Comparison of Neoplastic Hepatic Lesions in Man and Experimental Animals

H. Popper and I. J. Selikoff

Stratton Laboratory for the Study of Liver Disease and
Department for Environmental Medicine
Mount Sinai School of Medicine of the City University of New York
New York, New York 10029

C. Maltoni

Institute of Oncology, Bologna, Italy

R. A. Squire

Experimental Pathology Branch, National Cancer Institute
Bethesda, Maryland 20014

L. B. Thomas

Laboratory of Pathology, National Cancer Institute
Bethesda, Maryland 20014

Experimental models of cancers in animals serve, among other purposes, to investigate carcinogenesis and help to predict the potential of an agent, particularly an environmental one, to produce cancer in man. Hepatic tumors are a common model both in screening for carcinogenic potential and in the study of carcinogenesis. This study compares selected instances of human and experimental hepatic tumors to (1) illustrate the relevance of experimental neoplasms to human neoplasms, (2) review anatomic sequences in animal and man related to the morphology of hepatic carcinogenesis, and (3) call attention to some clinical features of hepatic tumors not commonly seen in man, including screening for their detection.

The sequences from nodule to carcinoma of neoplastic hepatic lesions in rodents have been studied frequently. These lesions are produced by a large number of agents, including some for which a carcinogenic potential in man is seriously doubted. An example is phenobarbital, which causes cancer in mice (Walker et al. 1973), but, on the basis of reliable epidemiologic studies, not in man (Clemmesen et al. 1974). Such observations have led to vigorous debates concerning extrapolation of rodent data to man (Tomatis et al. 1973; Epstein 1974). One argument against the application of rodent observations to man is the rarity of similar sequences in human primary hepatic carcinoma, which, in the majority of cases, develops in cirrhotic liver, where the one precursor lesion postulated in man (groups of dysplastic hepatocytes in normal or cirrhotic hepatic tissue outside the carcinoma [Anthony 1976]) is a feature not encountered in rodents. In the last few years, however, benign (Baum et al. 1973; Edmondson et al. 1976) and a few malignant (Davis et al. 1975) hepatocellular tumors in noncirrhotic livers have been observed in women of childbearing age taking contraceptive steroids and in men and women receiving anabolic steroids (Johnson et al. 1972; Farrell et al. 1975). This group of tumors invites comparison with rodent

tumors. Moreover, also in recent years, hepatic angiosarcoma has been observed in workers exposed to gaseous vinyl chloride during the process of its polymerization to the plastic polyvinyl chloride (Creech and Johnson 1974; Lloyd 1975), and an antecedent lesion, a peculiar hepatic fibrosis associated with portal hypertension, has also been identified (Müller et al. 1975; Thomas et al. 1975). Shortly before the association between vinyl chloride and angiosarcoma in man was recognized, Maltoni had produced angiosarcomas in rodents through exposure to vinyl chloride (Maltoni 1974; Maltoni and Lefemine 1974). This represents not only the first example of predicting hepatic malignant tumors in man, but also the first identification of the same type of hepatic tumor in man and rodents. It encourages a comparison of the sequences.

The material for this study consists of (1) rodent tumors produced by many agents and studied during the past 15 years; (2) human tumors associated with the intake of contraceptive and anabolic steroids taken from the files of the Mount Sinai School of Medicine; (3) rodent lesions following inhalation of vinyl chloride in the laboratories of Maltoni in Bologna and a few exposed in the Industrial Bio-Test Laboratories in Northbrook, Illinois (Gordon et al. 1974; Schaffner et al. 1976); and (4) biopsy and autopsy material of workers exposed to vinyl chloride which is on file in the pathological laboratories of the National Cancer Institute and at the Mount Sinai School of Medicine. Similar materials from persons with hepatic lesions attributable to inorganic arsenicals and Thorotrast and from angiosarcomas discovered in persons without known exposure to chemical agents were also studied for comparison. The human material, originally observed in many institutions, was made available for review or consultation. Since the emphasis in this study is on the morphological sequences rather than on individual cases, specific information about the cases is omitted. Detailed case information will be published elsewhere (L. B. Thomas, N. C. Telles and H. Popper, in prep.).

Nodular Lesions in Rodents

Because these lesions have in the past been designated with a variety of names, a recent workshop has recommended a standard nomenclature (Squire and Levitt 1975), at least for rats. This nomenclature will be followed here. Many toxic regimens produce uniform proliferation of the hepatocytes on the lobular periphery, a readily recognized response to the basic injury. By contrast, in otherwise normal livers, circumscribed alterations of hepatocytes are noted. These include: vacuolization of the cytoplasm, sometimes caused by fat deposition; ground-glass appearance, presumably the result of excess smooth endoplasmic reticulum; or increased basophilia. The nuclei vary from the surrounding parenchyma, often being larger. The cells are usually enlarged and occasionally in two-cell-thick plates. These lesions, designated as hyperplastic foci or areas, do not have a specific lobular location and frequently include the central vein. They are only recognized histologically. They differ from nodules designated previously as hyperplastic and now as neoplastic. These are larger, involve several lobules, may be grossly visible, and may deform the organ. They are often multiple and frequently accom-

panied by hyperplastic areas. The border toward the uninvolved parenchyma is distinct, and transition from uninvolved to neoplastic cells is observed in the same cell plate (Fig. 1a). They frequently exert pressure on the surrounding parenchyma. The latter's liver cell plates are then compressed and surround the nodule, but fibrosis is absent. The cytologic features are the same as described for the hyperplastic areas. The lobular architecture is distorted in that the relation of portal tract to hepatic veins is altered, with portal tracts being sparser than normal and often missing through large parts of the nodule. Isolated arteries may be found (Fig. 2a). The sinusoids are often conspicuously widened, proceeding to peliotic cysts. Circumscribed necrosis occurs in larger nodules. Focal variation in cell populations and in cytologic and architectural features produce "nodules in nodules" (Popper et al. 1960) (Fig. 3a). They are composed of hepatocytes, increased or reduced in size, which may show conspicuous basophilia, glycogen accumulation (Bannasch 1974), or nuclear hyperchromasia or polyploidy, and are arranged either in plates two or more cells thick or as acinar structures around dilated bile canaliculi containing proteinic material. These nodules in nodules exert pressure on the surrounding nodular parenchyma. They sometimes resemble frank carcinoma which arises in such nodular areas (Fig. 4a). The alterations described may be associated with histochemical variations, particularly with the loss of such enzymes as glucose-6-phosphatase (Kitagawa and Sugano 1973), as well as with abnormalities of ductular structures (oval cells or cholangiofibrosis). The sequences in mice are in principle the same. It should be noted that similar lesions have been reported in rodents after administration of sex steroids (Committee on Safety of Medicines 1972).

Steroid Hepatocellular Tumors in Man

In women of childbearing age who have been taking contraceptive steroids, three types of hepatic tumors have been reported: adenomas (Baum et al. 1973; Sherlock 1975; Nissen et al. 1975; Ameriks et al. 1975; Grabowski et al. 1975; Edmondson et al. 1976; McAvoy et al. 1976), focal nodular hyperplasia (Mays et al. 1974; O'Sullivan and Wilding 1974; Grabowski et al. 1975; McAvoy et al. 1976), and a small number of hepatocellular carcinomas (Hermann and David 1973; Davis et al. 1975; Mays et al. 1976). In men and women on prolonged therapy with anabolic steroids, adenomas (Sweeney and Evans 1976) and, more frequently, carcinomas have been reported (Johnson et al. 1972; Farrell et al. 1975).

The adenomas, more often in the right lobe and sometimes multiple, were composed of hepatocytes and differed cytologically from the surrounding parenchyma in that the hepatocytes were usually larger, rich in glycogen and sometimes also in fat, occasionally of ground-glass appearance, and often with large nuclei (Fig. 1b). Their cellular appearance was usually uniform. The lobular architecture was missing or distorted, and portal structures without bile ducts as well as an excess of isolated veins and arteries were seen (Fig. 5a). Radiographic investigation suggests a predominantly arterial blood supply (Ameriks et al. 1975). Irregular sinusoidal dilatation was frequent and often progressed to bloody cysts (Fig. 2b). In the adenomas associated with the intake of contraceptive steroids, the intima of arteries and veins was

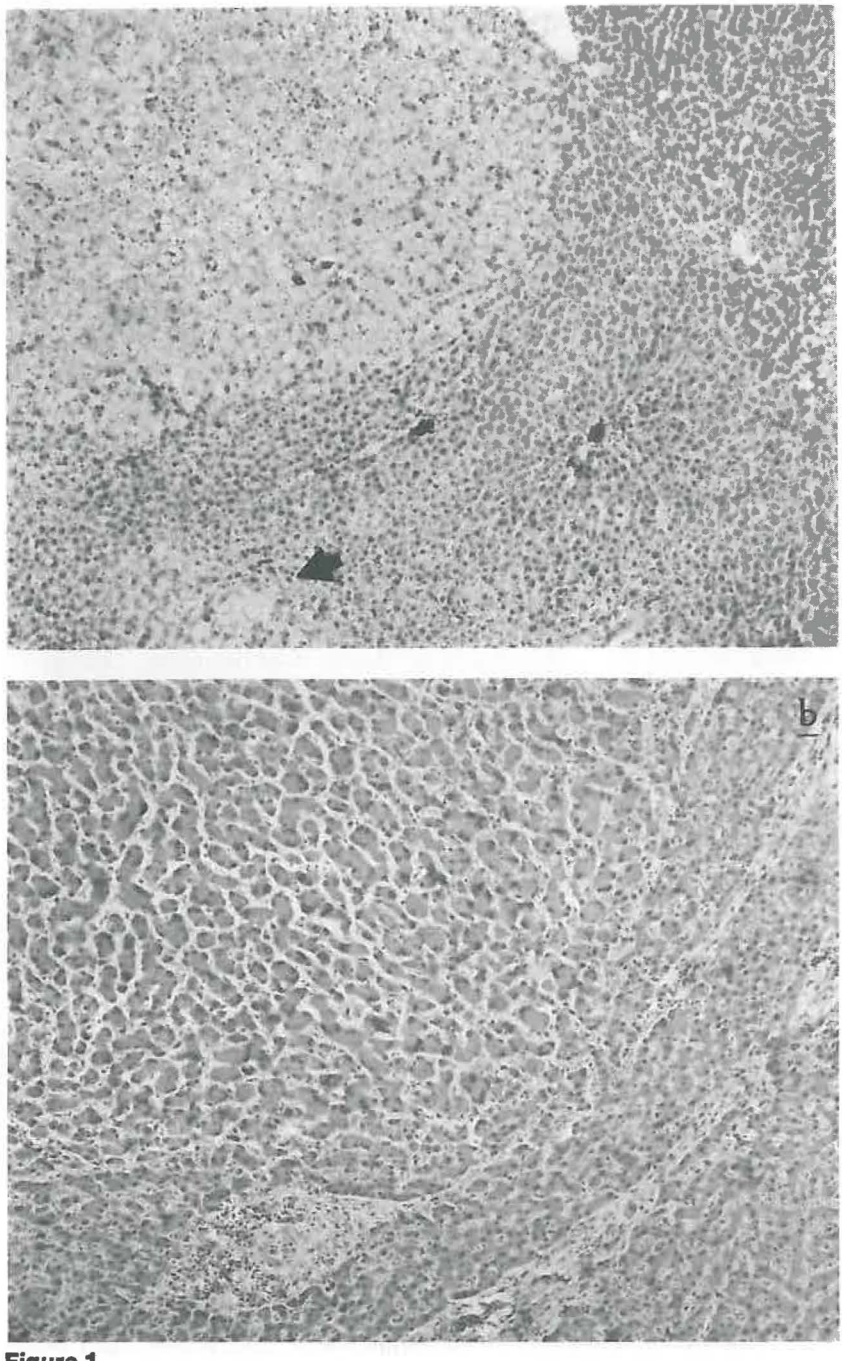

Figure 1

(*a*) Neoplastic nodule (*upper left*) in rat liver revealing transition from normal to tumorous hepatocytes in part of its periphery and compression in other areas. Arrow points to small hyperplastic area. (*b*) Hepatocellular adenoma (*upper left*) in man treated with anabolic steroids. Note transition to normal epithelium in some areas and compression of the parenchyma in other areas. Hematoxylin and eosin (H and E); magnification, 40×.

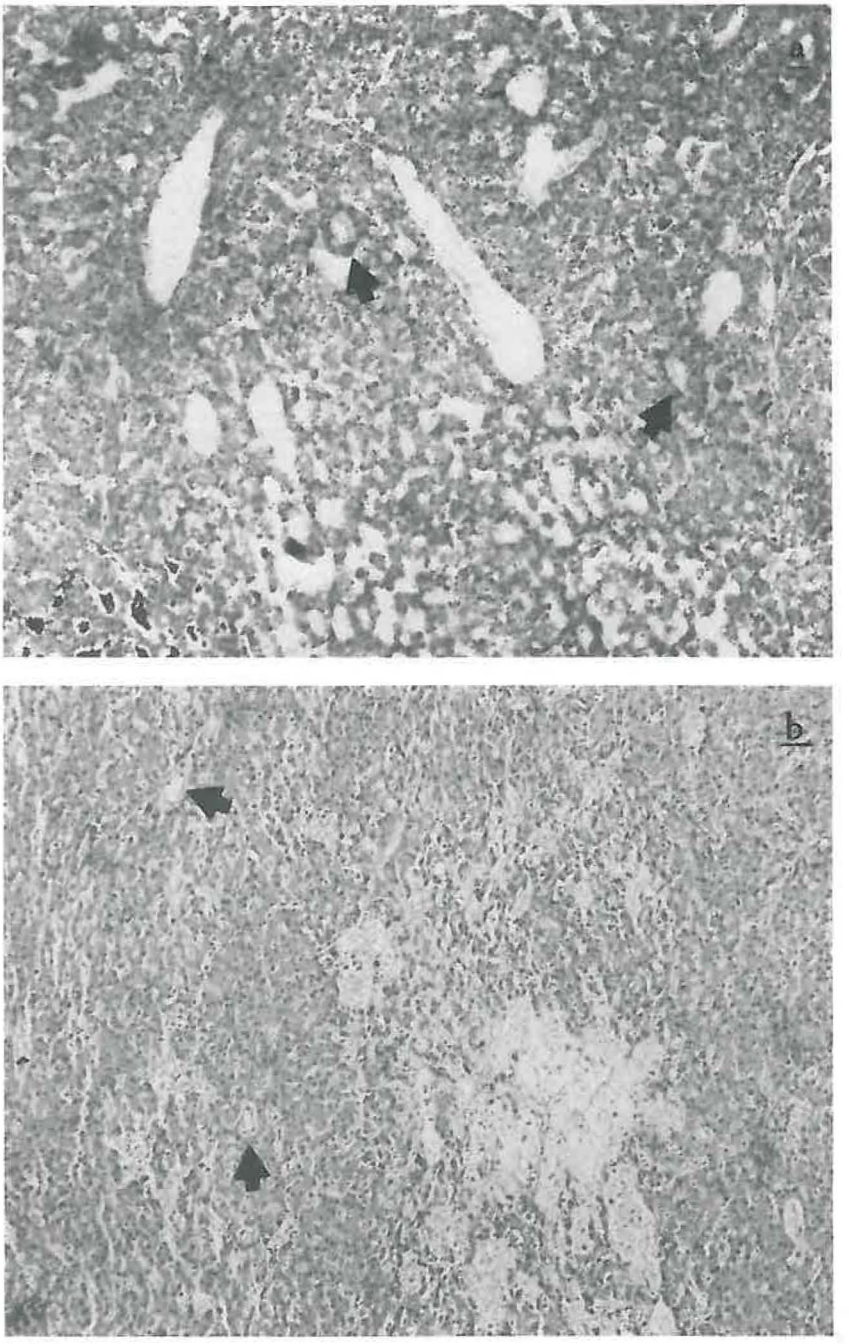

Figure 2

(*a*) Neoplastic nodule in rat liver with cystic dilatation of sinusoids and isolated arteries (*arrows*). (*b*) Hepatocellular adenoma in a woman who had taken contraceptive steroids. Note cystic dilatation of blood spaces (peliosis) and isolated arteries (*arrows*). H and E; magnification, 40×.

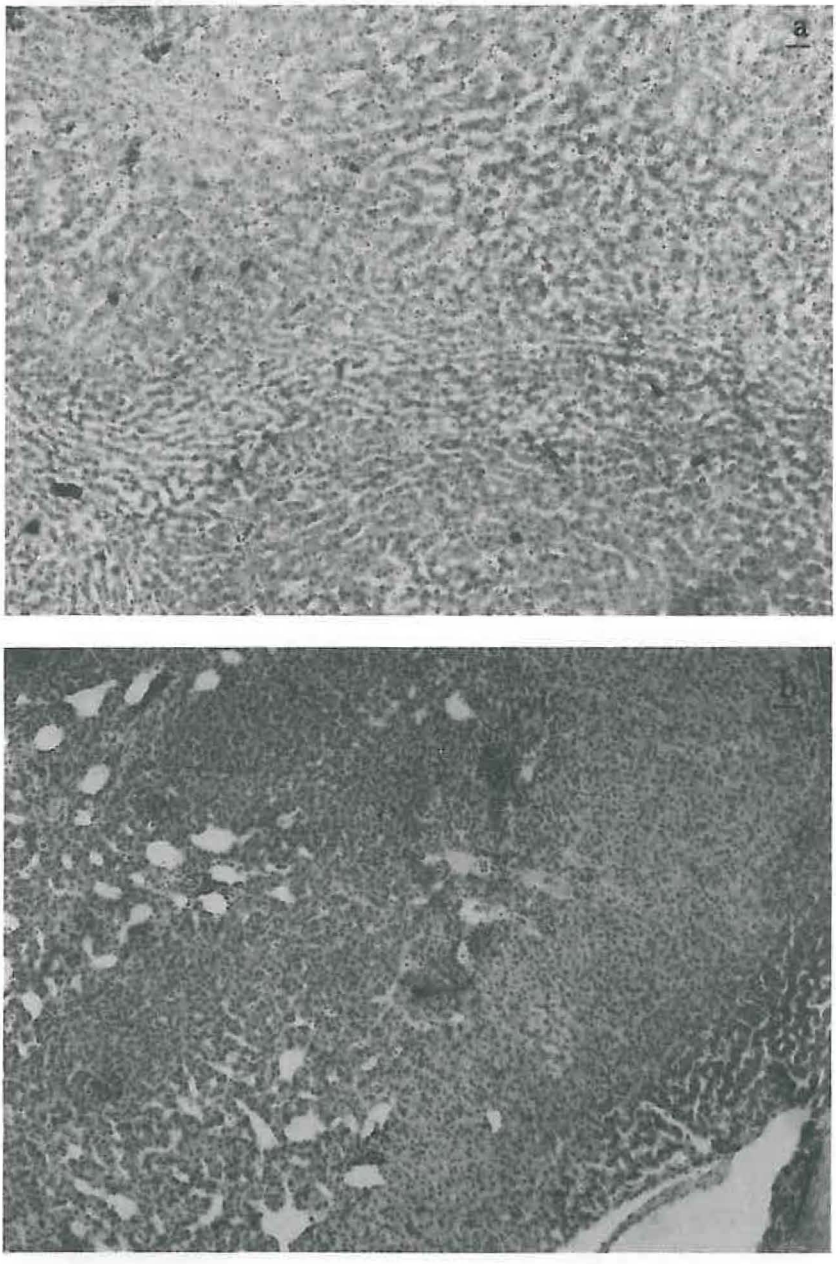

Figure 3.
(*a*) Nodular variations of hepatocytes in neoplastic nodule of rat liver (nodules in nodules). Note areas with two-cell-thick plates exerting pressure on surrounding nodular parenchyma. (*b*) Nodules in nodules and focal sinusoidal dilatation in hepatocellular adenoma in a woman who had taken contraceptive steroids. H and E; magnification, 40×.

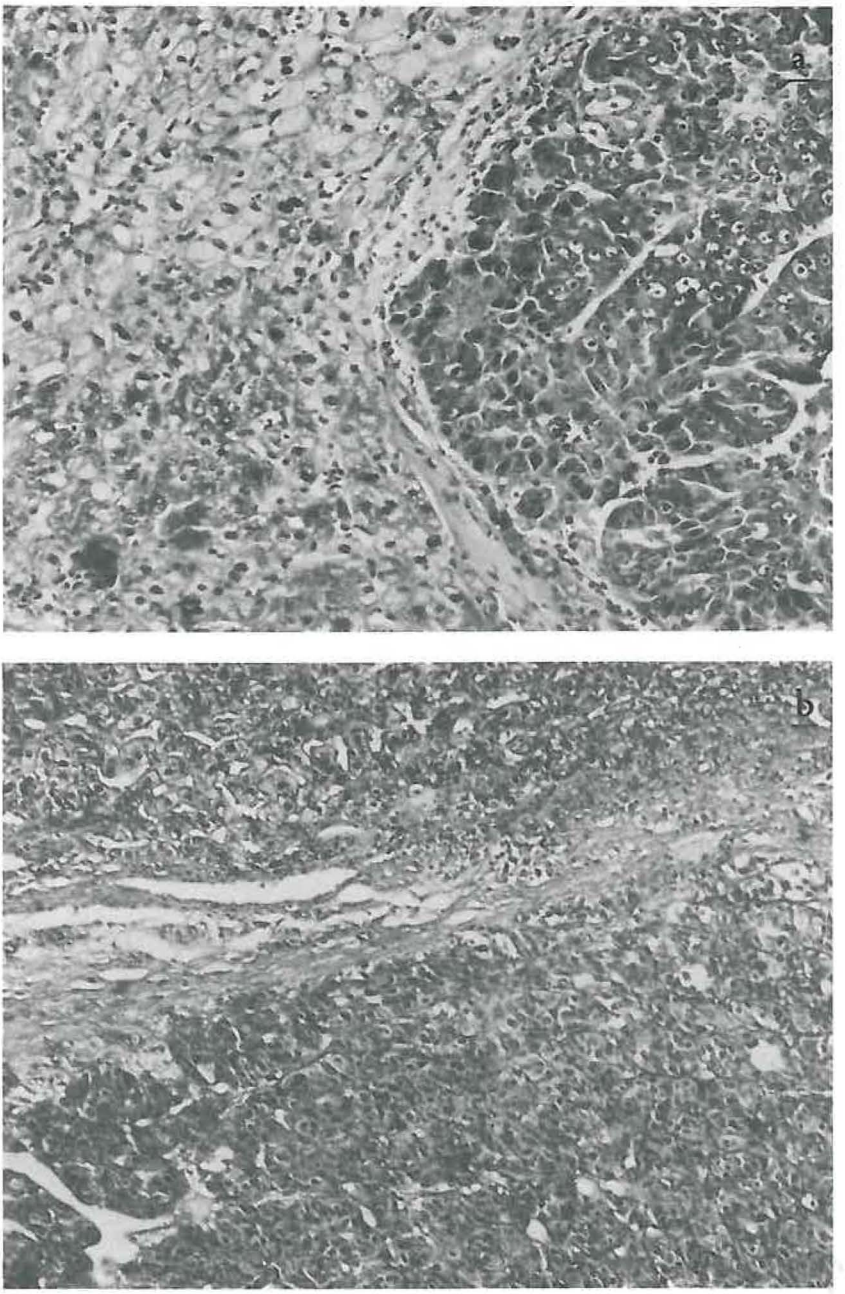

Figure 4

(*a*) Hepatocellular carcinoma (*right*) developing in neoplastic nodule in rat liver. Magnification, 100×. (*b*) Hepatocellular carcinoma (*lower half*) separated by connective tissue from surrounding normal parenchyma in a woman who had taken contraceptive steroids. H and E; magnification, 40×.

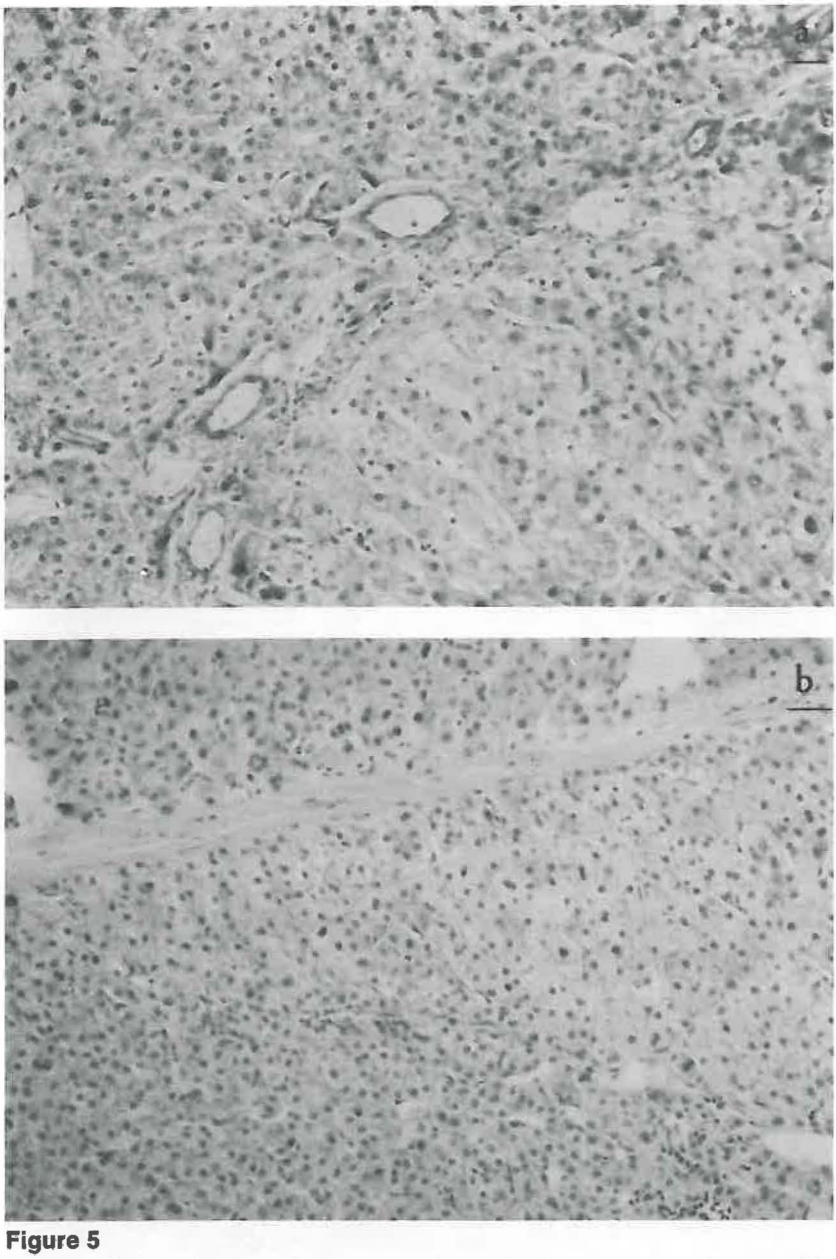

Figure 5
Hepatocellular adenomas in woman who had taken contraceptive steroids. (*a*) Note excess hepatic arteries on the border of the adenoma. (*b*) Connective-tissue band forming in nonadenomatous part of hepatic parenchyma. H and E; magnification, 100×.

thickened, and acid mucopolysaccharides accumulated, sometimes obstructing the lumen. Necrosis, hemorrhage, and fibrosis were frequent, resulting in septa near which hepatocytes assumed a ductular arrangement, as well as in hemorrhage extending into the surrounding parenchyma and into the peri-

toneal cavity. The borders of these tumors with the surrounding parenchyma were sharp. They can show transition from normal to adenoma cells in the same plate, or the tumor may exert pressure on the surrounding parenchyma (Fig. 1b). A capsule may have demarcated the tumor (Fig. 5b). This capsule formed within the surrounding parenchyma, which showed additional changes. They included, besides nonspecific portal inflammatory reaction, small irregularly distributed foci of hepatocytes different in structure, both far from the tumor and in its vicinity, periportal formation of two-cell-thick plates, and excess of arterial lumina in the portal tracts, explained by the tortuosity of the arteries. Large arteries in the periphery of the tumor may even be recognized by arteriography (Goldstein and Neiman 1974).

Sometimes the adenomas exhibited variations in cell populations (Goldfarb 1976) involving changes of cytoplasm and nuclei, including cholestasis and formation of acini containing bile plugs as well as hepatocytes arranged in plates two or more cells thick with thinning of the reticulum stroma. These irregularly distributed nodular changes within the adenoma, frequently following intake of anabolic steroids, raise the question of transformation to carcinoma, although, to date, follow-up of adenomas has not proved carcinomatous transformation. However, cases have been reported of frank carcinoma surrounded by a capsule within adenomas (Davis et al. 1975), and carcinomas, often multicentric (Fig. 4b), in otherwise normal livers have been reported in young women taking contraceptive pills (Hermann and David 1973; Mays et al. 1976).

Focal nodular hyperplasia, also called focal cirrhosis, is an established incidental finding at any age in both sexes, though somewhat more frequent in women (Ishak and Rabin 1975). It is only occasionally of clinical importance, i.e., when it is very large or, particularly in children, pedunculated. It is considered a hamartoma characterized by a central fibrotic core from which septa radiate into the surrounding nodular parenchyma. Bile ducts and ductules are often abundant in the septa and inflammation is frequent; even focal destructive cholangitis or excess hematic cells may be seen. Activated proliferation of hepatocytes, sinusoidal dilatation, and vascular alterations, as described above, as well as hemorrhage, occurred in focal nodular hyperplasia in association with contraceptive steroid intake (Stauffer et al. 1975; Mays et al. 1976), although there seems to be no record of this lesion following therapy with anabolic steroids.

Pathogenesis and Clinical Implications of the Steroid Nodular Lesion

There is great similarity in appearance between the hepatocytic nodular lesions in rodents and the steroid tumors in man, including cytology, architecture, and arrangement of vessels. There is, however, far greater fibrotic reaction in man, and the nomenclature is different: similar lesions are called adenomas in man and neoplastic nodules in rodents. The similarity also includes the nodules in nodules, although transition to carcinoma in the nodules in man has so far only been observed after intake of anabolic steroids. Both groups of steroids, which potentially induce the smooth endoplasmic reticulum and microsomal biotransformation, stimulate hepatocytic proliferation as in rodent nodules; many of the latter are also caused

by inducing agents. Both types of steroids produce sinusoidal dilatation, which has been observed in tumor-free livers in women on contraceptive steroids (Winkler and Poulsen 1975) and in men and women on anabolic steroids (Bagheri and Boyer 1974; Kühböck et al. 1975). Alterations of arteries and veins are associated only with contraceptives which are incriminated, in low incidence, in the production of thrombotic manifestations (Irey et al. 1970), including hepatic vein thrombosis of the Budd-Chiari type (Clubb and Giles 1968; Hoyumpa et al. 1971). In focal nodular hyperplasia, it is possible that steroid-induced stimulation of hepatocytic proliferation, as well as of sinusoidal and vascular alterations, may account for such acute manifestations as pain and hemorrhage in preexisting hamartomas. Regressive changes in preexisting adenomas, including septa formation and pseudoductular transformation of hepatocytes, however, may result in an appearance resembling focal nodular hyperplasia; this seems to be the case in some of the published reports. This secondary fibrosing process is well known in thyroid adenomas.

The causal relationship between steroids and hepatic tumors is statistically better established with the anabolic steroids, with their higher tendency to malignant degeneration, as illustrated by the high incidence in treated Fanconi anemia (Meadows et al. 1974). It is more dubious, indeed denied by some (Anthony 1975), for contraceptive steroid adenomas in view of their low incidence. To date, only some 200 cases have been reported, although it is estimated that ten million women in the United States take contraceptive pills. The following factors strongly support a causal relationship: The tumors occur almost exclusively in women in the earlier reproductive period; up to 10 years ago, there were only a few observations in children. In almost all cases, contraceptive steroids had been used; in a few others, the use of estrogens or a preceding pregnancy was reported (Mays et al. 1976). In the very few instances without an established cause, their recent occurrence raises the possibility of unidentified environmental factors now in existence. Hardly any cases have been reported from central Europe, where the widespread use of contraceptive pills is only of short duration. Highly convincing is the fact that in Japan, where hepatocellular carcinoma is frequent but contraceptive pills are outlawed, no adenomas have been observed despite a good reporting system. An increase of adenomas in central Europe in the future may confirm the causal correlation. Even more dubious is the relation of contraceptive drugs to hepatocellular carcinoma, which in the past occurred occasionally in noncirrhotic livers in this age group. Further observations are required, particularly since arrest or even regression of adenomas after cessation of therapy has been suggested.

Clinically, about one-third of the tumors were detected incidentally, one-third were presented as abdominal masses, sometimes with pain and fever from infarction, and in one-third hemorrhage resulted in a picture of abdominal catastrophy requiring surgical intervention. Peritoneal hemorrhage in women of reproductive age may thus be the result not only of a ruptured tube but also of a bleeding hepatic tumor, the management of which demands surgical expertise. Conventional hepatic tests are of little help in detecting the lesion. Often, but not always, it can be recognized on scanning. Peculiarly

enough, serum alpha-fetoprotein has not been elevated significantly in the cases so far reported, including the carcinomas.

Evolution of Vinyl-chloride-induced Lesions in Man and Rodents

An antecedent fibrotic lesion has been found in workers exposed to vinyl chloride (Müller et al. 1975; Thomas et al. 1975; Whelan et al. 1976). This has also been recognized in some persons exposed to inorganic arsenicals, mostly as a result of the previously popular treatment of psoriasis with Fowler's solution (Morris et al. 1974; Knolle et al. 1974; Huet et al. 1975), which also has been observed to cause angiosarcoma (Regelson et al. 1968). The same neoplasm was also observed in the 1950s in vineyard workers in Germany who were exposed to pesticides containing inorganic arsenicals (Roth 1957). Following the administration of Thorotrast for radiologic study, both hepatic fibrosis (da Silva Horta 1967) and angiosarcoma (Dahlgren 1961; Selinger and Koff 1975) have been found. The evolution from the antecedent lesion to angiosarcoma in man, described below, is based in large part on vinyl-chloride-induced lesions but applies equally, as judged from the study of a smaller number of biopsy and autopsy specimens, to the other agents, except that Thorotrast deposits are found in the last group. The initial lesion, more subtle in man than in rats exposed to vinyl chloride, was a focal hyperplasia of the hepatocytes often with plates two or more cells thick and with variation in cell size (Fig. 6a,b), corresponding to hyperplastic foci and nodules in rodents (Fig. 7a). In rats, frank carcinoma with necrosis (Maltoni 1976) developed within these nodules following formation of nodules in nodules (Fig. 7b). In man and rodents, the sinusoidal cells subsequently increased in number, initially as a variety of cell types, including endothelial cells, macrophages, lipocytes, and lymphocytes. In man, this was associated with plump lining cells with diffuse PAS reaction and with a nonconspicuous perisinusoidal fibrosis. On electron microscopy (Triche et al. 1975), an increase of collagen fibers in perisinusoidal and pericellular spaces was noted, as well as an increase in Ito cells (Ito 1973) or lipocytes (Bronfenmajor et al. 1966), which have been considered precursors of fibroblasts, producing type-III collagen or reticulum (Kent et al. 1976). The lack of liver cell degeneration and of lipofuscin distinguishes this lesion from common hepatitis. The portal tracts show a noncharacteristic fibrosis. In the vinyl chloride cases, a capsular and subcapsular nodular fibrosis produced a characteristic picture on laparoscopy (Marsteller et al. 1975). The spleen showed hyperplasia of both red and white pulp cells.

This antecedent stage proceeded to multicentric angiosarcoma through two precursor stages which were more cellular and fibrotic in man than in rodents. One was a further increase of the sinusoidal cells, some of which had polychromatic and even bizarre nuclei and flat cytoplasm. When they piled up, they were considered sarcomatous (Fig. 8a,b). They were free of PAS-positive or iron granules and of Thorotrast (in cases so exposed), all of which were usually abundant in macrophages. In both species, the sinusoidal lumen was only slightly dilated. In man, portal fibrosis and inflammation associated with bile-ductular proliferation was also prominent,

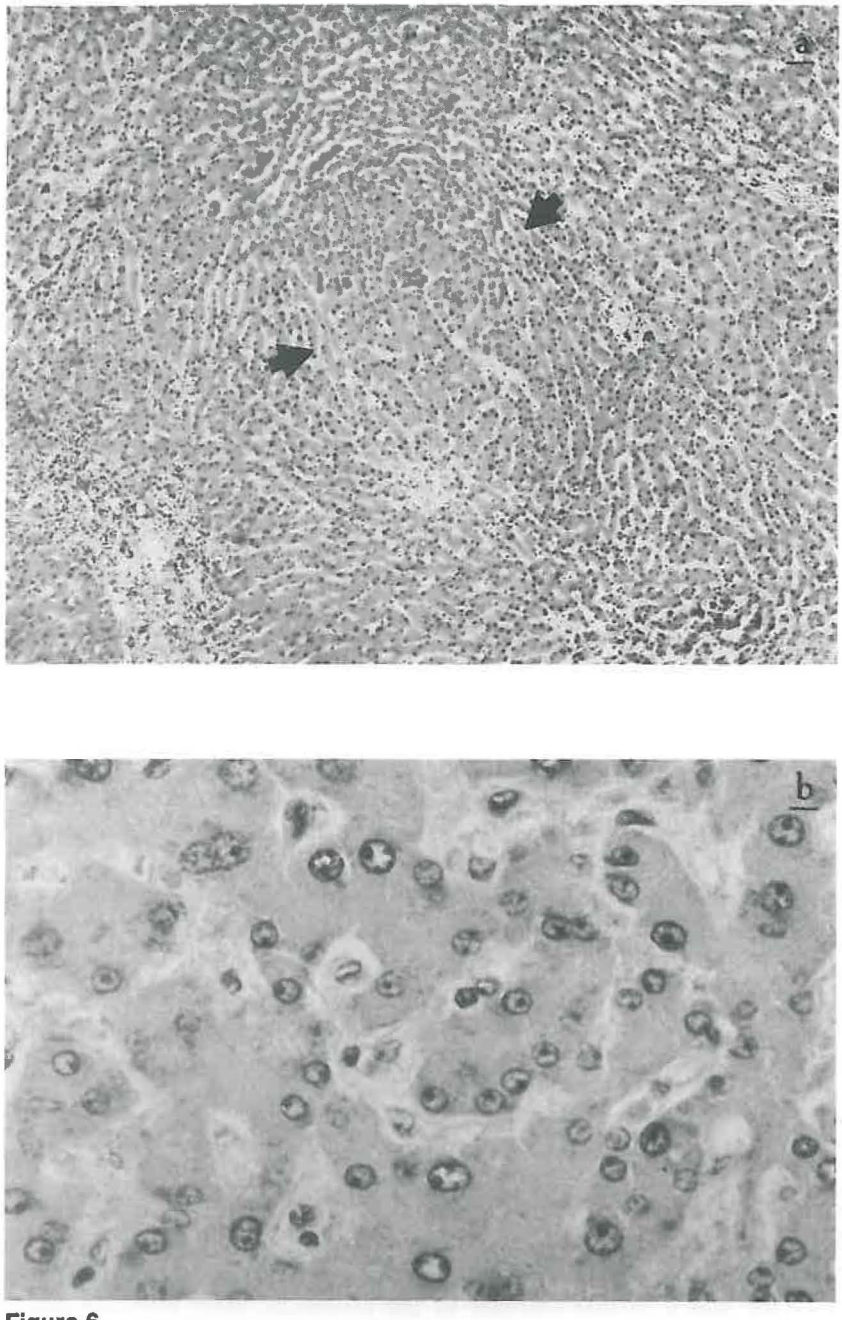

Figure 6
Liver of worker chronically exposed to vinyl chloride. (*a*) Hepatocytic hypertrophy and hyperplasia as well as sinusoidal cell proliferation in nodular portion of parenchyma (*arrows*). Fibrosis and bile ductular proliferation in portal tracts. Magnification, 40×. (*b*) Two-cell-thick hepatocytic plates with binucleated liver cells and proliferation of a variety of sinusoidal cells. H and E; magnification. 250×.

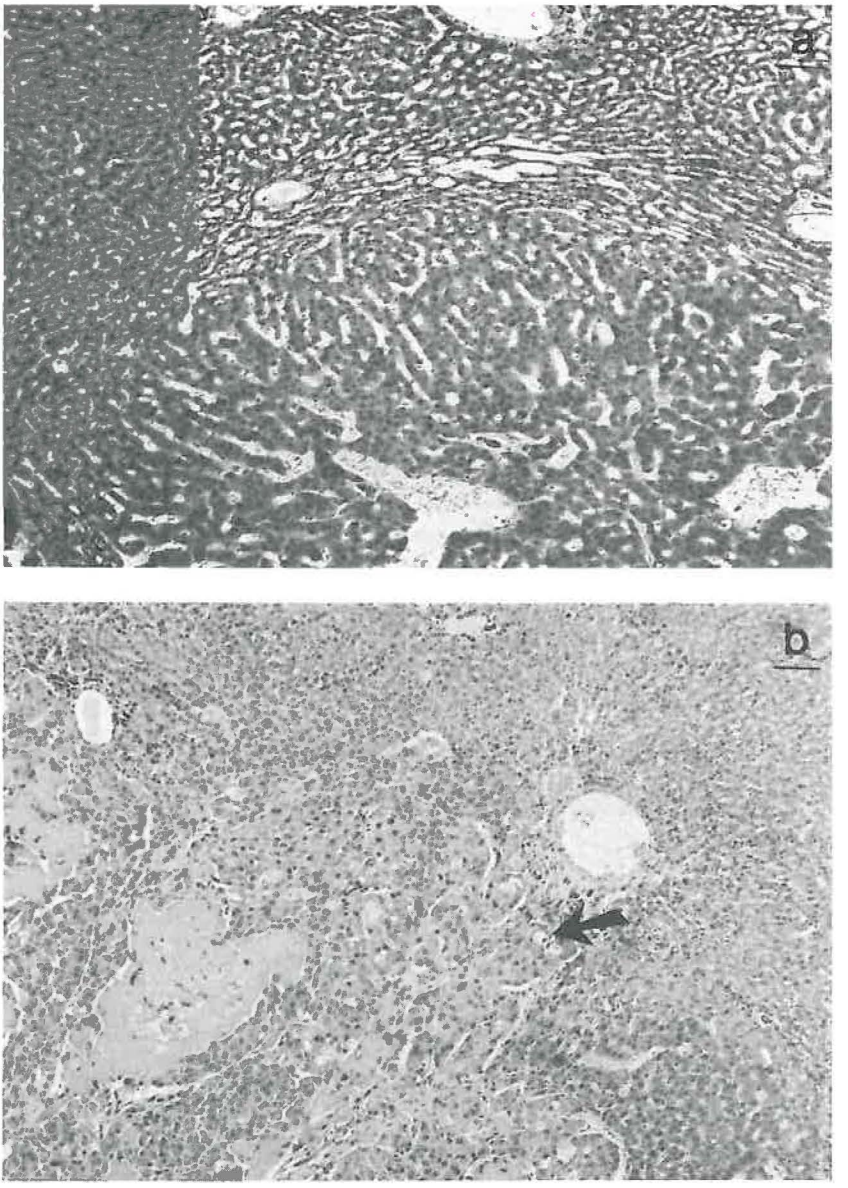

Figure 7
Rats chronically exposed to vinyl chloride. (*a*) Neoplastic nodule in lower portion of photomicrograph. Note the compression of the surrounding parenchyma. (*b*) Hepatocellular carcinoma (*lower left*) within a neoplastic nodule (*arrow*). H and E; magnification, 40×.

and connective-tissue septa formed around these bile ductules, which extended into the lobular parenchyma.

The other, more common precursor stage was focal dilatation of sinusoids unrelated to lobular architecture and thus not attributable to passive con-

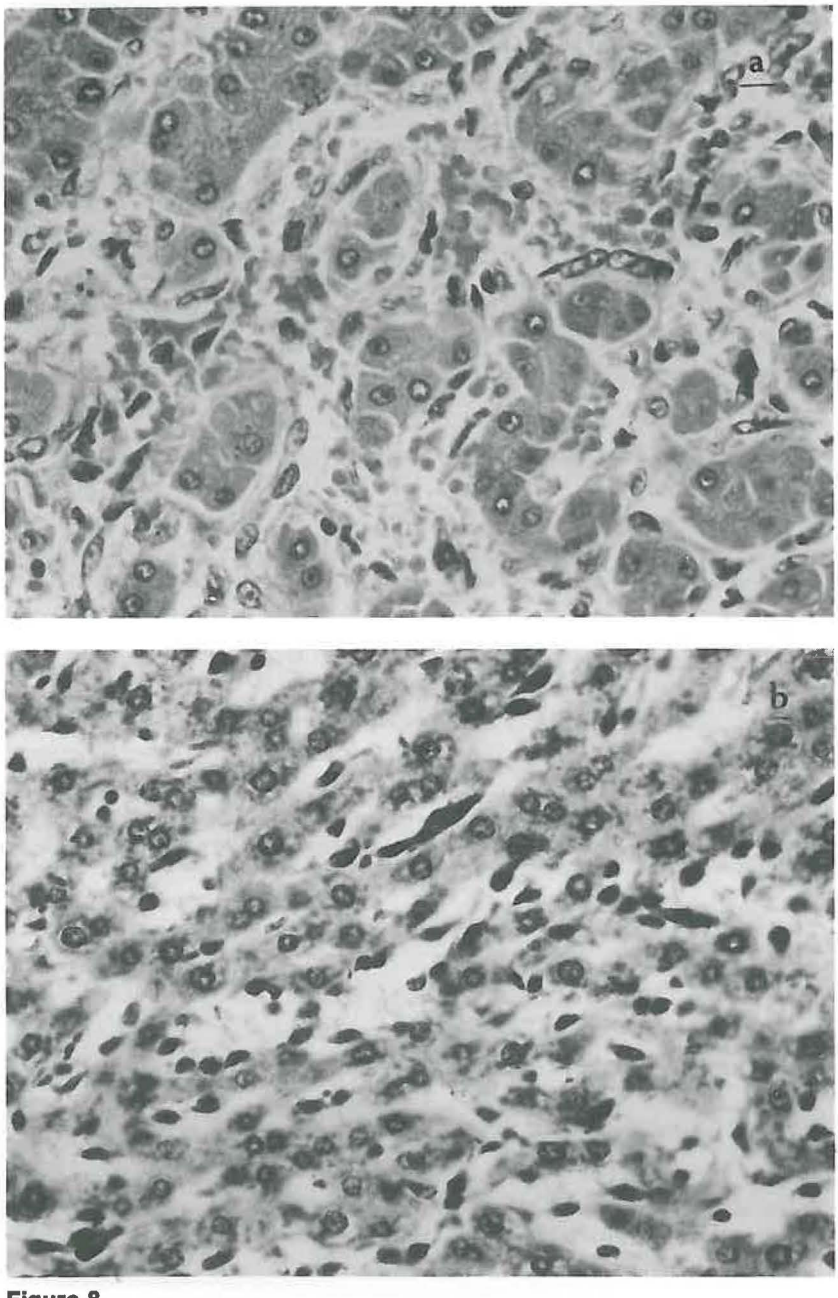

Figure 8
Intralobular angiosarcoma. Note sinusoidal lining cells with bizarre nuclei intermixed with inflammatory cells. The hepatocytes are hyperplastic. (*a*) Worker chronically exposed to vinyl chloride. (*b*) Rat exposed to vinyl chloride. H and E; magnification, 250×.

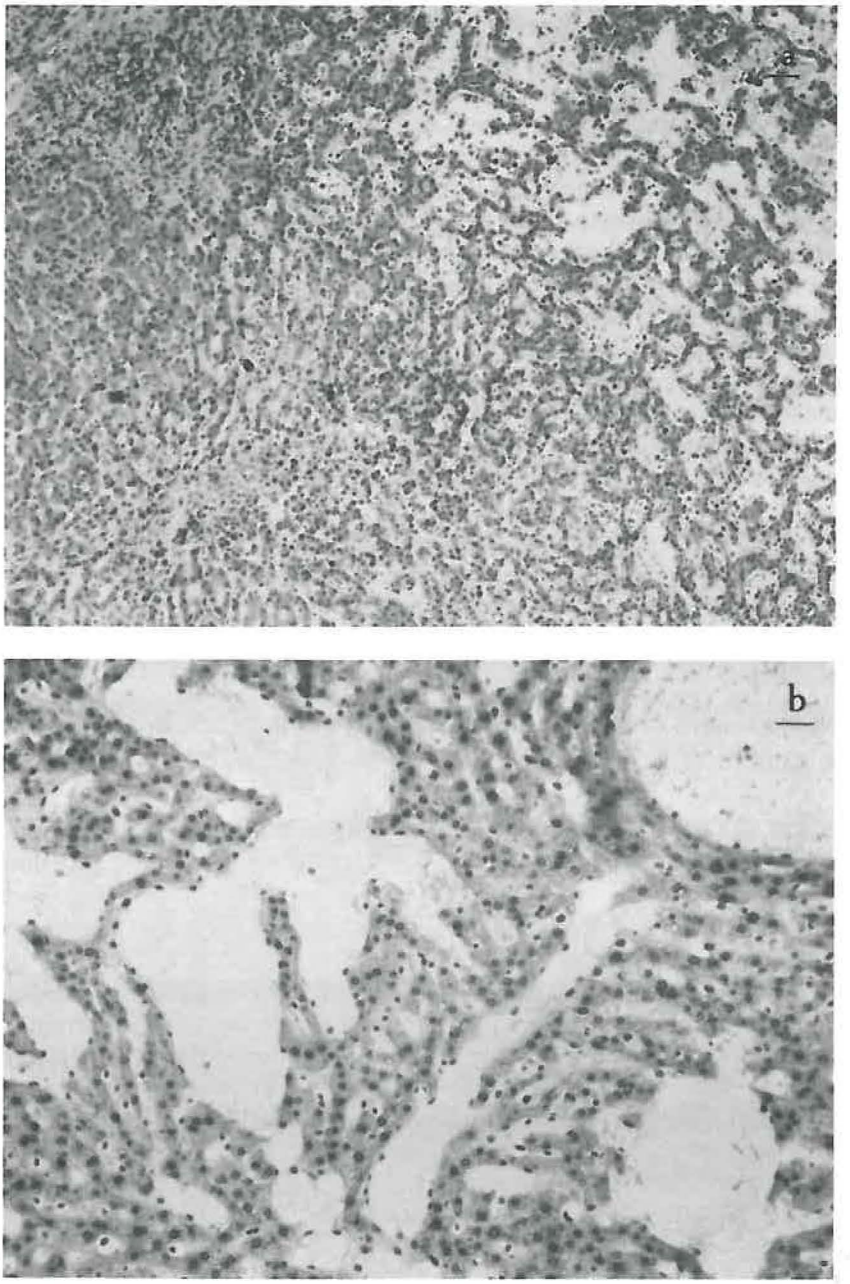

Figure 9
Sinusoidal dilatation with proliferation of lining cells and hepatocytic spurs extending into blood spaces. (*a*) Worker exposed to vinyl chloride. Magnification, 40×. (*b*) Rat exposed to vinyl chloride. H and E; magnification, 100×.

gestion. The sinusoidal lining cells became more prominent, and hematopoietic foci sometimes developed in man. Eventually, the dilatation proceeded to peliotic bloody cysts, which in man were sometimes recognized grossly. Blind ending spurs of hepatocytes indicated that the dilatation was due to widening of blood spaces rather than necrosis (Fig. 9a,b). The spurs of hyperplastic hepatocytes contained bile plugs and eventually were covered by proliferated lining cells which assumed an angiosarcomatous character by forming multiple layers with bizarre nuclei. The perisinusoidal spaces of Disse were wide and contained a variety of mesenchymal cells such as macrophages, which stored various pigments and fat droplets, lymphocytes, and even an occasional plasma cell or segmented leukocyte (Fig. 10). Lipocytes and fibroblasts were seen, and excess reticulum fibers traversed the Disse spaces. The most characteristic appearance is the plexiform arrangement of hyperplastic hepatocytic plates surrounded by angiosarcoma cells in blood spaces of various size often containing fibrin thrombi. The further evolution included increasing fibrosis of these trabeculae associated with their invasion by angiosarcoma cells, to terminate either in a cavernomatous picture or in progression to nodules consisting entirely of sarcoma cells, often spindle-shaped, in which vascular spaces were lined mostly by these cells and only occasionally by nontumorous endothelial cells. Both extensive fibrosis and formation of nodules were

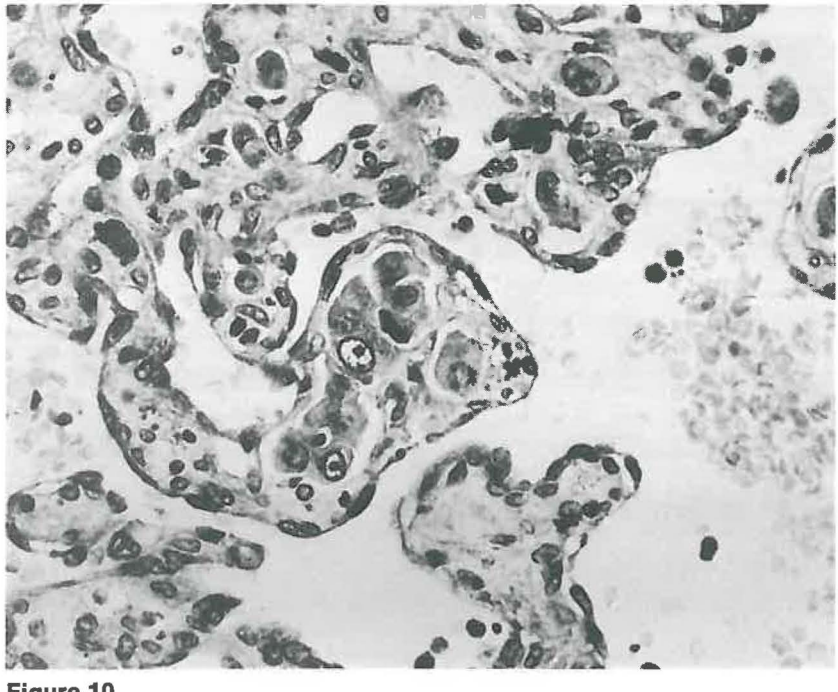

Figure 10
Hepatic angiosarcoma in worker exposed to vinyl chloride. Trabeculae covered by angiosarcoma cells extend into blood-filled cysts. Hyperplastic hepatocytes in the trabeculae are surrounded by a widened Disse space containing a variety of mesenchymal cells as well as increased connective-tissue fibers. H and E; magnification, 250×.

associated in man and rats with the disappearance of inflammatory cells, which are found only later as a reaction to necrosis (Fig. 11a,b).

Pathogenesis and Clinical Implications of Antecedent Lesions and Angiosarcoma Associated with Exposure to Chemicals

Three agents—vinyl chloride, inorganic arsenicals, and Thorotrast—produce a sequence in man which has been duplicated in mice and rats with vinyl chloride. The metabolic changes have been studied for vinyl chloride, and angiosarcoma has been induced experimentally by Thorotrast exposure in rabbits (Swarm et al. 1962), but nothing is known about similar lesions in animals chronically exposed to inorganic arsenicals. The initial lesion is a nodular hypertrophy and hyperplasia of the hepatocytes. This might be related to the metabolism of vinyl chloride in the biotransformation system of the smooth endoplasmic reticulum (Green and Hathway 1975; Watanabe et al. 1976), which is increased in mice exposed to vinyl chloride (Schaffner et al. 1976). Large doses produce an acute hepatic necrosis, particularly if the microsomal biotransformation system is induced, e.g., by phenobarbital (Reynolds et al. 1975), and covalent binding of the metabolite, presumably an epoxide (Kappus et al. 1975), as well as mutagenic effects of the metabolite (Malaveille et al. 1975; Greim et al. 1975), has been demonstrated. This focal hyperplasia may proceed in rats to carcinoma formation. This has not been established in man for vinyl chloride, but it has been for arsenicals in vineyard workers (Luechtrath 1972) and for Thorotrast (Dahlgren 1961). The hepatocytic hyperplasia is associated with a proliferation of sinusoidal cells of various kinds, including inflammatory cells in the liver and also of mesenchymal cells in the spleen. This antecedent fibrotic stage is characterized clinically by portal hypertension with splenomegaly sometimes causing bleeding esophageal varices and thrombocytopenia. Increased blood flow to the enlarged spleen results in the delivery of an amount of blood which the hepatic vascular bed has difficulty in accommodating, particularly since the combined sinusoidal, portal, and capsular fibrosis interferes with distention of the liver (Thomas et al. 1975). Thus, after exposure to vinyl chloride or inorganic arsenicals, a disease develops which is conventionally called Banti's syndrome, hepatoportal sclerosis, or idiopathic portal hypertension. It occurs sporadically in the United States but is found in higher incidence in India, Japan, and parts of Africa, and there the possibility of an unknown environmental toxic factor deserves consideration, although transition to multicentric angiosarcoma, as with the chemical agents, has not been reported. The stimulus for the proliferation of the mesenchymal cells is not established because the transfer of the active metabolite from hepatocytes to mesenchymal cells is difficult to understand and might require unidentified carrier proteins. It is accompanied initially by an inflammatory reaction for which an immunologic mechanism has been claimed (Ward et al. 1976). Full development of angiosarcoma is characterized by its escape from the inflammation. The pathogenesis of the vasodilatation, implying a weakening of the endothelial wall, is also uncertain. The origin of the angiosarcoma cells is not established, and there is little to support their relation to macrophages (Roth

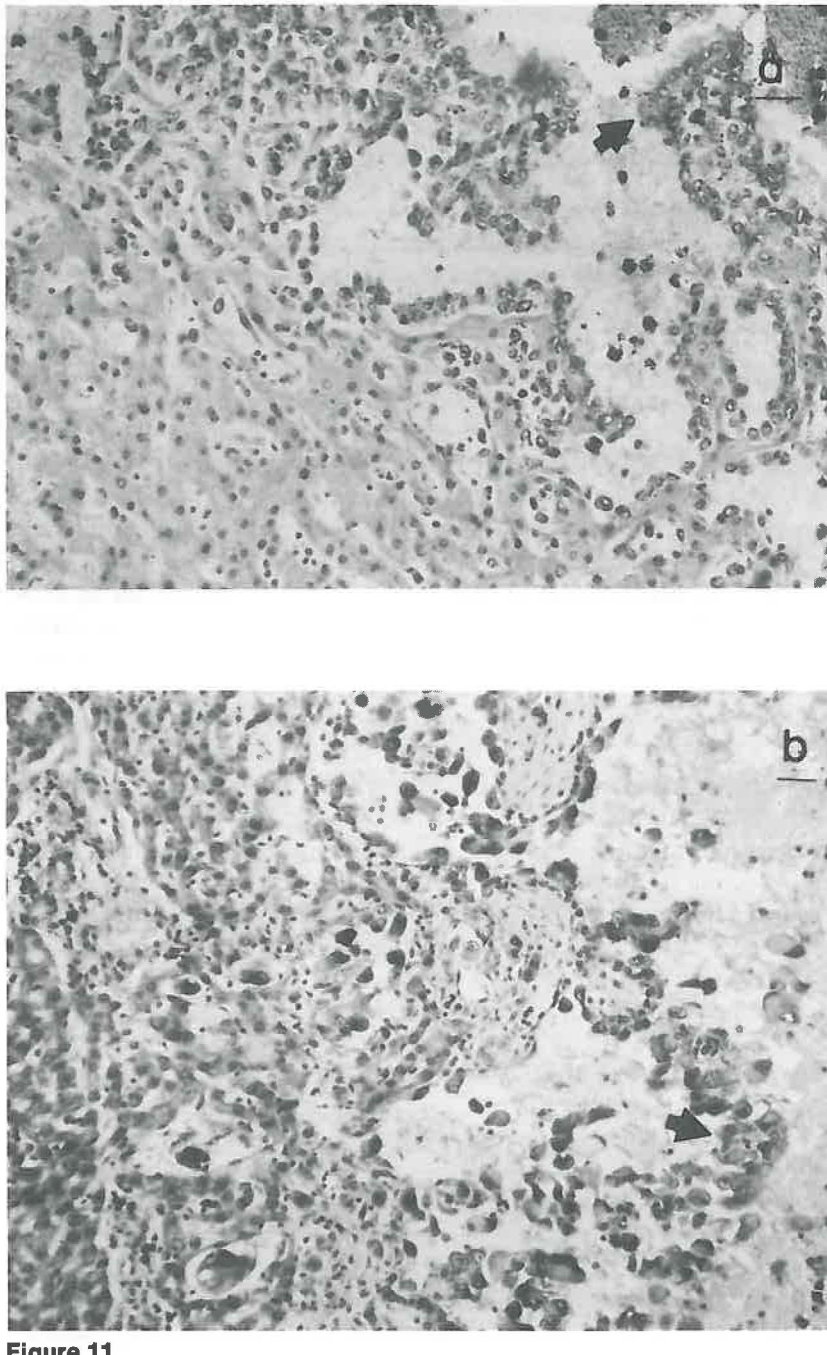

Figure 11
Hepatic angiosarcomas after exposure to vinyl chloride, showing trabeculae consisting of hepatocytes surrounded by fibrosis and covered by polyhedral angiosarcoma cells. They accumulate in multiple layers in the absence of inflammatory cells (*arrows*). (*a*) Man; (*b*) rat. H and E; magnification, 100×.

1957); ultrastructural studies, so far not available on angiosarcomas, may confirm the suspicion that they derive from endothelial cells, which would be in keeping with the more committed term "hemangio-endothelial sarcoma." Excessive fibroblastic stimulation may play a role in the few reported cases of cirrhosis following exposure to vinyl chloride (Smith and Williams 1974), to arsenicals in vineyard workers (Roth 1957), or to Thorotrast (Selinger and Koff 1975). Morphologic specificity of the angiosarcoma following exposure to vinyl chloride would be important from the epidemiologic viewpoint. Comparison with cases of angiosarcoma in men, women, and children without known relation to exposure to chemicals, although so far not complete (L. B. Thomas, N. C. Telles and H. Popper, in prep.), does suggest that portal origin or predominance of nodular angiosarcoma, sometimes single and capsulated, is more frequent in cryptogenic instances. The incidence of both the antecedent fibrotic and the angiosarcomatous lesions following vinyl chloride exposure is not established. Angiosarcoma seems to be far rarer in the material from Germany despite similar exposure. The recent enforcement of strict regulations, radically reducing the level of vinyl chloride in the air, raises the hope that no new cases will be induced in the plants. Since the duration of effective exposure stretches over many years, as epidemiologic studies on these and other carcinogens have indicated, additional cases are still to be expected in the foreseeable future. This calls for screening procedures (Mokk et al. 1974). The conventional hepatic tests, though widely used, seem to have little diagnostic value. A test to measure active fibroplasia by biochemical or immunologic techniques would be desirable, as it would also be for monitoring chronic hepatitis. However, these techniques still represent experimental rather than clinical procedures. Today, periodic scanning to establish both enlargement of the liver and alterations of the blood flow, confirmed by angiographic techniques in suspicious cases, is a mainstay of diagnosis (Tamburro et al. 1976).

DISCUSSION

Comparison of hepatocytic nodules produced in rats with the human hepatic tumors associated with the use of contraceptive and anabolic steroids, which share a common 17-alkylated ring structure, reveals similarities, as do the vinyl-chloride-induced lesions in man and rodents. The major difference is the greater tendency to inflammatory reaction and, more important, to fibrosis in man. The normal rat liver has a far less developed reticulum framework than the human liver, and its hydroxyproline content, as a measure of collagen, is one-third that of the human adult liver (Kent et al. 1959). In effect, the severely cirrhotic rat liver has a hydroxyproline content identical to that of a normal human adult liver. Inflammatory reactions are also far less conspicuous in experimental than in human cirrhosis (Popper 1977a). The similarities between steroid and rat nodular proliferation also concern vascular alterations, bespeaking increased arterial blood supply, and the variations in cell populations (nodules in nodules), which have a potential to proceed to cancer. This development of cancer in preformed nodules is strong morphologic support for Farber's hypothesis of hepatocarcinogenesis (Farber 1974)

with sequential formation of multiple cell populations. They differ in susceptibility to toxic agents, in loss of enzymes, in dependence on regulation, and in increasing irreversibility; eventually benign tumor and subsequently carcinoma develop. Whether the pathogenesis of the steroid nodular lesions differs from that of other types of hepatocellular carcinoma remains to be established. They are not associated with cirrhosis or with a significant rise of alphafetoprotein serum levels, which occurs with most but not all experimental chemical hepatocellular carcinomas, nor are they associated with foci of dysplastic cells (Anthony 1976) which accompany hepatocellular carcinoma with hepatitis-associated antigen, the *s* component of which is often abundant in these cells (Popper 1977b). By contrast, the transformation to cancer is indicated more by architectural than by cytologic features in the examples studied here.

The lesion produced by vinyl chloride in rodents and man, as well as by inorganic arsenicals and Thorotrast in man, exhibits the simultaneous proliferation of both hepatocytic and sinusoidal cells, with the latter more frequently proceeding to malignancy. However, at least in experimental animals, including primates, proliferation of sinusoidal cells potentially proceeding to angiosarcoma has been observed with several hepatocarcinogens, such as dimethylnitrosamine (Herrold 1967). Not only do epithelial and angiosarcomatous tumors result, but they may also be combined, at least in primates as observed by Adamson (1972); in man, we saw this combination in only one patient without known exposure. Whether the suggested increase in cryptogenic hepatic angiosarcoma indicates increasing exposure to unknown environmental agents remains to be established.

SUMMARY

The evolution of hepatic nodular proliferations in rodents is briefly reviewed and that in man following exposure to contraceptive and anabolic steroids is more thoroughly reviewed. Both are compared. Similarly, the almost identical sequences from antecedent fibrotic lesions to angiosarcoma in man following exposure to vinyl chloride, inorganic arsenicals, and Thorotrast and in rodents treated with vinyl chloride are described. Clinical implications and methods of detection are referred to. In both groups of lesions, the similarity of their evolution supports the predictive nature of animal experimental studies for carcinogenesis in man, as proposed for hepatic nodules in rodents. The major difference is the greater fibroplastic reaction in man in both examples studied. The development of hepatocellular carcinoma within the nodules of man and rodents supports the concept of multiple cell populations in hepatocarcinogenesis. In the vinyl-chloride-associated lesions, the simultaneous proliferation of hepatocytes and sinusoidal cells, the latter potentially terminating in angiosarcoma, is stressed, although the metabolic processes underlying this evolution remain to be established.

Acknowledgment

This research was supported by National Institute of Occupational Safety and Health Grant Fund No. G-4-4641.

REFERENCES

Adamson, R.H. 1972. Long-term administration of carcinogenic agents to primates. In *Medical primatology* (ed. E.I. Goldsmith and J. Moor-Jankowski), p. 216. Karger, Basel.

Ameriks, J.A., N.W. Thompson, C.F. Frey, H.D. Appelman and J.F. Walter. 1975. Hepatic cell adenomas, spontaneous liver rupture, and oral contraceptives. *Arch. Surg.* **110:**548.

Anthony, P.P. 1975. Hepatoma associated with androgenic steroids. *Lancet* **i:**685.

———. 1976. Precursor lesions for liver cancer in humans. *Cancer Res.* **36:**2579.

Bagheri, S.A. and J.L. Boyer. 1974. Peliosis hepatis associated with androgenic-anabolic steroid therapy. *Ann. Intern. Med.* **81:**610.

Bannasch, P. 1974. Carcinogen-induced cellular thesaurismoses and neoplastic cell transformation. *Recent Results Cancer Res.* **44:**115.

Baum, J.K., F. Holtz, J.J. Bookstein and E.W. Klein. 1973. Possible association between benign hepatomas and oral contraceptives. *Lancet* **ii:**926.

Bronfenmajor, S., F. Schaffner and H. Popper. 1966. Fat-storing cells (lipocytes) in human liver. *Arch. Pathol.* **82:**447.

Clemmesen, J., V.F. Frederiksen and C.M. Plum. 1974. Are anticonvulsants oncogenic? *Lancet* **i:**705.

Clubb, A.W. and C. Giles. 1968. Budd-Chiari syndrome after oral contraceptives. *Br. Med. J.* **1:**252.

Committee on Safety of Medicines. 1972. *Carcinogenicity tests of oral contraceptives.* Her Majesty's Stationery Office, London.

Creech, J.L., Jr. and M.N. Johnson. 1974. Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J. Occup. Med.* **16:**150.

Dahlgren, S. 1961. Thorotrast tumours. A review of the literature and report of two cases. *Acta Pathol.* **53:**147.

da Silva Horta, J. 1967. Late effects of thorotrast on the liver and spleen, and their efferent lymph nodes. *Ann. N. Y. Acad. Sci.* **145:**676.

Davis, M., B. Portmann, M. Searle, R. Wright and R. Williams. 1975. Histological evidence of carcinoma in a hepatic tumour associated with oral contraceptives. *Br. Med. J.* **4:**496.

Edmondson, H.A., B. Henderson and B. Benton. 1976. Liver-cell adenomas associated with use of oral contraceptives. *N. Engl. J. Med.* **294:**470.

Epstein, S.S. 1974. Environmental determinants of human cancer. *Cancer Res.* **34:**2425.

Farber, E. 1974. Pathogenesis of liver cancer. *Arch. Pathol.* **98:**145.

Farrell, G.C., D.E. Joshua, R.F. Uren, P.J. Baird, K.W. Perkins and H. Kronenberg. 1975. Androgen-induced hepatoma. *Lancet* **i:**430.

Goldfarb, S. 1976. Sex hormones and hepatic neoplasia. *Cancer Res.* **36:**2584.

Goldstein, H.M. and H.L. Neiman. 1974. Angiographic findings in benign liver tumors. *Diagnost. Radiol.* **110:**339.

Gordon, D.E., L.B. Thomas, G. Kent, J. Calandra, R. Bahu and H. Popper. 1974. Hepatic angiosarcoma in man and rodents following prolonged exposure to vinyl chloride. *Gastroenterology* **67:**A-17.

Grabowski, M., U. Stenram and A. Bergqvist. 1975. Focal nodular hyperplasia of the liver, benign hepatomas, oral contraceptives and other drugs affecting the liver. *Acta Pathol. Microbiol. Scand. Sect. A* **83:**615.

Green, T. and D.E. Hathway. 1975. The biological fate in rats of vinyl chloride in relation to its oncogenicity. *Chem.-Biol. Interact.* **11:**545.

Greim, H., G. Bonse, Z. Radwan, D. Reichert and D. Henschler. 1975. Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* **24:**2013.

Hermann, R.E. and T.E. David. 1973. Spontaneous rupture of the liver caused by hepatomas. *Surgery* **74:**715.

Herrold, K. McD. 1967. Histogenesis of malignant liver tumors induced by dimethylnitrosamine. An experimental study in Syrian hamsters. *J. Natl. Cancer Inst.* **39:**1099.

Hoyumpa, A.M., Jr., L. Schiff and E.L. Helfman. 1971. Budd-Chiari syndrome in women taking oral contraceptives. *Am. J. Med.* **50:**137.

Huet, P.-M., E. Guillaume, J. Côté, A. Légaré, P. Lavoie and A. Viallet. 1975. Noncirrhotic presinusoidal portal hypertension associated with chronic arsenical intoxication. *Gastroenterology* **68:**1270.

Irey, N.S., W.C. Manion and H.B. Taylor. 1970. Vascular lesions in women taking oral contraceptives. *Arch. Pathol.* **89:**1.

Ishak, K.G. and L. Rabin. 1975. Benign tumors of the liver. *Med. Clin. North Am.* **59:**995.

Ito, T. 1973. Recent advances in the study on the fine structure of the hepatic sinusoidal wall: A review. *Gunma Rep. Med. Sci.* **6:**119.

Johnson, F.L., J.R. Feagler, K.G. Lerner, P.W. Majerus, M. Siegel, J.R. Hartmann and E.D. Thomas. 1972. Association of androgenic-anabolic steroid therapy with development of hepatocellular carcinoma. *Lancet* **ii:**1273.

Kappus, H., H.M. Bolt, A. Buchter and W. Bolt. 1975. Rat liver microsomes catalyse covalent binding of ^{14}C-vinyl chloride to macromolecules. *Nature* **257:**134.

Kent, G., I.G. Fels, A. Dubin and H. Popper. 1959. Collagen content based on hydroxyproline determinations in human and rat livers. Its relation to morphologically demonstrable reticulum and collagen fibers. *Lab. Invest.* **8:**48.

Kent, G., S. Gay, T. Inouye, R. Bahu, O.T. Minick and H. Popper. 1976. Vitamin-A containing lipocytes and formation of type III collagen in liver injury. *Proc. Natl. Acad. Sci.* **73:**3719.

Kitagawa, T. and H. Sugano. 1973. Combined enzyme histochemical and radioautographic studies on areas of hyperplasia in the liver of rats fed *N*-2-fluorenylacetamide. *Cancer Res.* **33:**2993.

Knolle, J., E. Förster, A. Roessner, H. Themann, P. Höhn and K.-H. Meyer zum Buschenfelde. 1974. Die nicht-zirrhotische portale Fibrose (hepatoportale Sklerose) nach chronischer Arsenvergiftung. Klinische und pathologisch-anatomische Befunde. *Dtsch. Med. Wochenschr.* **99:**903.

Kühböck, J., T. Radaszkiewicz and H. Walek. 1975. Peliosis hepatis, eine Komplikation der Anabolikatherapie. *Med. Klin.* **70:**1602.

Lloyd, J.W. 1975. Angiosarcoma of the liver in vinyl chloride/polyvinyl chloride workers. *J. Occup. Med.* **17:**333.

Luechtrath, H. 1972. Chirrhosis of the liver in chronic arsenic poisoning of vintners. *German Med. Month.* **2:**127.

Malaveille, C., H. Bartsch, A. Barbin, A.M. Camus, R. Montesano, A. Croisy and P. Jacquignon. 1975. Mutagenicity of vinyl chloride chloroethyleneoxide, chloroacetaldehyde and chloroethanol. *Biochem. Biophys. Res. Commun.* **63:**363.

Maltoni, C. 1974. Occupational carcinogenesis. In *Proceedings of the 2nd International Symposium on Cancer Detection and Prevention,* Bologna, April 9–12, 1973, p. 19. Excerpta Medica, Amsterdam.

———. 1976. Predictive value of carcinogenesis bioassays. *Ann. N. Y. Acad. Sci.* **271:**431.

Maltoni, C. and G. Lefemine. 1974. Carcinogenicity bioassays of vinyl chloride. 1. Research plan and early results. *Environ. Res.* **7:**387.

Marsteller, H.J., W.K. Lelbach, R. Müller, P. Gedigk and C.E. Lange. 1975.

Klinische und laparoskopische Aspekte der Leberschaeden bei Chemiearbeitern in der Vinylchlorid-Polymerisation. *Leber Magen Darm* **5**:196.

Mays, E.T., W.M. Christopherson and G.H. Barrows. 1974. Focal nodular hyperplasia of the liver. Possible relationship to oral contraceptives. *Am. J. Clin. Pathol.* **61**:735.

Mays, E.T., W.M. Christopherson, M.M. Mahr and H.C. Williams. 1976. Hepatic changes in young women ingesting contraceptive steroids. *J. Am. Med. Assoc.* **235**:730.

McAvoy, J.M., R.K. Tompkins and W.P. Longmire, Jr. 1976. Benign hepatic tumors and their association with oral contraceptives. Case reports and survey of the literature. *Arch. Surg.* **111**:761.

Meadows, A.T., J.L. Naiman and M. Valdes-Dapena. 1974. Hepatoma associated with androgen therapy for aplastic anemia. *J. Pediatr.* **84**:109.

Mokk, L., J.L. Creech, J.G. Whelan and M.N. Johnson. 1974. Liver damage and angiosarcoma in vinyl chloride workers. A systematic detection program. *J. Am. Med. Assoc.* **230**:64.

Morris, J.S., M. Schmid, S. Newman, P.J. Scheuer and S. Sherlock. 1974. Arsenic and noncirrhotic portal hypertension. *Gastroenterology* **64**:86.

Müller, R., H. Bechtelsheimer, P. Gedigk, H.J. Marsteller and W.K. Lelbach. 1975. Das morphologische Bild der Leberschaedigung nach chronischer Vinylchlorid-Exposition. *Leber Magen Darm* **5**:204.

Nissen, E.D., F. Kent and D.R. Kent. 1975. Liver tumors and oral contraceptives. *Obstet. Gynecol.* **64**:460.

O'Sullivan, J.P. and R.P. Wilding. 1974. Liver hamartomas in patients on oral contraceptives. *Br. Med. J.* **3**:7.

Popper, H. 1977a. Cancer of the liver. An overview. In *Proceedings of the 3rd International Symposium on Detection and Prevention of Cancer* (ed. H.E. Nieburgs). Marcel Dekker, New York. (In press.)

———. 1977b. Experimentelle Leberzirrhose. In *Verhandlungen der Deutschen Gesellschaft fuer Innere Medizin,* vol. 82, p. 271. Bergmann, Munich.

Popper, H., S.S. Sternberg, B.L. Oser and M. Oser. 1960. The carcinogenic effect of aramite in rats. A study of hepatic nodules. *Cancer* **13**:1035.

Regelson, W., U. Kim, J. Ospina and J.F. Holland. 1968. Hemangioendothelial sarcoma of liver from chronic arsenic intoxication by Fowler's solution. *Cancer* **21**:514.

Reynolds, E.S., M. Treinen Moslen, S. Szabo, R.J. Jaeger and S.D. Murphy. 1975. Hepatotoxicity of vinyl chloride and 1,1-dichloroethylene. Role of mixed function oxidase system. *Am. J. Pathol.* **81**:219.

Roth, F. 1957. Arsen—Leber—Tumoren (Haemangioendotheliom). *Z. Krebsforsch.* **61**:468.

Schaffner, F., H. Popper and I.J. Selikoff. 1976. Initial features of vinyl chloride (VC) hepatic injury. *Gastroenterology* **71**:A-35. (Abstr.)

Selinger, M. and R.S. Koff. 1975. Thorotrast and the liver: A reminder. *Gastroenterology* **68**:799.

Sherlock, S. 1975. Hepatic adenomas and oral contraceptives. *Gut* **16**:753.

Smith, P.M. and D.M.J. Williams. 1974. Vinyl chloride and cirrhosis. *Digestion* **10**:125.

Squire, R.A. and M.H. Levitt. 1975. Report of a workshop on classification of specific hepatocellular lesions in rats. *Cancer Res.* **35**:3214.

Stauffer, J.Q., M.W. Lapinski, D.J. Honold and J.K. Myers. 1975. Focal nodular hyperplasia of the liver and intrahepatic hemorrhage in young women on oral contraceptives. *Ann. Int. Med.* **83**:301.

Swarm, R.L., E. Miller and H.J. Michelitch. 1962. Malignant vascular tumors in rabbits injected intravenously with colloidal thorium dioxide. *Pathol. Microbiol.* **25**:27.

Sweeney E.C. and D.J. Evans. 1976. Hepatic lesions in patients treated with synthetic anabolic steroids. *J. Clin. Pathol.* **29**:626.

Thomas, L.B., H. Popper, P.D. Berk, I. Selikoff and H. Falk. 1975. Vinyl chloride induced liver disease. From idiopathic portal hypertension (Banti's syndrome) to angiosarcoma. *N. Engl. J. Med.* **292**:17.

Tomatis, L., C. Partensky and R. Montesano. 1973. The predictive value of mouse liver tumour induction in carcinogenicity testing—A literature survey. *Int. J. Cancer* **12**:1.

Triche, T., K. Nanba, K. Ishak, A. Wolkoff and P.D. Berk. 1975. Hepatic ultrastructural changes in vinyl-chloride (VC) workers. *Clin. Res.* **23**:259A.

Walker, A.I.T., E. Thorpe and D.E. Stevenson. 1973. Toxicology of dieldrin (HEOD). 1. Long-term oral toxicity studies in mice. *Food Cosmet. Toxicol.* **11**:415.

Ward, A.M., S. Udnoon, J. Watkins, A.E. Walker and C.S. Darke. 1976. Immunological mechanisms in the pathogenesis of vinyl chloride disease. *Br. Med. J.* **1**:936.

Watanabe, P.G., G.R. McGowan and P.J. Gehring. 1976. Fate of [^{14}C]vinyl chloride after single oral administration in rats. *Toxicol. Appl. Pharmacol.* **36**:339.

Whelan, J.G., Jr., J.L. Creech and C.H. Tamburro. 1976. Angiographic and radionuclide characteristics of hepatic angiosarcoma found in vinyl chloride workers. *Radiology* **118**:549.

Winkler, K. and H. Poulsen. 1975. Liver disease with periportal sinusoidal dilatation. A possible complication to contraceptive steroids. *Scand. J. Gastroenterol.* **10**:699.

Origins of Human Cancer

BOOK C
Human Risk Assessment

edited by

H. H. Hiatt
Harvard School of Public Health

J. D. Watson
Cold Spring Harbor Laboratory

J. A. Winsten
Harvard School of Public Health

COLD SPRING HARBOR CONFERENCES ON CELL PROLIFERATION
VOLUME 4

Cold Spring Harbor Laboratory
1977

Origins of Human Cancer

International Standard Book Number 0-87969-119-0
Library of Congress Catalog Card Number 76-57915

Printed in the United States of America

Cover and book design by Emily Harste

This publication was supported in part by
grants from the Rita Allen Foundation and
the Charles E. Merrill Trust.