusoidal cells requires elucidation. (Am J Pathol 92:349–376, 1978)

THE RECOGNITION of the association between exposure to gaseous vinyl chloride during its polymerization to the common plastic polyvinyl chloride and the appearance of hepatic angiosarcoma[1] has increased the interest in this tumor. This concern was accentuated by its production in rodents exposed experimentally to vinyl chloride before the human lesion was recognized.[2] Until then, hepatic angiosarcoma had been considered rare in man, although it has been known that exposure to thorium dioxide (Thorotrast)[3] and inorganic arsenicals[4] may be followed by de-

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velopment of the same tumor. By contrast, in domestic and experimental animals hepatic angiosarcoma is more common. In humans exposed to vinyl chloride, [5,6] arsenicals,[7] and Thorotrast,[8] a peculiar hepatic fibrosis often associated with portal hypertension has been observed; transition of this antecedent lesion to angiosarcoma has been postulated. It therefore appeared important to study available cases of angiosarcoma associated with exposure to vinyl chloride, Thorotrast, or inorganic arsenicals and cases without known etiologic factors in an attempt to a) ascertain differences in pattern and evolution associated with various etiologic factors; b) describe a focal hyperplastic precursor lesion; c) trace the evolution of the precursor lesion and of the angiosarcoma; d) stress the connection of sinusoidal cell hyperplasia with the proliferation of the hepatocytes, since hepatocellular carcinoma has also been documented to result from exposure to Thorotrast [9] and inorganic arsenicals [4] and has been found in rodents exposed to vinyl chloride [10] while in humans this association with vinyl chloride exposure is only suggested by anecdotal observations;[11] e) study the relation of a mixed mesenchymal cell reaction to intralobular fibrosis; and f) provide diagnostic criteria for both the precursor stage and angiosarcoma useful in epidemiologic surveys.

There were relatively few reports of hepatic angiosarcoma of unknown etiology [12-15] before the vinyl chloride experience raised interest in the tumor. They are now supplemented by several others,[16-18] but even large-scale surveys [19,20] emphasized the rarity of the hepatic tumor. This tumor has been reported in all age groups, including children, and has no established sex predilection. These lesions have to be differentiated from benign but actively proliferating vascular tumors such as capillary hemangioendothelioma,[21] particularly its infantile variety,[22] as well as from malignant mesenchymal tumors with conspicuous participation of capillaries such as malignant mesenchymoma,[22] hemangiopericytoma,[23] and Kaposi's sarcoma, all of which have been found occasionally in the liver. Highly vascularized hepatocellular carcinomas sometimes also present diagnostic problems.

The proper designation of the tumor under discussion has been argued in cases of unknown etiology and in those associated with arsenic and Thorotrast exposure; terms such as "hemangioendothelial sarcoma,"[22] "Kupffer cell sarcoma,"[24] "malignant hemangioendothelioma," and "hemangiosarcoma" have been used. In recent years, however, it has become the custom to use "angiosarcoma" as the most descriptive term although the recent nomenclature proposal for hepatic lesions [25] lists most of these terms with equal emphasis. For simplicity, the term "angiosarcoma" is used here.

Review of Available Data

The first association of hepatic angiosarcoma with vinyl chloride was reported in the work force of one of the oldest polymerization plants in the United States [1] and additional cases have subsequently been reported and well-described histologically.[26-28] Since then more instances have been reported in the United States [6] as well as in Canada,[29] England,[30,31] Germany,[11,32-35] and France.[36-38] The fibrotic antecedent lesion, initially recognized in Bonn, Germany,[5] was well documented by laparoscopic and scintigraphic observations [35,39-41] and subsequently reported in this country [6] and in England [42] in workers exposed to gaseous vinyl chloride. The German investigators were stimulated by the recognition of splenomegaly in workers with acro-osteolysis following exposure to high concentrations of vinyl chloride.[43,44] Characteristic changes in the spleen associated with the antecedent fibrotic lesion were described as resembling the changes initially associated with the Banti syndrome.[6] These observations confirmed previous reports of nonspecific hepatic lesions in vinyl chloride workers.[45,46] Rare instances of cirrhosis were also reported.[47] Idiopathic portal hypertension following the long-term exposure to inorganic arsenicals in Fowler's solution used in the treatment of psoriasis has been reported,[7,48-50] a treatment which also has been rarely followed by hepatic angiosarcoma.[51,52] The largest series of angiosarcomas following exposure to arsenicals in pesticides, which also cause hepatocellular carcinoma and cirrhosis, although less frequently, comes from vineyard workers in the Rhineland.[4,53,54] Hepatic angiosarcomas and carcinomas and, apparently, the fibrotic antecedent lesion following Thorotrast exposure have been well-described in Portugal.[55,56] The number of angiosarcomas following Thorotrast exposure has increased in recent years,[57-61] and the lesion has been produced experimentally in rabbits.[62] Finally, inorganic copper has also been incriminated.[63]

The initial recognition that vinyl chloride has a carcinogenic potential in rodents [64] stimulated the extensive investigations of Maltoni,[2] and changes in the livers of workers exposed to vinyl chloride have been compared with those in rodents.[65] Angiosarcoma is more frequent in animals, both domestic and experimental, than in humans. It occurs spontaneously in dogs [66] and in some strains of inbred mice,[67] and it has been observed in rodents and primates exposed to carcinogens, particularly those which also produce hepatocellular carcinoma [68-71] and after infection of hamsters by polyomavirus.[72]

Materials and Methods

This study is based on review of material which became available primarily for consulta-

tion at the Laboratory of Pathology of the National Cancer Institute and at the Mount Sinai School of Medicine. Most of the material was submitted as a result of an extensive epidemiologic investigation of angiosarcoma in the United States from 1964 to 1974 by the Center for Disease Control, Atlanta, Georgia. Additional material came from the Environmental Sciences Laboratory of the Mount Sinai School of Medicine. Some material of vinyl-chloride–related cases was provided for study directly by pathologists in Louisville, Kentucky; other material came from many pathologists from this country and Europe. Eight of the Thorotrast cases were obtained from the Armed Forces Institute of Pathology.[73]

In addition, cases were pulled from the files at the National Cancer Institute and at Mount Sinai Hospital. In all instances, histologic slides were studied, and, in some, blocks or wet tissue were also available. Where possible, additional special stains were performed such as Mallory's trichrome, chromatrop aniline blue, silver for reticulum (mainly collagen Type III)[74] without toning to leave hard collagen (Type I) yellow-brown (otherwise stained by conventional connective tissue stains of the aniline blue variety), PAS reaction after removal of glycogen by diastase to identify increased phagocytosis, iron reaction, and Shikata stain to demonstrate elastic tissue and the s component of hepatitis B antigen.

The material studied as well as sex and age distribution are listed in Table 1.

In the vinyl-chloride–associated material is a series of cases in which multiple biopsies or biopsies and subsequent autopsies could be studied. The fibrotic precursor lesion was identified in biopsies and occasionally in autopsy specimens in areas sufficiently removed from the angiosarcoma to reasonably exclude mechanical or other effects. These were male patients, and the estimated exposure to vinyl chloride varied from 12 years to 28 years; the average exposure was 19.6 years. The latent period between first exposure and diagnosis of angiosarcoma ranged from 12 to 38 years; the average latent period was 22.3 years.

The arsenic-associated lesions were the result of exposure to Fowler's solution for many years for psoriasis or asthma; in the 4 cases for which detailed information is available, the duration of exposure ranged from 10 to 17 years, with a mean of 14 years. Thorotrast had been given for radiologic visualization of, primarily, brain lesions many years ago. When Thorotrast administration could be verified, records indicated that it had been given 16 to 40 years before the patient sought medical care for a hepatic disease.[73]

The patient material designated "unknown" represents the histologically completed part of a survey of cases of hepatic angiosarcoma of unknown etiology retrospectively reviewed after death certificate, autopsy, or surgical biopsy recorded the above diagnosis. Among 117 cases, 4 were children. In this survey the cases not conforming to this diagnosis were eliminated, and in the confirmed cases a search for etiologic factors was initiated but is not complete.[75] Among the factors under consideration are previous liver disease, other chemicals, and various sources of exposure to known etiologic agents such as arsenic.

Results

Features Distinguishing Etiologic Factors

The same pattern and evolution were encountered in all the cases studied, independent of the etiology, with hardly any exceptions. The most prominent feature of the Thorotrast-associated lesions was deposition of large amounts of Thorotrast in dense connective tissue in the capsule and in the portal tracts but often also within the parenchyma. Proliferation of bile ductules was absent or only rarely seen in the Thorotrast-associated cases, in contrast to the vinyl chloride and arsenical as well as cryptogenic cases, in which marked bile duct proliferation was common. Hematopoietic foci were found in cases of each etiology. Therefore,

in the following presentation of the observations, little reference will be made to etiology. The same holds true with few exceptions for the legends of the illustrations.

Precursor Stage

This stage was characterized by hepatocytic proliferation associated to varying degrees with sinusoidal lining cell proliferation and with focal sinusoidal dilatation. The hepatocytic hyperplasia was observed in two forms which varied in size and degree of demarcation, uniformity of hepatocellular hyperplasia, and extent of associated sinusoidal lining cell activation and fibrosis.

In the first form, multiple, poorly circumscribed foci of hepatocytes exhibited variations from the surrounding parenchyma. The cells varied in size and formed two-cell–thick plates with their nuclei adjacent to the sinusoidal border. Binucleated and even multinucleated hepatocytes were seen (Figure 1). The bile canaliculi were sometimes dilated but devoid of bile plugs. Lipofuscin pigment was often increased. The sinusoidal lining cells in these foci were increased in number and varied in morphologic appearance. They included normal endothelial cells, plump cells with spindle-shaped nuclei and with diffuse PAS-positive diastase-resistant reaction of the cytoplasm, a few macrophages, and many lipocytes, identified by small fat droplets. The silver-impregnated reticulum framework was barely increased.

The second form was observed when transformation to angiosarcoma was present or subsequently established. A uniform, conspicuous hyperplasia and hypertrophy of hepatocytes in contiguous, almost nodular areas, was associated with an even more conspicuous increase in various sinusoidal cells (Figure 2). The sinusoidal cell proliferation included normal and enlarged endothelial cells, macrophages with PAS-positive diastase-resistant granules, lymphocytes, and occasionally plasma cells or segmented leukocytes (Figure 3). This sinusoidal cell reaction differed from that seen in hepatitis in that it was not associated with degeneration and necrosis of hepatocytes. The reticulum framework was increased in silver stains and showed an excess of both longitudinal and cross fibers. The irregular areas with increased reticulin were recognized under low-power magnification (Figure 4). The areas of hepatocytic and sinusoidal cell hyperplasia and hypertrophy distorted the lobular architecture. The areas usually involved only part of the lobule but occasionally extended into a neighboring one. They were usually closer to the portal tract than to the central zone and were not round but garland-shaped. The surrounding parenchyma, also curved, was either normal or compressed (Figure 5). The hepatocytes in the compressed areas often revealed degeneration,

and PAS-positive macrophages accumulated while the reticulin framework was condensed. Sometimes these compressed zones were hyperemic or hemorrhagic. After Thorotrast exposure, Thorotrast granules were found in the macrophages of the compressed areas; they were absent or infrequent in the hyperplastic areas. Bile plugs were noted on the border between the hyperplastic and the compressed areas.

The mixed hyperplastic areas differed from nodules in multiple nodular hyperplasia of humans and from the hyperplastic areas and neoplastic nodules of rodents by a) proliferation of various sinusoidal cells, b) increase of the reticulin framework, and c) garland-shaped outline.

Focal sinusoidal dilation observed frequently in the precursor stage involved in part portions of the mixed hyperplastic areas and in part the surrounding parenchyma, without relation to lobular topography. The cells lining the irregularly dilated sinusoids were increased in number (Figure 6) and the surrounding reticulin framework was thickened.

Nonspecific alterations in the surrounding parenchyma included occasional focal steatosis and clumping of hepatocytic cytoplasm. The portal tracts showed varying degrees of fibrosis which sometimes disrupted the limiting membrane and extended into the periportal parenchyma. Portal fibrosis was occasionally associated with proliferation of bile ductules and, in a few instances, with accumulation of lymphoid cells around bile ducts (Figure 7). This pericholangitis was usually accompanied by diffuse canalicular cholestasis.

Where it was possible to study the capsule, it showed focal capsular and subcapsular fibrosis in vinyl-chloride–associated cases. In the Thorotrast cases, these fibrotic areas contained Thorotrast deposits. Inflammatory cells were not noted in the areas of capsular fibrosis.

Transition to Angiosarcoma

Five processes participated to varying degrees in the transition to angiosarcoma: 1) proliferation with increasing anaplasia of intralobular endothelial cells; 2) initial hyperplasia of hepatocytes followed by atrophy and disappearance; 3) increasing fibrosis in perisinusoidal spaces; 4) progression of sinusoidal dilatation to peliosis; and 5) sarcomatous transformation of lining cells of sinusoids and of portal capillaries. The combination of the processes resulted in three pathways to angiosarcoma:

1. *Intralobular growth with fibroplasia.* The number of sinusoidal cells in the hyperplastic areas increased further so that they were in places arranged in more than one layer, although flat endothelial cells lined part of the sinusoidal spaces. The conspicuous hepatocytic hyperplasia might result in hepatocytic trabeculae lined by increased sinusoidal cells (Figure

8). The Disse space was widened and filled with many reticulum fibers in layers of variable thickness since cross fibers were also irregularly increased. It contained fibroblasts and many lipocytes. Some sinusoidal lining cells had a bulky cytoplasm and hyperchromatic nuclei; similar cells were found also in the Disse spaces and indented adjacent hepatocytic plates. The sinusoids were either narrowed or somewhat dilated in places and contained an increased number of inflammatory cells. Eventually, spindle-shaped lining cells predominated, while lymphocytes, macrophages, and other inflammatory cells disappeared. Atypical cells formed multiple uniform layers lining the sinusoids and, because of increasing atypia and anaplasia, were considered to be angiosarcoma cells. Further progression was reflected in several features which occurred coincidentally with the previously described changes: a) Hard connective tissue (Type I collagen) associated with elastic fibers increased conspicuously around angiosarcoma cells within and around vascular spaces and compressed adjacent hepatic plates which atrophied and resembled bile ductules (Figure 9) and finally also disappeared. Fibrotic areas often became hyalinized and replaced many lobules. b) The angiosarcoma cells filled and extended the sinusoidal spaces to interfere with microcirculation, and the hepatic parenchyma showed anoxic necrosis (Figure 10). c) The angiosarcoma cells formed large clusters and, occasionally, solid nodules (Figure 11) composed mostly of spindle-shaped and sometimes of polyhedral cells.

2. *Intralobular growth with sinusoidal dilatation.* This pattern was an accentuation of the sinusoidal dilatation which was noted mainly in the mixed hyperplastic areas. It was characterized by further proliferation of sinusoidal and perisinusoidal cells. With increasing dilatation, adjacent hepatic plates were disrupted so that small cystic spaces formed by confluence of dilated sinusoids. These irregular spaces were separated by plates of hyperplastic hepatocytes (Figure 12) and the surrounding framework was remarkably thickened (Figure 13). Often, hematopoietic cells accumulated in the dilated sinusoids. Into larger spaces, blindly ending spurs of hepatocytes extended, covered by atypical sinusoidal or angiosarcoma cells. Eventually, the diffuse irregular loosening of the parenchymal architecture by some uniform and some irregular sinusoidal dilatation produced a plexiform, trabecular arrangement of the hepatocytes, with great variations in the quantitative relation between hepatocytes and sinusoidal cells. Initially the hepatocytes appeared hyperplastic and arranged in two and more cell layers around bile canaliculi which often contained bulky bile thrombi (Figure 14). When the hyperplastic hepatocytes predominated, the picture resembled the trabecular type of he-

patocellular carcinoma although the hepatocytes were not anaplastic. The considerably widened Disse spaces around the trabeculae contained a variety of mesenchymal cells, including segmented leukocytes, macrophages, lipocytes, and lymphocytes, as well as spindle-shaped angiosarcoma cells (Figure 15) and a significantly increased, irregularly arranged reticulum framework. The angiosarcoma cells, often in two layers, did not contain PAS-positive glycogen-resistant granules indicative of phagocytosis, nor iron granules or Thorotrast, even when the latter was found in other locations. The destruction of the lobular architecture by the described loosening need not involve the portal and the central canals. They remained as beam-like trabecular structures containing vessels and bile ducts and traversed the enlarging blood spaces. They were covered by layers of angiosarcoma cells, which also infiltrated these beams which underwent hyalinizing fibrosis (Figure 16). Bile ductules were similarly initially preserved and appeared also as trabeculae. The blood spaces eventually became grossly visible blood cysts in which fibrin clots formed.

Another variant of the trabecular form of angiosarcoma was gradual fibrosis in the hepatocytic cords while inflammatory cells disappeared (Figure 17). Hard, Type I collagen, intermixed with elastic fibers, formed in the reticulin matrix and predominated in later stages.

A third variant was relatively thin fibrotic stalks lined by spindle-shaped angiosarcoma cells, presenting a papillary arrangement (Figure 18).

In all three variants, angiosarcoma cells also formed solid clusters and, eventually, nodules.

3. *Portal growth.* Mainly spindle-shaped angiosarcoma cells lined capillaries or lymphatics of the portal tracts or appeared single or in small nests in their connective tissue (Figure 19). This was associated, even in small lesions, with involvement of the periportal parenchyma. Only in few instances was angiosarcoma restricted to the portal tracts. Thus, portal involvement appeared to be caused by spread of intralobular angiosarcoma. This growth pattern was accompanied by proliferation of bile ductules surrounded by inflammatory cells intermixed with angiosarcoma cells. Considerable fibroplasia, mainly of reticulin character, but, subsequently, also hyalinized collagen, produced masses of fibrous tissue, with varying amounts of either ductules or angiosarcoma cells together with occasional solid nests of predominantly spindle-shaped angiosarcoma cells.

Portal angiosarcoma was accompanied in a few instances by connective tissue septums containing proliferated bile ductules, some venules and arterioles, and angiosarcoma cells intermixed to various degrees with inflammatory cells. These septums formed bridges connecting portal

tracts with each other or with central canals. They subdivided the lobules to create the picture of a cirrhosis (Figure 20). In the thus formed parenchymal nodules the hepatocytic plates were rearranged, usually in layers two and more cells thick, but, in contrast to common cirrhosis, they had an increase in reticulin and in sinusoidal cells surrounding the hepatocytes.

Variations in Angiosarcoma Pattern

Clusters and nodules were composed of angiosarcoma cells of two types. One type was spindle-shaped and had large nuclei and distinct cytoplasmic extensions (Figure 11). The nuclei were often hyperchromatic and sometimes multiple, but nucleoli were small. Few vascular spaces were lined by angiosarcoma cells but, occasionally, by flat endothelial cells. The other type of cell was large and had abundant cytoplasm. These polyhedral cells were often multinuclear and showed greater tendency for anaplasia than the spindle-shaped cells (Figure 21). The polyhedral cell nodules had few vascular spaces, while necrosis and hemorrhage were common. Transitions between and admixtures of both types of cells were frequent. The polyhedral cells were not intermixed with inflammatory cells except following necrosis. Sometimes they formed multiple layers lining blood-filled cystic cavities (Figure 22).

Both types of nodules, which also were grossly visible, compressed the surrounding parenchyma and had often a connective tissue pseudocapsule. They were frequently associated with invasion of veins by tumor cells, mainly of branches of the portal vein. Extensive necrosis was common with or without vein invasion. With silver stains, some areas of necrosis showed persisting hepatic trabeculae and portal tracts, while others failed to reveal remnants of hepatic structure.

Specific developmental patterns were distinguished to trace the evolution of the multicentric angiosarcomas to sinusoidal, trabecular, papillary, portal, and nodular end stages.[6] Combinations, however, of several patterns were usually seen in different parts of the same liver. For instance, fibrosing areas around hepatocytes, presumably derived from intralobular lesions, merged with others around proliferated ductules originating in portal tracts. Two cases of unknown etiology had a combination of both angiosarcoma and hepatocellular carcinoma. In one of them, nodules of these two types of malignant growth were separated by a fibrous pseudocapsule (Figure 23).

Additional Features

Foci of hematopoietic cells, mainly nucleated red cells but also mega-

karyocytes, were frequent in all forms of angiosarcoma (Figure 24). The hematopoietic cells were also in the perisinusoidal space but more frequently in the spaces lined by the angiosarcoma cells. The portal tracts showed variable amounts of lymphocytic infiltration. Bile ducts and ductules showed not only proliferation of their epithelial cells, associated with hyperchromasia and formation of several layers, but also excess mucus formation, irregular dilatation, and out-pouching of epithelium into the surrounding, often hyalinized, connective tissue.

Extrahepatic metastases (although multicentric origin is not excluded) of either spindle-shaped or polyhedral cell character were reported in 69% of the cases (Table 1). There was a lower percentage of metastases in those of known etiology, notably Thorotrast, than in those of unknown etiology, but the differences are probably not significant. The autopsy records of the entire series list involvement of other organs as follows: lung and pleura (35%), spleen (28%), lymph nodes (25%), bone (23%), adrenal glands (14%), diaphragm (9%), heart (8%), and brain (8%). Many other organs were involved rarely. Spleen involvement was recorded far more frequently (36%) in the group of unknown etiology than in the vinyl chloride (13%) or Thorotrast group (11%).

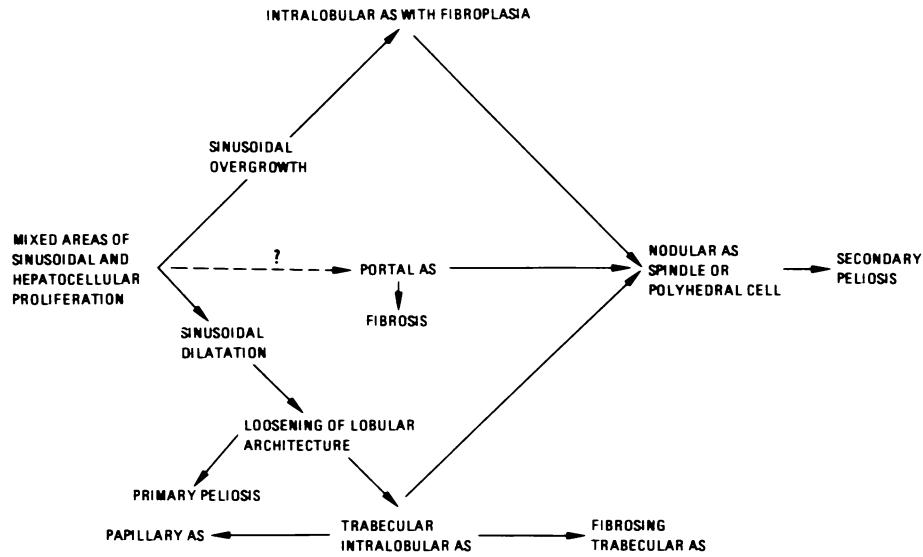
Diagnosis

Diagnostic criteria are particularly important in the evaluation of biopsy specimens. The following features proved useful in the survey of the material. The trabecular pattern with sinusoidal dilatation was most diagnostic when plates of hyperplastic hepatocytes were covered by single or multiple layers of angiosarcoma cells and hepatocytic spurs extended into blood-filled spaces. A second diagnostic feature was anaplasia of sinusoidal lining cells with or without an associated inflammatory reaction, especially when accompanied by sinusoidal dilatation and perisinusoidal fibrosis. The diagnosis of angiosarcoma was more difficult when only a few anaplastic cells were observed in the sinusoids since lymphoma, myeloma, or single metastatic carcinoma cells had to be excluded. The association with inflammation is characteristic for angiosarcoma. A third feature, more readily overlooked especially in small biopsy specimens, is angiosarcoma cells mixed with inflammatory cells and proliferated bile ductules in portal spaces. Solid tumor nodules offered the greatest differential diagnostic problem; particularly, polyhedral cells are difficult to distinguish from hepatocellular carcinoma and other types of sarcoma. Connective tissue stains, including silver stains, assist in the distinction from hepatocellular carcinoma. Frequent misdiagnoses were encountered in the material available for study because lymphoma, heman-

Table 1—Reviewed Hepatic Angiosarcoma Material

Etiology	No.	Sex		Age at death (yrs)		Race		Specimens			No. of cases with metastases
		M	F	Average	Range	W	B	Biopsy	Autopsy	Biopsy and autopsy	
Vinyl chloride	19	19	—	52	36-67	19		13	17	11	10/16*
Thorotrast	26	19	7	54	32-73	25	1	9	19	2	8/19
Fowler's solution	5	4	1	49	41-58	5		2	5	2	3/5
Unknown				4 mo							
				3							
Children	4	1	3	3	—	4		4	4	4	3/4
				13							
Females	28	—	28	57	18-82	23	3	13	18	3	16/18
Males	85	85	—	61	18-89	81	3	22	70	7	50/68*
Total	167†	128	39	—	—	157	7	63	133	29	90/130

* Information about metastases not available for 1 vinyl chloride and 2 male cases.
† Includes 2 orientals and 1 whose race was unknown.



TEXT-FIGURE 1—Proposed schema of evolution of hepatic angiosarcoma (AS).

giopericytoma, mixed mesenchymoma, hemorrhagic hepatocellular carcinoma, or various types of metastatic tumor were diagnosed as angiosarcoma.

The mixed hyperplastic areas are not readily diagnosed in needle biopsy specimens. Silver stains are then most helpful.

Discussion

Morphologic study of a large number of cases of angiosarcoma and their developmental stages revealed few, if any, differences between the cases induced by agents such as vinyl chloride, arsenic, Thorotrast and those in which no etiologic agent has been established. Intensive study of a far larger number of cases did not confirm our early impression that vinyl-chloride–related angiosarcoma differs morphologically from cryptogenic cases.[6] This suggests that in cryptogenic cases, environmental factors are responsible, the nature of which is being sought by intensive epidemiologic studies.[75] The available surveys [19,20] indicate a recognized environmental etiology in only a small percentage of cases of hepatic angiosarcoma.

The observations provided information of potential assistance in the diagnosis of the lesion, including the precursor stage. This information should be helpful in the diagnosis of industrial hazards. The enforcement of strict hygienic regulations has reduced the levels of vinyl chloride in the polymerization plants or other places where polyvinyl chloride is proc-

essed.[41,76] Presumably no additional instances of initiation will occur. However, in view of the long period of promotion of tumors, additional cases of angiosarcoma, unfortunately, have to be expected in the future. The differential diagnostic criteria may have practical importance if other environmental and industrial hazards produce the same morphologic sequences.

The diagnostic significance of the characteristic precursor stage of mixed hyperplasia and hypertrophy of hepatocytes and sinusoidal cells, frequently associated with sinusoidal dilatation, may be helpful in screening, although its specificity requires further study. It is best recognized in silver stains even with low-power examination.

The second purpose of this paper is an attempt to better understand the development of hepatic angiosarcoma (Text-figure 1). Characteristically it entails, at least in early stages, a proliferation and hyperplasia of both hepatocytes and sinusoidal cells. The metabolic factors accounting for this interplay remain to be established. The appearance points to a proliferative, potentially carcinogenic stimulus for the hepatocytes. This is supported by occasional primary hepatocellular carcinomas seen after arsenic and Thorotrast and, in rare cases, after vinyl chloride exposure. In young rats with less-developed microsomal biotransformation, including lesser metabolite degrading activity,[77] vinyl chloride exposure leads more frequently to hepatocellular carcinoma than in adult rats.[10] This observation points to the possibility that variations in enzymatic biotransformation may determine the type of tumor that develops. In experimental animals, both angiosarcoma and hepatocellular carcinoma have been produced, sometimes simultaneously.[78] In human hepatocellular carcinoma, varying degrees of sinusoidal cell activity are noted, also expressed in variations of the reticulum framework around the trabecular form of this tumor. This suggests variable stimulation of sinusoidal cells even in frank hepatocellular carcinoma. Cases of both tumors in humans have been seen very rarely. Thus, the interaction between carcinogenic effects on sinusoidal cells and hepatocytes is a promising area for further study. Peculiarly, in Thorotrast-induced epithelial tumors, bile duct carcinoma may be more frequent than hepatocellular carcinoma.[79] The described alterations of the bile ducts in the angiosarcomas studied in this series suggest a carcinogenic stimulus on bile duct cells, but the possibility that this is only a secondary reaction to the presence of a tumor in the liver cannot be excluded.

Reports of intensive investigations of the metabolism of vinyl chloride are available.[41,80–83] It is transformed by hepatic microsomal action to mutagenic [84–86] and potentially oncogenic [87] metabolites which covalently bind to DNA [88,89] and are injurious to hepatocytes in animals exposed to

very large doses of vinyl chloride.[90] These metabolites are then rendered inactive by enzymatic or nonenzymatic action or by binding to glutathione.[81]

In the precursor stage, significant degenerative alterations of the hepatocytes, such as steatosis, focal necrosis, and lipofuscin deposits which are not age-dependent, have only been recognized histologically after short intervals between exposure and biopsy; these features subsequently regress.[91] This explains why conventional liver function tests are of little diagnostic value in this stage. Only with progression of the process to tumor formation appear measurable alterations of hepatic function. Angiographic [92] and scintigraphic [93] demonstration of the lesion has greater diagnostic value and the lesions are also reflected in hemodynamic alterations.[94]

A predominant early alteration is the sinusoidal cell proliferation associated with sinusoidal dilatation and fibrosis. While in such stages these cells are intermixed with various inflammatory cells, a transformation to angiosarcoma cells is associated with a disappearance of the other mesenchymal cells in all growth patterns. Hypothetically, the inflammation may have a suppressing effect from which the angiosarcoma cells seem to escape. Azoxymethane-induced angiosarcoma growth is stimulated by antilymphocytic serum which has no effect on the hepatocellular carcinoma produced by this agent.[95] An immune complex disorder has been claimed as the basis of the vinyl chloride injury.[96] This could explain the inflammatory reaction in both parenchyma and portal tracts.

Transition to angiosarcoma cells from the sinusoidal lining cells could be traced. They appeared to be derived from endothelial cells, not only because they were devoid of any phagocytic activity but also because they were clearly distinguished from phagocytic macrophages. Their origin from endothelial cells was also confirmed by electron microscopic study.[97] However, better confirmation by use of histologic markers of endothelial cells, for instance, Weibel–Palade bodies,[98] or of catalase and peroxidases [99] is desirable. The angiosarcoma cells exhibited various forms of arrangement to include a) lining or tectorial, b) enveloping hepatocytes or bile ductules, c) vasoforming, or d) solid. They were either spindle-shaped with relatively little cytoplasm or polyhedral. The latter sometimes resembled epithelial cells and thus created differential diagnostic problems. The solid or nodular forms developed from almost any growth pattern, including the intralobular, the portal, and the trabecular.

Angiosarcoma may develop in the lobular parenchyma and in the portal tracts. The primary growth in the parenchyma appears to be far more frequent, with the invasion of the portal tracts being secondary. In the

fibrotic stages particularly, both growth patterns are combined, reflected in the presence of both hepatocytes and ductules.

The sinusoidal dilatation found in the majority of instances is not explained by passive congestion because of its irregular location in the lobule. Similar forms of sinusoidal dilatation without angiosarcoma have been reported in women taking oral contraceptive drugs [100] and in men and women receiving large doses of anabolic steroids.[101] In the latter cases, the peliosis may be sufficiently advanced to produce hepatic failure. In these instances, it has been explained by injury to the sinusoidal lining [102] and this was also demonstrated in vinyl-chloride–intoxicated mice.[97] The observations here are in keeping with this assumption of a sinusoidocidal effect. In contrast to this, primary peliosis, the progression of which to large bloody cysts is associated with persistence of hyalinized connective tissue beams, best seen in silver stains, is a secondary peliosis occurring within angiosarcoma nodules undergoing extensive central necrosis and hemorrhage. In these instances no septums remain in the silver stains. Necrosis with hemorrhage is the predominant gross manifestation of these tumors.

The relation of the sinusoidal cell hyperplasia to fibroplasia represents an interesting problem. The excess of perisinusoidal and sinusoidal cells of several types, accompanied at least initially by hepatocytic hyperplasia, was regularly associated with excess reticulum fibers. This reticulin increase was also found in the presence of sinusoidal dilatation and was a useful diagnostic criterion. The morphologic appearance suggests a fibroblastic activity of some of the perisinusoidal cells.[103] Increased and enlarged Ito cells [104] or lipocytes,[105] small fat droplets containing perisinusoidal cells, were observed in light and in electron microscopic studies,[91,97,106,107] particularly in the fibrotic precursor stage. They are considered precursors of fibroblasts.[108,109] Their transformation to fibroblasts is associated with formation of collagen Type III, mainly reticulum.[109] Also, the splenic enlargement after vinyl chloride exposure results partially from proliferation of fibroblastic cells.[110] Subsequent stages of the hepatic lesion are associated to a varying degree with desmoplasia, either in the form of a creeping fibrosis around hepatocytes, often in pseudoductular arrangement or around bile ductules, or as massive hyalinized fibrosis. Then hard, collagen Type I, a different gene product than collagen Type III, develops within the reticulum and eventually replaces it. This implies a significant desmoplastic potential of the tumor, although the role of the fibroblasts in the later stages has not been established. A peculiar form is the septal fibrosis which leads to an almost cirrhotic picture.

The characteristic abundance of hematopoietic cells, including megakaryocytes, in the developing angiosarcoma, both intrasinusoidal and perisinusoidal, may reflect a local formation of these cells in the activated mesenchyme [103] or bone marrow irritation.

The great variability in appearance of the tumor is accounted for by the variable growth potential of hepatocytes and sinusoidal cells and by the irregular tendency for fibrosis and sinusoidal dilatation. However, in virtually all cases a multicentric angiosarcoma of variable type results.

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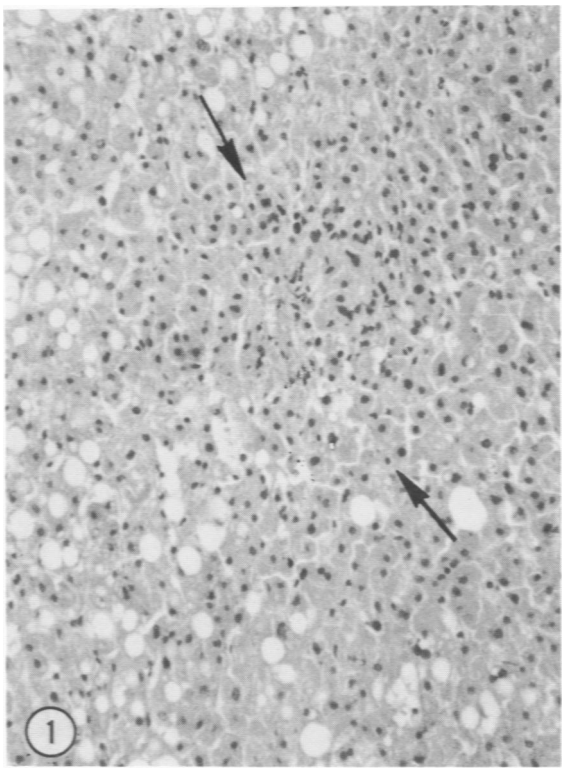
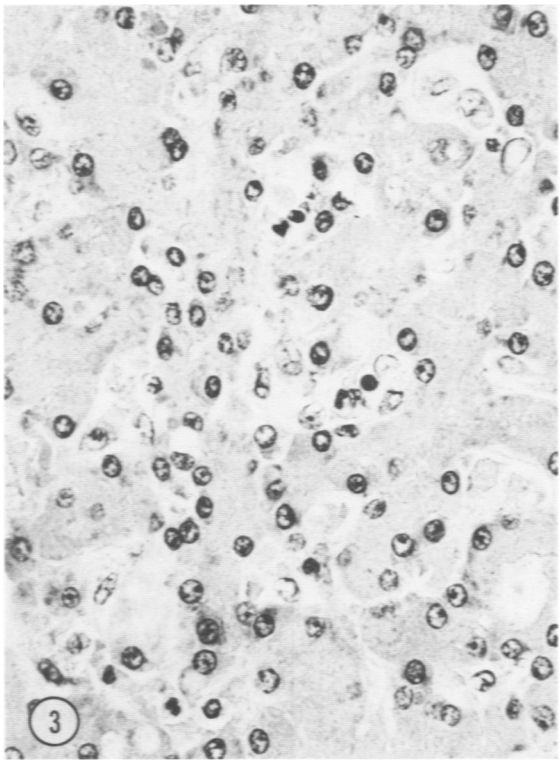
[*Illustrations follow*]





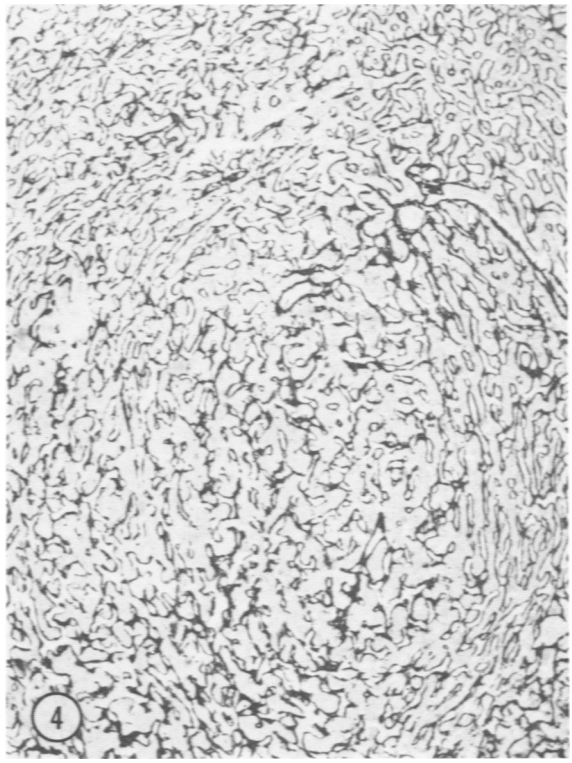
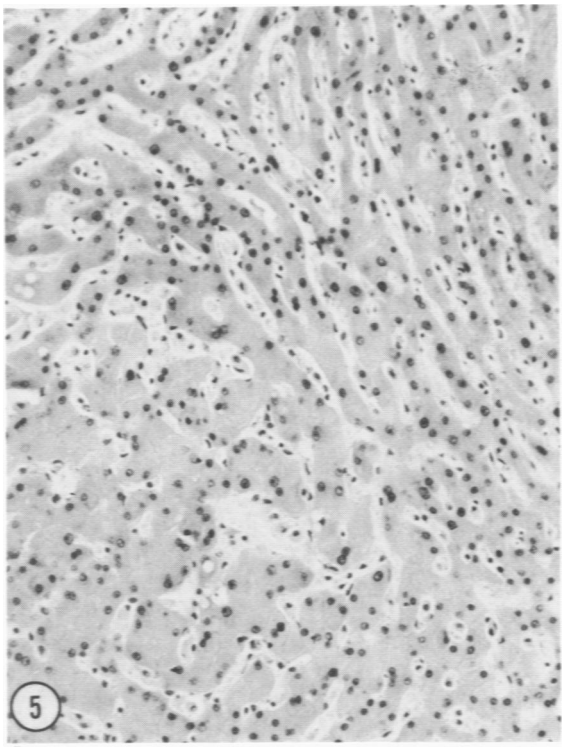
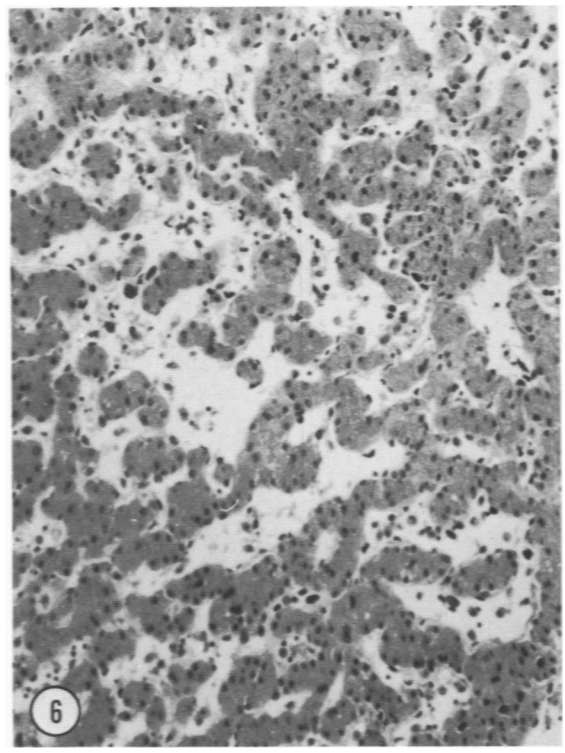
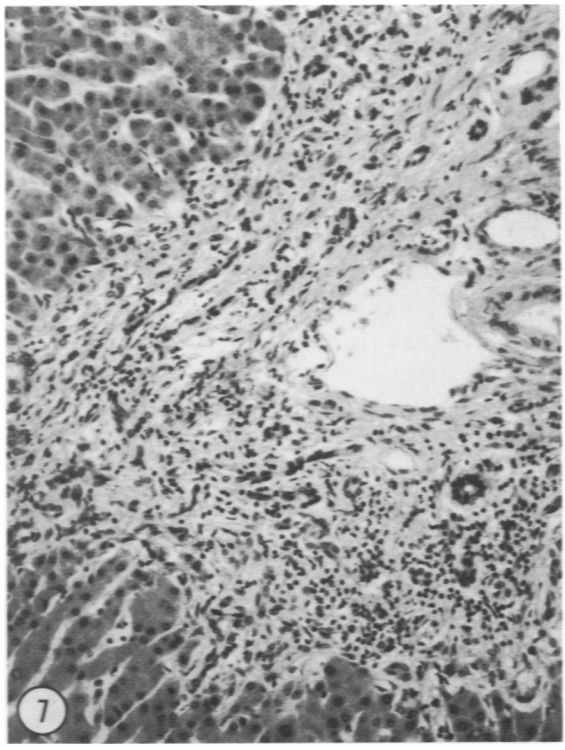


Figure 1—Focal variation in size of hepatocytes within lobular parenchyma (*between arrows*). Note small and large cells and large-droplet steatosis in surrounding parenchyma. Liver biopsy (S74-415) from worker exposed to vinyl chloride. (H&E, ×100) **Figure 2**—Hepatocytes in more than one-cell-thick plates surrounded by increased sinusoidal cells form an almost nodular area which exerts pressure on the surrounding parenchyma. Autopsy (L7374) specimen from worker exposed to vinyl chloride. (H&E, ×40) **Figure 3**—Mixed hyperplastic area. Hepatocytes, usually in two-cell-thick plates, indicated by sinusoidal position of nuclei and excess of sinusoidal cells of various types. Autopsy (L7206) specimen from worker exposed to vinyl chloride. (H&E, ×250) **Figure 4**—Increased reticulin in mixed hyperplastic area. Autopsy (A74-163). (Silver stain, ×60)







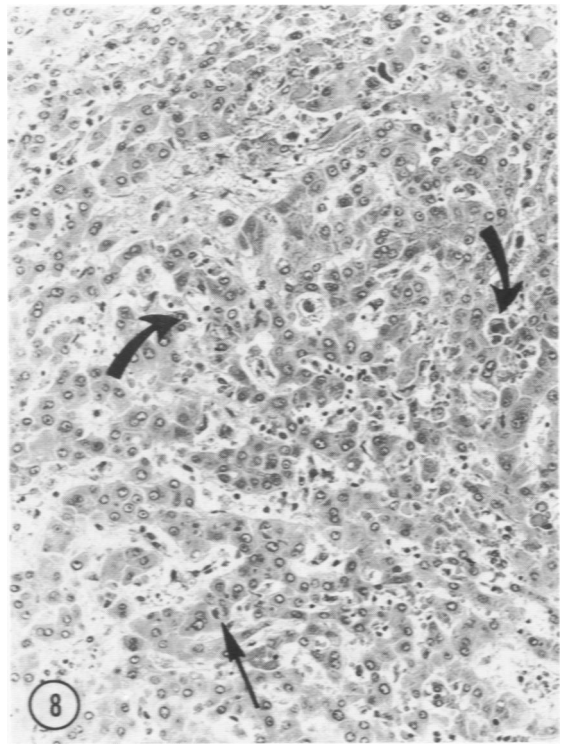
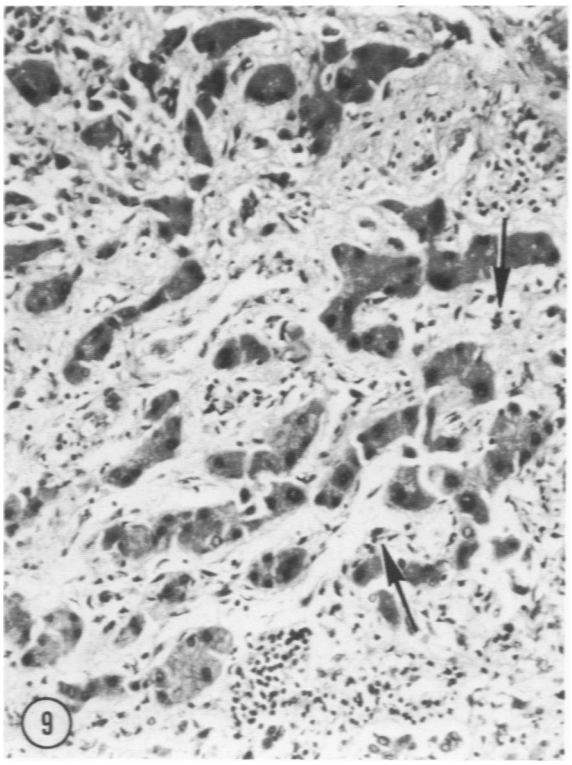
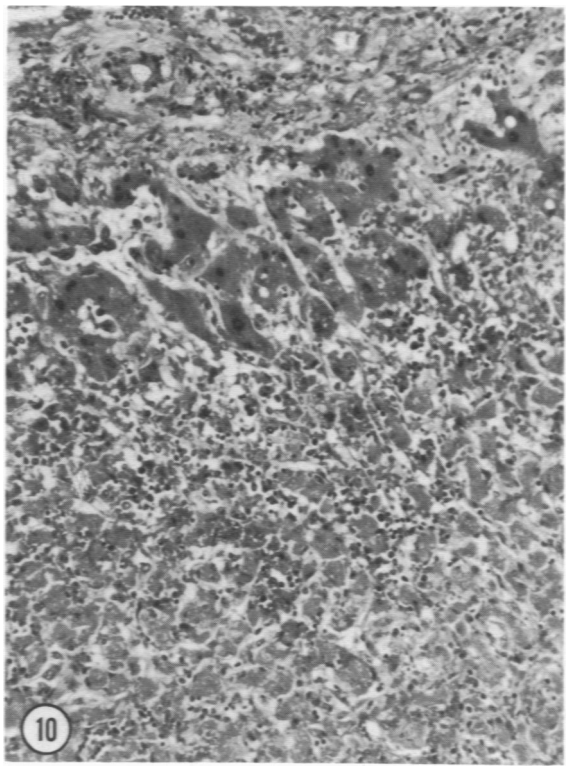
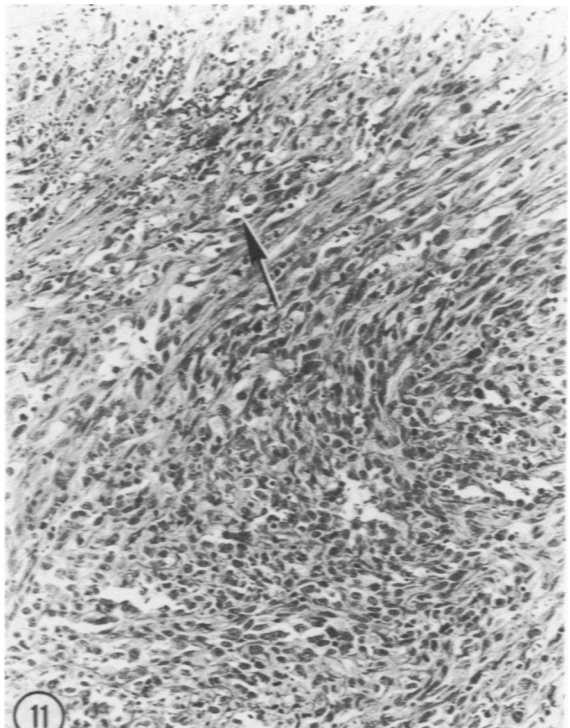


Figure 5—Mixed hyperplastic area in *left lower corner* characterized by hepatocytes in two-cell-thick plates and increased sinusoidal cells. The surrounding parenchyma reveals one-cell-thick plates and appears compressed, with the hepatocytic plates bent. Autopsy (A75-75). (H&E ×100) **Figure 6**—Irregular dilatation of sinusoids, the lining cells of which are increased. Note hepatocytic nuclei near sinusoidal border as indication of two-cell or more-thick plates. Autopsy (A74-281). (H&E, ×100) **Figure 7**—Portal fibrosis in part extending into periportal parenchyma with loss of hepatocytes in the limiting plate. Note accumulation of lymphocytes in *right lower aspect*. Autopsy (A74-163). (H&E, ×100) **Figure 8**—Hepatocytes in two-cell or more-thick plates, sometimes containing bile plugs in dilated bile canaliculi (*straight arrow*). Sinusoidal and perisinusoidal spaces contain a variety of cells, some showing beginning anaplasia and forming multiple layers (*curved arrows*). Autopsy (A74-31). (H&E, ×100)







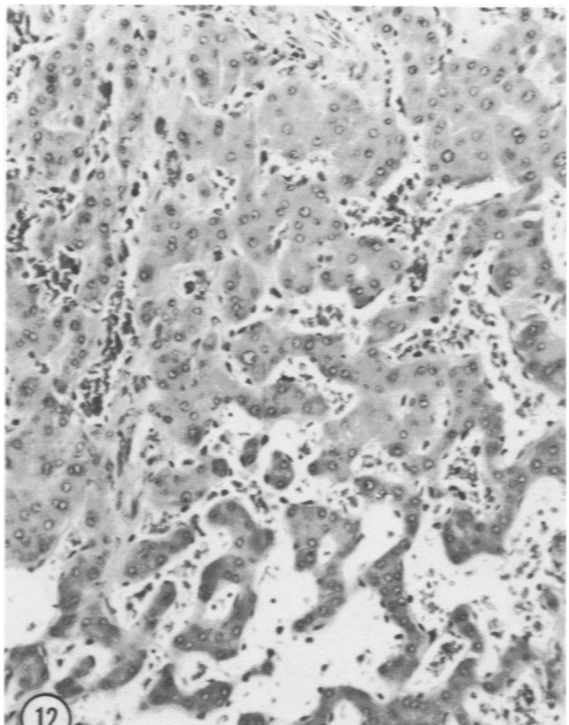
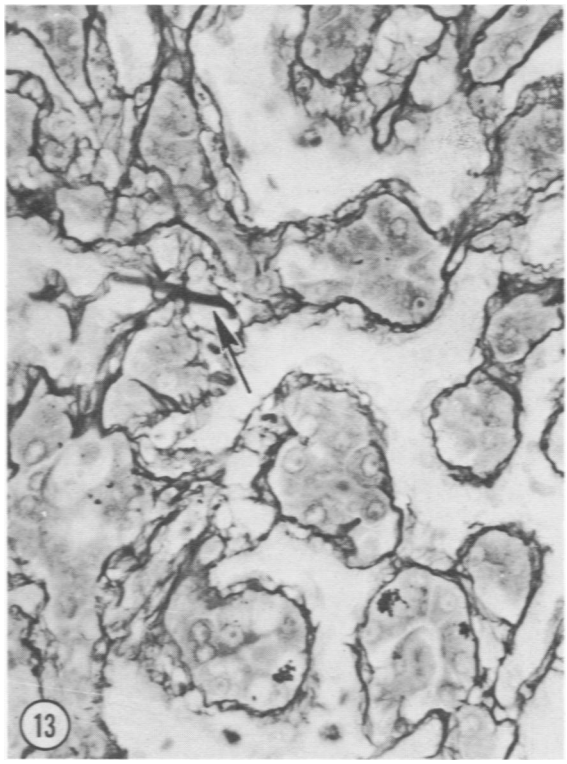
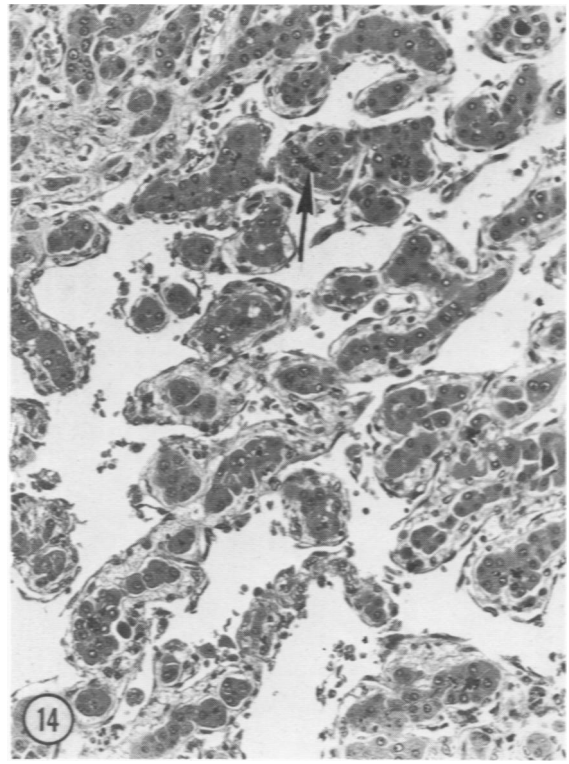
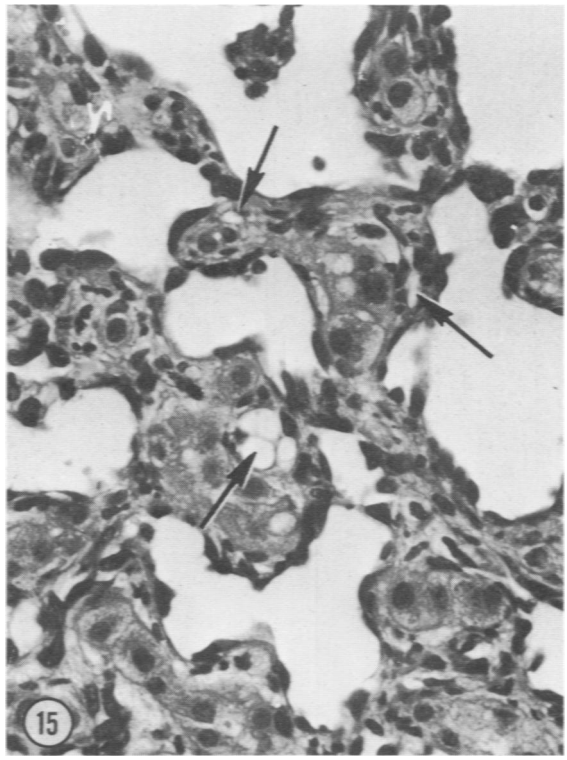


Figure 9—Hepatocytes in pseudoductular arrangement surrounded by excess fibrous tissue and vascular spaces lined by angiosarcoma cells, often in multiple layers (*arrows*). Accumulation of lymphocytes on *lower border.* Autopsy (L7493). (H&E, ×100) **Figure 10**—Angiosarcoma cells filling sinusoids, in *upper aspect* between hyperplastic and in *lower aspect* between necrotic hepatocytes. Autopsy (A77-301). (H&E, ×100) **Figure 11**—Nodule composed of spindle-shaped angiosarcoma cells in places lining vascular spaces which are also lined by nontumorous endothelial cells (*arrow*). Biopsy (S74-816). (H&E, ×160) **Figure 12**—Loosening of lobular architecture by irregular dilatation of sinusoids containing angiosarcoma cells intermixed with sinusoidal lining cells of other types. Note conspicuous hyperplasia and hypertrophy of hepatocytes in *upper aspect.* Autopsy (A74-287). (H&E, ×100)







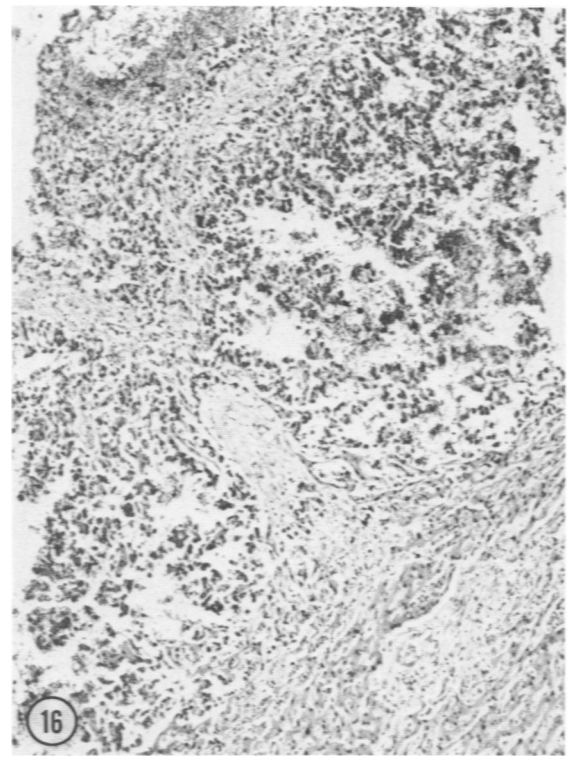
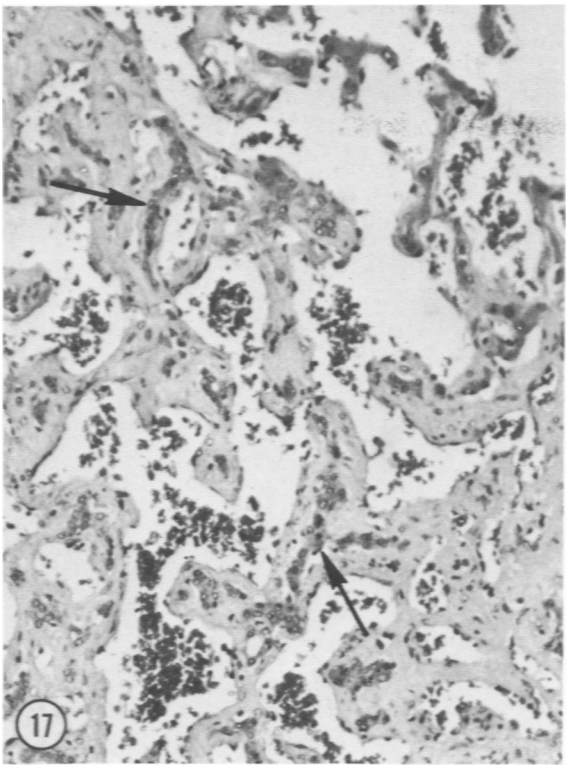
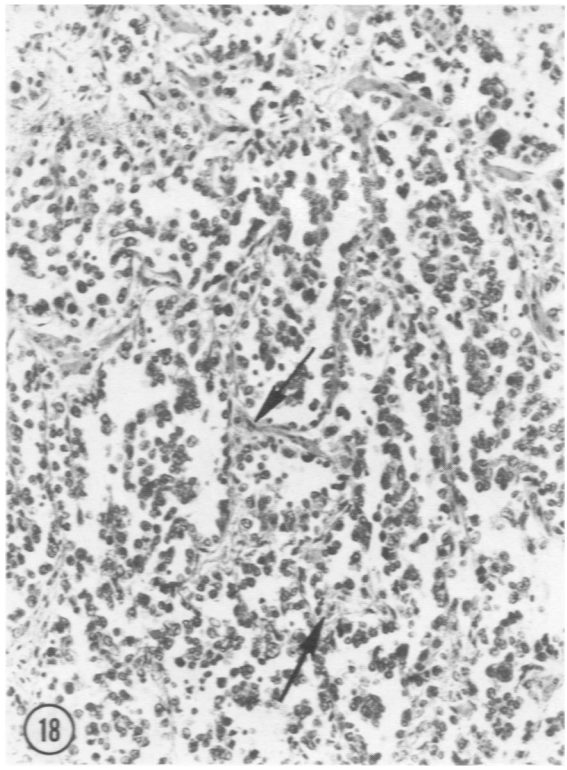
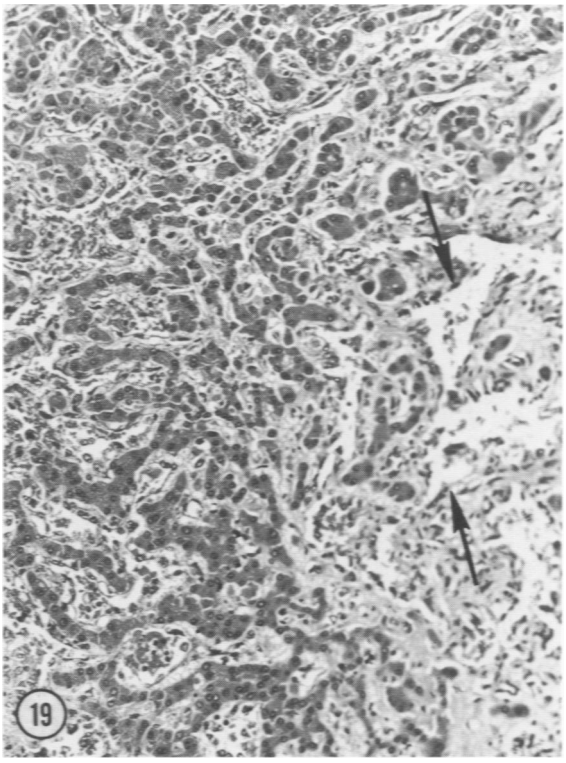


Figure 13—Increased reticulum framework around hyperplastic hepatocytes in places associated with sinusoidal dilatation. Note bundles of hard collagen (*arrow*). Autopsy (A74-31). (Silver stain, ×250) **Figure 14**—Trabecular form of angiosarcoma. Note hyperplastic hepatocytes in *upper aspect,* in part around thick bile plugs (*arrow*) and fibrosis of Disse space compressing hepatocytes. The Disse spaces are widened and contain a variety of cells. The sinusoidal spaces are lined by angiosarcoma cells. Autopsy (A74-31). (H&E, ×160) **Figure 15**—Trabecular form of angiosarcoma with spindle-shaped sarcoma cells covering hepatic trabeculae. Note hyperplastic hepatocytes and a variety of cells in sinusoidal spaces, some of them fat storing (*arrows*). Biopsy (S74-3014). (H&E, ×250) **Figure 16**—Empty blood spaces (primary peliosis) traversed by fibrosed and hyalinized beams representing persisting portal tracts and central canals. Fibrosing, trabecular angiosarcoma in *right upper* and *left lower* corners. Autopsy (L7489). (Silver stain, ×40).







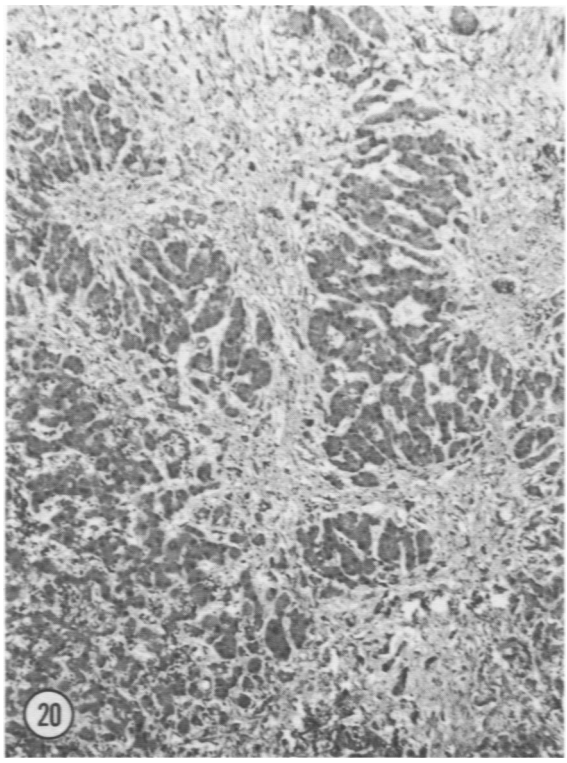
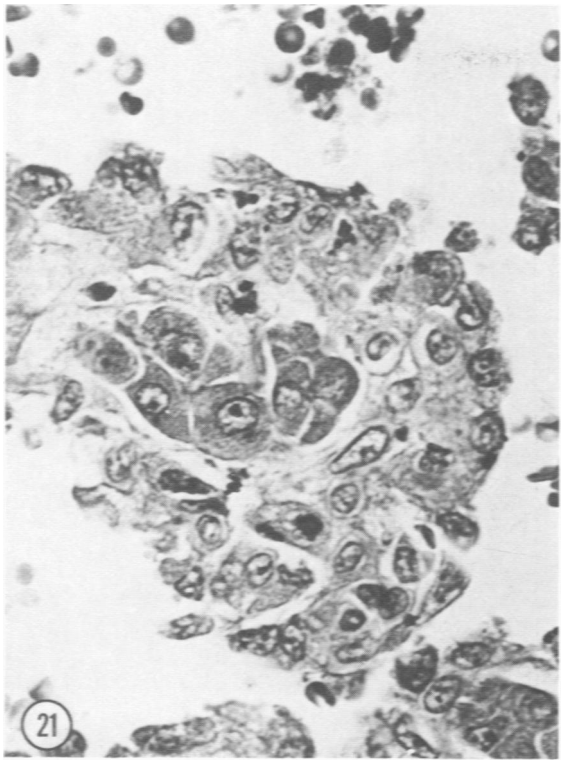
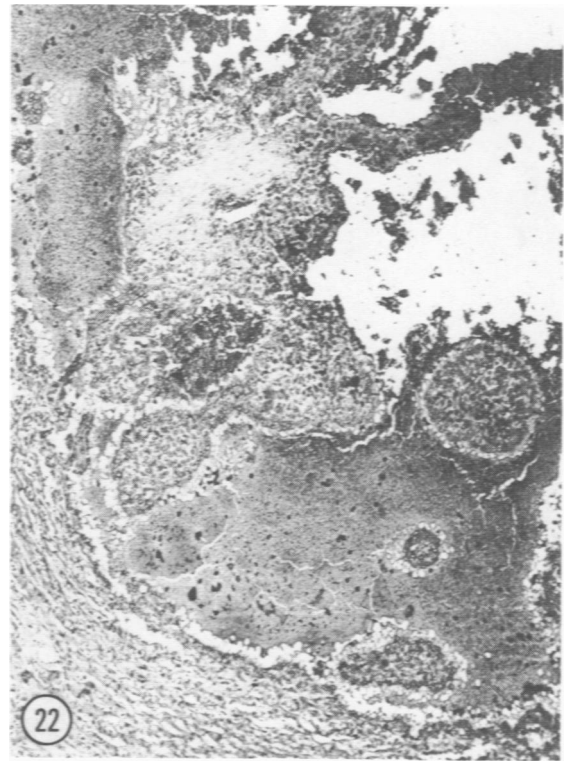
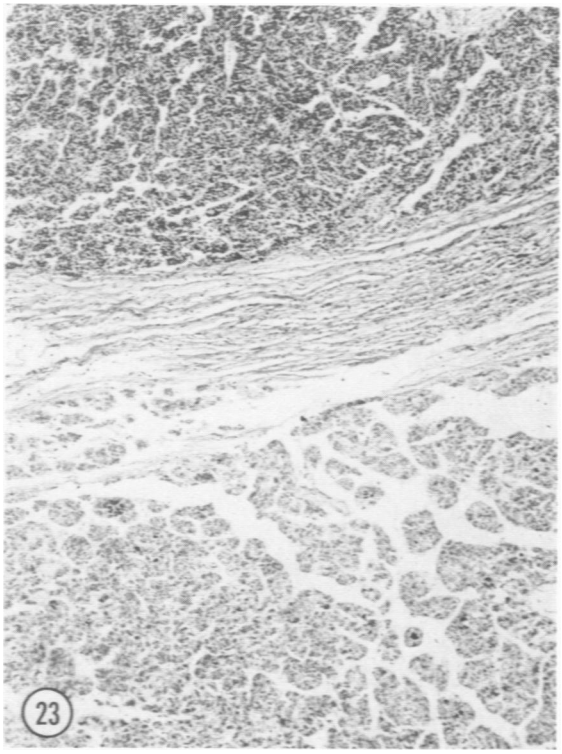


Figure 17—Progressive fibrosis of trabecular angiosarcoma with compressed, atrophic hepatocytes (*arrows*). Autopsy (A74–287). (H&E, ×100) **Figure 18**—Papillary angiosarcoma. Note tumor cells around thin connective tissue stalks and within stalks (*arrows*). Autopsy (A74–89). (H&E, ×160) **Figure 19**—Angiosarcoma filling sinusoids of lobular parenchyma and in and around vascular spaces in portal tracts (*arrows*). Autopsy (A74–31). (H&E, ×100) **Figure 20**—Connective tissue septums linking portal with portal and central canals, containing angiosarcoma cells and surrounding nodules consisting of hyperplastic hepatocytes, in part separated by angiosarcoma, to produce the picture of cirrhosis. Autopsy (A74–133). (H&E, ×40)







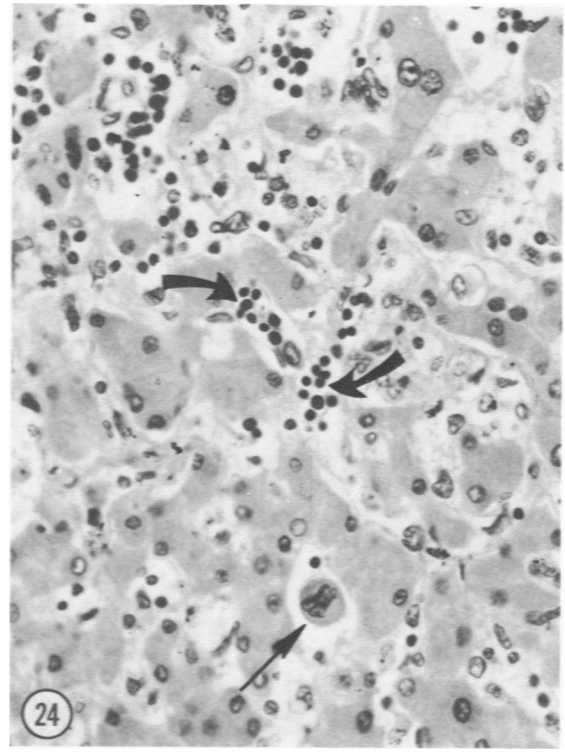


Figure 21—Multiple layers of polyhedral angiosarcoma cells surrounding hepatocytes. Autopsy (A74-31). (H&E, ×400) **Figure 22**—Nodules composed of polyhedral cells with necrosis and hemorrhage (secondary peliosis). Autopsy (A74-78). (H&E, ×40) **Figure 23**—Solid angiosarcoma above and trabecular hepatocellular carcinoma below, separated by a pseudocapsule, in autopsy specimen of case without established etiology. Autopsy (A75-175). (H&E, ×25) **Figure 24**—Accumulation of hematopoietic cells within sinusoids in angiosarcoma (megakaryocytes, *straight arrow*; nucleated red cells, *curved arrows*). Autopsy (A74-281). (H&E, ×250)