THE PATHOGENESIS OF TUMORS OF THE LIVER PRODUCED BY BUTTER YELLOW*

By EUGENE L. OPIE

(From the Laboratories of The Rockefeller Institute for Medical Research)

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Tumors produced by dimethylaminoazobenzene (butter yellow) occur in an organ which in the presence of injury may undergo rapid regenerative changes affecting both terminal ducts and livers cells. Study of these tumors shows that there is an orderly sequence of changes with proliferation of bile ducts, liver parenchyma, and supporting fibrous tissue, terminating in the formation of tumors that are characteristically malignant and astonishingly complex in structure. The sequence of events leading to the production of tumors is profoundly modified by diet (1), which is the essential factor in determining the presence or absence of cirrhosis. The study further gives opportunity to investigate the relation of tumor formation to cirrhosis, a subject that has long been discussed with reference to human pathology.

Sasaki and Yoshida (2) found following administration of *o*-aminoazotoluene progressive hyperplasia of liver cells resulting in the formation of adenomas that might become malignant. The earliest tumors appeared after 196 days. If administration of the agent was discontinued after 4 months, tumors were formed later.

Orr (3) used the rice diet of earlier observers because it produced tumors more rapidly than similar diets consisting in large part of maize, wheat, or oats. Nodular cirrhosis was found after 4 months of feeding, and at this time tumors began to appear. He found considerable difficulty in determining whether the earliest accumulation of mononuclear cells and disintegration of liver cells was about the central vein or about the portal spaces, and even after 4 months found 9 animals with periportal changes, 3 with changes about the central vein, and 3 with the two combined. Proliferation of bile ducts was found after 3 months and was conspicuous after 4 months, but even at this time formation of collagen fibrils in the newly formed cellular tissue was scant. He designated tumors as bile duct carcinomas arising in areas of bile duct proliferation, bile duct cyst adenomata containing cavernous spaces surrounded by cuboidal epithelium, and liver cell carcinoma. The latter resembled nodules of regenerating liver cells save for the absence of glycogen and the occurrence of atypical mitoses. Tubular acini may suggest origin from bile ducts but the characters of the cells were those of liver cells.

Maruya (4) came to the conclusion that cancer of the liver with administration of butter yellow was produced by proliferation of liver cells, and regarded the cho-

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langioma-like carcinoma as an atypical hepatoma. The tumor which he designated fibro-adenoma did not produce malignant growth, but with increase of fibrous tissue underwent atrophy.

In some rats that had developed tumors after 3 months of feeding with butter yellow, Sigiura and Rhoads (5) found in small cirrhotic livers a few cancer nodules. In other animals the liver reached great size and contained large tumor masses. When animals were given brown rice alone, about 75 per cent of tumors were cholangiomas with glandular alveoli, whereas with the addition of carrots the liver was larger and contained large nodules designated hepatomas, in which the cells resembled those of the liver. In both instances the lesions were regarded as cancers, but metastases accompanied only one-fifth of them.

Tumors of the liver produced by butter yellow were classified by Edwards and White (6) as hepatomas, Type I, hepatomas, Type II, and adenocarcinoma. The first consisted of cords of cells which were acidophilic and resembled closely those of the liver. Gland-like alveoli and cysts were formed by cells with close resemblance to liver cells. Cells forming hepatomas, Type II, stained faintly and were usually basophilic with poorly defined outlines. Sinuses separating cords of cells were in places cavernous and in places collapsed. Adenocarcinoma was characterized by cubical or columnar cells arranged to form glandular alveoli, but in nearly all of these tumors there was tissue resembling that of the hepatomas. Their alveoli were distinguished from proliferating bile ducts by cellular stratification and by numerous atypical mitoses, the presence of solid masses of cells, papillary structures, invasion of blood vessels, and metastases.

Methods and Material

In accord with the procedure that has been followed by many investigators, *p*-dimethylaminoazobenzene has been added to diets in the proportion of 0.06 per cent, 20 cc. of a 3 per cent solution of butter yellow in olive oil being added to 1000 gm. of the diet.

Diets which with butter yellow produce nodular cirrhosis within 4 or 5 months (1) are as follows: (1) Diet consisting of rice supplemented by a small quantity of carrot; (2) diets consisting of casein, forming 11.5 per cent of the caloric value of the diet, fat (Crisco), and sugar, with adequate quantities of mineral salts and vitamins.

Diets which have produced tumors in the absence of significant cirrhosis have been as follows: (3) Diets in which rice has been given in association with abundant supply of vitamins (Vegex and Oleum percomorphum); (4) diets in which with adequate vitamins the protein level has varied from 12 to 40 per cent and the fat level from 44 to 88 per cent.

It is significant that in the presence of cirrhosis tumors appear more rapidly than in its absence, but cirrhosis is not essential to the production of tumors.

In some experiments animals have been allowed to live until their death has been caused by toxic action of butter yellow in association with unfavorable diets, by tumors, or, in a few instances, by intercurrent disease. In other experiments animals have been killed or the liver examined by parts removed at operation 5 months after the beginning of feeding.

The most satisfactory methods of staining have been with methylene blue and phloxine, or with Giemsa's stain following fixation with Zenker's fluid.

The Portal Unit

Attention has been directed repeatedly to the almost universally accepted but anomalous definition of the liver lobule. The unit of structure of glandular

organs, save for the liver, consists of acini grouped about a terminal duct and more or less outlined in sections by fibrous tissue. The lobule of the liver, in accordance with accepted nomenclature, has at its center the central vein and at its periphery several portal spaces each with its bile duct. With embryological development tubules radiate from a terminal duct and form the parenchyma that surrounds the duct.

Sabourin (7) and some others have defined the lobule as the hepatic tissue about a terminal bile duct. To avoid confusion that might result if the attempt were made to discard the well established lobule which has a radicle of the hepatic vein at its center, Mall (8) designated as a "portal unit" the tissue that surrounds a portal space and pours its secretion into the corresponding duct. The shortest capillaries according to Mall pass directly from the portal to the hepatic (central) vein, but in the intermediate regions the radiating capillaries to reach this vein must bend in greater degree, as their distance from the straight line between portal and hepatic vein increases. As the result of this arrangement, there are points on the periphery of the portal unit intermediate between two hepatic (central) veins where the deflected capillaries of several adjoining units come into close approximation. Mall has designated these as nodal points.

The penetration of wide capillaries from the portal veins in septal spaces between adjacent vascular lobules, and the radiation of capillaries from them toward the central vein has been observed by Géraudel (9), Debeyre (10), and others, and is doubtless a more accurate description of the vascular supply of the lobule than that of Mall. Twigs from the hepatic artery and terminal branches of the bile ducts have been described accompanying the dilated capillaries between the vascular lobules (Pfuhl (11)).

Certain pathological lesions emphasize the significance of the relation of the duct to the surrounding parenchyma. With chronic passive congestion accompanied by diminished aeration of blood in capillaries about the terminal branches of the hepatic veins (central veins), liver cells atrophy and disappear. With poisons such as chloroform or carbon tetrachloride, necrosis occurs about these hepatic veins and as Whipple and Sperry (12) showed for chloroform, and Cameron and Karunaratne (13) for carbon tetrachloride, regeneration proceeds peripherally from the intact parenchyma about the terminal duct, and under favorable conditions restores the liver to normal. Even more significant is the relation of the changes that will be described in association with cirrhosis to the structural unit which has the terminal duct as its center.

In a section of the normal liver of the rat, as in other mammals, capillaries radiate from the so called central vein and columns of liver cells appear to have a similar relation to the vein. Nevertheless with inflammatory edema of the rat's liver (Fig. 1) the portal fibrous tissue is distended and spaces in the intervening parenchyma that were previously inconspicuous or absent are opened up so that islands of parenchyma with bile duct, portal vein, and hepatic artery in the center (Fig. 2) are now clearly marked out. These now isolated islands of parenchyma correspond to the portal units of Mall. The terminal branches of duct, of artery, and of portal vein enter the isolated unit from a larger portal space which lies at its periphery. Also at the periphery of this portal unit defined by edema are the terminal hepatic (central) veins so that both portal spaces and terminal hepatic veins lie within the edematous tissue that now outlines the primary unit of parenchyma.

Cirrhosis Produced by Butter Yellow

The changes that lead to cirrhosis are first evident about the terminal branches of the hepatic veins (central veins) but later involve the portal space where fibrous stroma is more abundant than elsewhere. Cameron has shown that carbon tetrachloride which first causes necrosis—designated central in accordance with usual nomenclature, that is, about terminal hepatic veins—produces cirrhosis which begins in this location and ends in typical nodular cirrhosis, not infrequently but inexactly designated portal cirrhosis.

The relation between portal spaces, larger than those that penetrate into portal units, and the radicles of hepatic veins (central veins) well seen in the edematous liver of the rat, defines the path by which fibrous tissue and sprouting ducts find their way from the larger portal spaces to the radicles of the portal vein and explains why the lesion which begins with "central" atrophy and necrosis ends with new formation of fibrous tissue about both portal spaces and hepatic veins. Following administration of butter yellow congestion of capillaries and edema are sometimes seen in the vascular septa between portal units (Fig. 3).

A characteristic feature of nodular cirrhosis is parenchymatous hyperplasia which is a compensatory process following destruction of liver tissue. Hyperplasia with human or experimental cirrhosis evidently maintains no constant relation to the original architecture of the liver which becomes much distorted. The conventionally accepted liver lobule is further disturbed because the new bands of fibrous tissue following the path that has been described unite subterminal portal spaces and terminal hepatic veins (central veins).

When animals that have received butter yellow die within the first few months of its administration, liver cells about the radicles of the hepatic veins (central veins) are diminished in size and often form straight narrow columns. Atrophy has occurred and portal spaces are closer together. These animals have died from intoxication, body fat has almost completely disappeared, and atrophy of liver tissue is probably hastened by inanition. When pieces of liver are removed by operation from well nourished animals receiving butter yellow, fatty degeneration may be found in the parenchyma surrounding hepatic veins (central fatty degeneration). In other instances liver cells in the same situation have undergone disintegration and disappeared.

In animals that have received butter yellow the earliest changes suggesting cirrhosis occur about hepatic veins and their smallest branches. Disintegration and disppearance of liver cell columns is associated with the accumulation of mononuclear cells. The largest of these are mononuclear phagocytes (histiocytes) which, often containing hemosiderin, are concerned in removal of disintegrating liver cells. Lymphocytes, fibroblasts, and endothelial cells are seen in greater or less abundance. The latter, sometimes seen in double rows, evidently form new capillaries. Though fibroblasts are often abundant, col-

lagen fibrils are at first not increased, and for convenience the lesion in this stage will be designated "incipient cirrhosis." It may be found after 2 or 3 months of feeding with diets rich in protein and earlier with diets of rice. The changes are not limited to the neighborhood of the hepatic veins but, as already indicated, affect the structures in and about the portal spaces as well, and accumulating mononuclear cells and sprouting capillaries are seen here. The appearance of collagen fibers in the new connective tissue marks another stage in the progress of cirrhosis and for convenience cirrhosis not recognizable by gross examination but with new formation of collagen fibrils will be designated "early cirrhosis." Cirrhosis is first evident on gross examination when the surface of the liver becomes indented and nodules appear at first obscurely and later definitely defined. "Nodular cirrhosis" in these rats has the characters of typical nodular or Laennec's cirrhosis of human pathology.

New Formation of Bile Ducts

Coincident with the earliest stages of cirrhosis produced by butter yellow and characterized by localized disappearance of parenchymatous cells and the accumulation of mononuclear cells belonging to connective tissue, newly formed bile ducts make their appearance and soon may be recognizable wherever the new connective tissue is found. They are narrow tubular structures with low, approximately cubical epithelium and resemble closely the terminal bile ducts. They are seen about bile ducts that lie in the portal spaces at the periphery of the portal units, about hepatic veins, and their smallest branches (central veins), and in the intervening proliferating connective tissue. They are not, at least in the early stages of cirrhosis, found about the terminal bile ducts that with terminal branches of the portal vein and hepatic artery penetrate to the centers of the portal units.

When bile ducts appear immediately about hepatic veins and their terminal branches, usually designated central veins, it is obvious that they are newly formed. The question has long been discussed whether they sprout from preexisting ducts or are formed by atrophy of liver cell columns. If attention is focused upon the ducts at the periphery of the portal units it is evident in sections at appropriate levels that the new ducts sprout from them along the preexisting septa (Fig. 3) that lie midway between central veins as previously described, and decreasing in size reach the neighborhood of the hepatic veins (Fig. 4). The extent of new duct formation varies widely and is in some instances truly astonishing, for with butter yellow administration in wide areas of liver tissue, including many portal units, columns of parenchymatous cells may be almost completely replaced by newly formed bile ducts.

The new ducts are found in continuity with liver cell columns. They sprout in large number along new connective tissue in the direction of the corresponding defects in the parenchyma and presumably establish new outlets for the secretion of isolated columns of liver cells.

Hyperplasia of Liver Cells

A significant feature of the changes in the liver that follow administration of butter yellow is the occurrence of hyperplasia of hepatic cells. It is characterized by changes in the size, staining, and arrangement of cells, and may occur diffusely with no sharp demarcation, or in foci so that well defined nodules are formed. Hyperplasia may occur in the absence of cirrhosis or other change that has destroyed liver tissue, but is more conspicuous and widespread in association with nodular cirrhosis.

When butter yellow is administered with diets containing abundant protein and fat, with vitamins in adequate quantities, hyperplasia associated with little or no cirrhosis is found, occasionally 3 months after the beginning of butter yellow administration, but usually after 5 months. Focal hyperplasia is found in almost all animals that have lived from 6 to 8 months. Hyperplasia of liver parenchyma occurs earlier with diets that rapidly produce cirrhosis and is a characteristic feature of the nodular cirrhosis that ensues.

With hyperplasia, liver cells are considerably enlarged and the cytoplasm usually stains palely with acid dyes. The nuclei are large and though the nucleolus is conspicuous the nucleus is usually vesicular and pale. Hyperplasia occurs with no constant relation to the lobule. The transformation of liver cell columns into tubular structures with nuclei arranged in two rows near the margin of the columns is sometimes associated with hyperplasia. When these tubules are cut in cross-section a narrow central lumen may be seen.

In some places close to hyperplastic liver tissue are groups of cells equally large or larger and with large deeply stained nuclei. Such cells and their nuclei may reach great size, having diameters 2 or 3 times that of normal liver cells and the nucleus is occasionally lobulated. The cells with hyperchromatic nuclei seem to have undergone some injury during hyperplasia.

With focal hyperplasia the changes that have been described occur in sharply defined round areas in which swollen acidophile cells are in sharp contrast with the smaller faintly basophile cells of the surrounding parenchyma. Liver cell columns and the capillaries between them situated just outside of the focus may be compressed and distorted by its expansile growth.

Mitotic figures are seldom found in areas where cells are conspicuously acidophilic and it is probable that multiplication of cells is not proceeding actively. Mitotic division has perhaps ceased or is temporarily suspended. Mitotic division of nuclei is more likely to be found in cells with basophile than in those with acidophile cytoplasm, and is perhaps referable to the presence of ribonucleic acid in the cytoplasm (Caspersson and Schultz (14)). With transformation of liver cell columns into tubular structures the cytoplasm of the cells may be faintly basophile and mitotic figures may be found.

Though hyperplastic nodules are usually composed of swollen cells that stain palely with acid dyes, similar foci may be characterized by deep basophile

staining of the cytoplasm, best seen with the methylene blue of the Giemsa stain. Foci of basophile hyperplasia are usually composed of cells larger than those about them. Their nuclei are large and have a large deeply stained nucleolus. Broad columns in normal relation to the intervening capillaries are formed by cells with sharply defined boundaries. The cell column has sometimes assumed a tubular form with a small lumen. In contrast to nodules composed of cells with acidophile cytoplasm, these basophile nodules contain cells undergoing mitosis, usually in considerable number.

Hyperplasia of Newly Formed Ducts

Newly formed ducts as well as parenchymatous cells undergo hyperplasia. This is well seen in foci of hyperplasia of liver cells for when newly formed ducts are present between columns of liver cells in such foci, they take part in the process. They become much increased in size and their lumina are enlarged. Their cells become high and finally columnar with nuclei at their bases.

With advanced nodular cirrhosis produced after 4 or 5 months by butter yellow in combination with the rice-carrot or other diet favorable to the production of cirrhosis, hyperplasia of liver parenchyma is advanced and similar changes affect the newly formed bile ducts then present in great number (Fig. 4). They are transformed from narrow channels formed by low cubical cells into wide tubules with high columnar cells, and wide lumina. The change affects preexisting ducts of portal spaces and spreads outward from them so that in wide areas hyperplastic ducts and cellular fibrous tissue may replace the liver parenchyma.

Cystic Ducts

Cysts evidently formed by dilatation of small bile ducts may make their appearance as early as 3 months after the beginning of feeding with butter yellow and occur with increasing frequency in animals that have lived longer. In sections a group of small dilated channels (Fig. 5) with low cubical or even flat epithelium represents a tortuous dilated duct or a group of ducts cut many times by the plane of the section. In areas of varying extent these cystic ducts may replace more or less completely the parenchymatous cells of the liver and may form, often situated just below the surface of the liver, grossly recognizable cysts several millimeters or occasionally a centimeter in diameter. The groups of dilated ducts are not infrequently in contact with a portal space and below the liver capsule may extend as a triangular area from a portal space at its apex to the surface of the liver which forms its base.

The position of these cystic ducts in the interior of portal units and their number in some instances indicate that they are derived in great part at least not from normal bile ducts but from those that have been newly formed. Their contents is usually clear, there is no inflammatory reaction about them,

and no increase of fibrous stroma between them. It is not evident how new ducts are occluded so that cysts are formed. Columns of liver cells may retain their position in the interstices between the cystic ducts and the hyperplastic changes which these cells and the cells of the dilated ducts themselves undergo (Fig. 6) will be described later.

Cholangiofibrosis

The occurrence of isolated foci in which new bile ducts formed by a single layer of cubical epithelial cells have widely dilated lumina and are surrounded by dense fibrous tissue has been repeatedly observed. Orr gives a photograph of this lesion with the label "bile duct carcinoma" and Sigiura and Rhoads (5) designate it "bile duct adenomatosis." Edwards and White (6) do not regard the lesion as neoplastic and point out that with advanced fibrosis ducts may atrophy and disappear.

In the livers of rats that have ingested butter yellow administered in conjunction with diets that produce no nodular cirrhosis as well as with those that produce advanced nodular cirrhosis, a lesion that may be designated cholangiofibrosis (Fig. 7) often precedes the appearance of tumors. It occurs about newly formed ducts and is accompanied by localized new formation of fibrous tissue. Small white, sometimes pearl-white nodules, usually measuring 2 to 5 mm. in diameter, are seen within the liver tissue frequently below the surface, and smaller foci of similar structure are not infrequently recognized microscopically. They consist of dilated tubules within abundant fibrous stroma. The tubules resemble newly formed bile ducts but have low cubical epithelium and a wide lumen which is filled in part by homogeneous or streaked material. Their contents are evidently chemotactic for polymorphonuclear leucocytes which are present in many tubules in variable number. Leucocytes are usually found in the surrounding cellular fibrous stroma. The lesion that is designated cholangiofibrosis (Fig. 7) is characterized by: (1) dilatation of newly formed bile ducts which may contain mucus-like material; (2) inflammation with accumulation of granulocytes within the lumina of the ducts and in the interstitial tissue between them; (3) abundant formation of fibrous tissue.

A small focus of cholangiofibrosis may be found in contact with a portal space and the adjacent duct may be slightly dilated, but the mechanism of this duct obstruction is not evident. Accumulation of inflammatory exudate and mucus-like material in ducts may have part in its production. Newly formed dilated ducts presumably contain some irritant material that causes an inflammatory reaction followed promptly by new formation of fibrous tissue. This tissue is cellular at first and concentrically arranged about the ducts, but may later lose its cells and become hyaline immediately about the dilated ducts. In some instances the epithelial cells of the ducts may atrophy and disappear within dense fibrous stroma.

Tumors Produced by Butter Yellow

Tumors that are produced by butter yellow may be classified as follows: (1) Trabecular hepatomas, (2) adenohepatomas, (3) Cyst-adenomas, and (4) Cholangiomas. The occurrence of deaths with tumors at intervals during the administration of butter yellow is shown in Fig. 1 of a preceding paper (1).

These terms are in part descriptive of the histological characters of the lesions and in part indicate their origin. The term hepatoma has been applied to tumors composed in large part of cells resembling those of the parenchyma of the liver, whereas cholangiomas maintain the form of bile ducts. Cyst-adenomas are derived from the cystic ducts that have been described, and have been regarded as neoplasms only when atypical proliferation of epithelium of cystic ducts has been demonstrable.

It is probable that a large part of these tumors should be regarded as benign, because with few exceptions they are not evidently invasive and do not form metastases. As histological structure is an uncertain index of malignancy, tumors with metastases have been regarded as decisively malignant. They reproduce their distinctive characters in the metastases that are formed.

(*a*) Trabecular and adenomatous hepatomas arise from foci of hyperplasia of liver tissue; (*b*) cyst-adenomas are formed from cystic ducts, and; (*c*) cholangiomas arise from foci of cholangiofibrosis. Though these are their usual modes of origin, other possibilities cannot be excluded.

Tumors may reach considerable size with diameters from 3 to 6 cm. and occasionally two of these large tumors of the same or of different type may occur in the same liver. In most of the animals that survive during 4 months, and are allowed to die as a result of butter yellow administration, multiple tumors are present, and these vary in size from microscopic lesions to conspicuous macroscopic nodules. The continuity of tumor alveoli with surrounding liver cell columns, and the relation of the tumor to lesions that are precursors of tumors, indicate that the nodules are not metastases, but multiple growths. In each of 40 animals of Table IV (1), more than one tumor has been identified in the liver; in 12 instances, the multiple tumors of an animal have belonged to one of the four types enumerated above, but in 24 instances, two, and in 4 instances three different types have been recognized in the same liver.

A conspicuous feature of the tumors produced by butter yellow under conditions that prevent advanced cirrhosis (Table IV) has been their localization in the right half of the liver, and especially in the upper lappet of the right lobe. This observation is not in accord with that of Kinosita (15) who recorded the localization of hepatic tumors produced by butter yellow given with a rice-carrot diet, and did not find them more frequently in one lobe than in another. The liver of the rat consists of (*a*) a right lobe with upper and lower lappets, (*b*) a central lobe divided by the suspensory ligament and a cleft on its under surface into right and left lappets, (*c*) the caudate lobe with right and left lappets, and, (*d*) the large left lobe. The percentages of the

weight of the liver of these parts are approximately as follows: Right upper lappet, 17; right lower lappet, 8; right central lappet, 24; left central lappet, 10; caudate lobe, 9; left lobe, 32.

Of tumors with diameters from 10 to 66 mm. in animals of Table IV (1) 45 were in the right upper lappet which weighs less than a fifth of the organ; 5 in the right lower lappet; 13 in the right central lappet; 4 in the left central lappet; 2 in the caudate lobe, and 5 in the left lobe. The frequency of smaller macroscopic nodules was approximately the same as that of larger tumors.

It is probable that Kinosita (15) observed the position of tumors in animals given diets consisting chiefly of rice that produced cirrhosis. In animals of my experiments examined after 4 or 5 months of butter yellow administration, the location of tumors in animals with no nodular cirrhosis was as follows: right upper lappet, 6; right lower lappet, 1; right central lappet, 1; left central lappet, 0; caudate lobe, 0; left lobe, 0. In animals with nodular cirrhosis the location of tumors was: right upper lappet, 9; right lower lappet, 0; right central lappet, 11; left central lappet, 2; caudate lobe, 0; left lobe, 3. In the presence of nodular cirrhosis, the predominance of tumors in the right upper lappet has disappeared.

Trabecular Hepatomas

The simplest type of tumor produced by butter yellow is what may be designated trabecular hepatoma because it consists of regularly disposed anastomosing trabeculae of liver cells much broader than normal columns of liver cells and separated by capillaries or sinusoids similar to those of liver tissue (Figs. 8 to 10). The cells resembling liver cells have abundant cytoplasm which is usually acidophile and stains brightly with acid dyes such as eosin or phloxin, but may take with varying intensity a diffuse basophile stain. The tumor cells, like those of the liver, may undergo fatty degeneration and in wide areas may contain small fat droplets. In hepatomas with basophile stain (Fig. 10) mitotic division of cells is unusually active.

The gross appearance of these growths indicates that they are neoplasms for they are sharply defined, usually of yellowish white color, rounded in form, and often reaching a diameter of 2 or more cm. They occur in the absence of cirrhosis but when it is present they are readily distinguishable from hyperplastic nodules of liver parenchyma by their gray or yellowish gray color and usually by their greater size. In some of these hepatomas a part of the trabeculae are regularly tubular with cells on longitudinal section in two parallel rows and nuclei near the margins of the tubule. On cross-section a narrow lumen may be seen.

It is noteworthy that these changes though more advanced are similar to those that occur with focal hyperplasia of parenchymatous cells, and transitions from one to the other, characterized by increasing width and complexity of the anastomosing liver cell columns and local obliteration of architecture of the liver, are found. When a trabecular hepatoma occurs in one part of the liver, multiple foci of hyperplasia are usually present and a sharp separation of parenchymatous hyperplasia and neoplasm is not always possible. At-

tempts to transmit these tumors to other rats of the same inbred strain, or when removed in part by operation to grow them in the subcutaneous tissue of the animal that harbors them, are in progress.

Adenohepatomas

Adenohepatomas are in gross appearance indistinguishable from trabecular hepatomas but are in large part composed of structures that resemble glands, because cells are more or less regularly arranged about a lumen (Figs. 11 and 12). Their characteristic feature is the formation of these adenomatous alveoli by cells that are still recognizable as liver cells. Adenomatous and trabecular structures may be mingled in the tumor and in different parts one or the other may predominate. In some of these tumors are found columns of liver cells of tubular form like those seen with hyperplasia and they may be found in continuity with tumor alveoli. Broad trabeculae, much wider than normal columns of liver cells, may contain a lumen which is inconspicuous in one place and opens into a wide lumen elsewhere. These glandular alveoli may be formed by a single layer of cells or by superimposed cells and cells may be desquamated into the wide lumen that is sometimes formed. The tumor cells have abundant cytoplasm which, like that of liver cells, stains with acid dyes. The part projecting into the lumen is often rounded, but columnar cells in palisade-like formation may occur. With multiplication of cells, these adenomatous alveoli vary much in size and shape, as the result in part of infolding of the epithelium, and in part of localized proliferation of cells producing papillary ingrowths.

Adenohepatomas have varied in size, from small nodules about 1 mm. in diameter, only recognizable microscopically, to large oval tumor masses 4 cm. in long diameter. There are low bosses on the surface suggesting that the tumor consists of confluent nodules and multiple tumors of this type, of approximately the same size, are not infrequently found in different lobes of of the same liver.

Cyst-Adenoma

The cystic ducts that have been described consist of dilated tubules uniformly lined by low cubical epithelium (Fig. 5). The number of cystic ducts assembled in one spot and their individual size varies widely but usually there is no evidence that cellular proliferation has occurred. Nevertheless, in some instances proliferation of epithelial cells and new formation of cysts is so active and atypical that the lesion assumes the appearance of a neoplasm (Figs. 13 to 15). Cysts vary much in size and microscopically are seen to be filled by homogeneous material often containing red blood corpuscles in considerable number. The low cubical cells may become columnar with their nuclei at the bases of the cells. Proliferation may produce multiple layers of cells. It may be more active in some places than in others so that heaped up epithelial

cells may form papillary projections into the cavity of the cyst. Multiplication of epithelial cells within a circumscribed space may produce infolding of the epithelium and much irregularity in the shape of the cystic alveoli that make up the tumor. Proliferation of cells may occur upon the outer aspect of the walls of cysts and produce solid masses of epithelial cells. These cells resemble those that form the cysts and are small and cubical or polygonal.

Groups of cystic ducts are often found in the liver tissue surrounding a cyst-adenoma and may lie just outside of it. In the peripheral part of the tumor cysts may be lined by a single layer of cells, whereas in adjacent cysts, proliferative changes producing atypical epithelium may be in progress. In the part of a cyst wall next to the tumor proliferative changes may be in progress, though the outer aspect of the same cyst is formed by low cubical cells (Fig. 14).

Tumors with the characters that have been described contain numerous cysts recognizable by gross examination, and the tissue between the cysts is grayish-white. Lesions with the gross appearance of simple cysts may be found microscopically to have become neoplastic. Cyst-adenomas may be oval with a long diameter of 3 to 5 cm. but smaller nodules, occasionally two or three in the same liver, are more frequent.

In the interstices between the cysts of these tumors, liver parenchyma may persist and undergo hyperplasia (Figs. 14 and 15) similar to that which has been noted in some instances in association with cystic ducts that have assumed none of the characteristics of neoplasms (Fig. 6).

Cholangiomas

Cholangiomas (Figs. 16 to 18) are in large part composed of glandular alveoli with cubical, or even flat epithelium and wide lumina, resembling newly formed ducts that have undergone dilatation, and all transitions to the lesion that has been designated cholangiofibrosis are found. These tumors consist of gray white tissue and are firm in texture because dense fibrous stroma is abundant. In tumors from 10 to 15 mm. in diameter the surface is irregular or nodular and near its center may be indrawn or umbilicated.

Tumor alveoli vary widely in size and shape. The epithelium may be cubical on one side of an alveolus and columnar with basally placed nuclei on the other. The lumen may increase in size, and proliferation of cells one above the other may produce an epithelial lining of considerable thickness. With formation of cyst-like alveoli epithelium folds inward and papillae formed by local proliferation of cells may project into its cavity. Cells may multiply upon the external aspect of glandular or cyst-like structures and penetrate into the surrounding stroma, thus forming solid masses of cells (Fig. 18). These cells are small and polygonal and their cytoplasm takes a diffuse stain with basic dyes. Among them mitotic figures are readily found. Giant cells with lobed hyperchromatic nuclei or with several nuclei may be formed. A characteristic feature of the cells in solid masses is their tendency to rearrange themselves so that without other separation from adjacent cells they surround a circular space or lumen.

Tumors such as those just described with few exceptions contain areas with the characteristic features of cholangiofibrosis and these correspond to the depressed spots seen upon the surface of the tumor. Microscopically in these areas there are small dilated ducts, of which the number and relation to one another shows that they are newly formed, polymorphonuclear leucocytes and proliferation of fibrous tissue. In small tumors the transformation from cholangiofibrosis to neoplasm is especially evident. The epithelium on one side of a dilated duct may produce multiple layers of small polygonal cells with basophile cytoplasm. Dilated ducts with almost uniform structure in one place may assume much irregularity of size and shape in another. In some places cysts may be formed by dilatation of the lumen and papillae formed by proliferating epithelial cells may project into them. The solid masses of small polygonal cells with basophile cytoplasm and cells in mitotic division described above, are seen in some small tumors that still retain the characteristic features of cholangiofibrosis, and cells in these masses may have begun to rearrange themselves about well defined lumina.

Malignant Hepatomas and Cholangiomas

Focal hyperplasia, cystic ducts, and cholangiofibrosis are precursors of hepatic tumors produced by butter yellow. Though the latter are characterized by the gross and microscopic features of neoplasms, it is uncertain whether they have acquired the potentiality of indefinite autonomous growth, and analogy with the skin papillomata of carcinogenic hydrocarbons studied by Rous and Kidd (16) suggests the possibility that this property is lacking. It has seemed desirable to decisively identify as malignant growths produced by butter yellow only those accompanied by metastases and with the aid of the histological characters they exhibit to consider whether other tumors have acquired the potentiality of autonomous growth though metastases are not discoverable by procedures that are necessarily somewhat superficial.

In animals that have died or have been allowed to survive for at least 12 months of butter yellow administration (Table IV (1)), metastases have accompanied hepatomas in 10 instances (see Fig. 1 of preceding paper (1)). Hepatomas that are recognized as malignant because they are accompanied by metastases have been large soft gray-white or yellowish white tumors with hemorrhagic spots, and have varied in diameter from 30 to 60 mm. They have had their origin in the right lobe of the liver and usually in its upper lappet. The characters of liver cells are usually well preserved in broad trabeculae which are separated by capillaries or more or less widely dilated sinusoids, but in the tumors with metastases and in some with none there are homogeneous masses of small polygonal cells in which division is evidently proceeding rapidly for mitotic figures are readily found.

Metastases occur as rounded nodules 2 or 3 mm. in diameter, scattered often

in great abundance in the omentum or attached to the diaphragm. They are occasionally found at the hilum of the spleen, invading the organ. The histological structure of the metastases (Fig. 19) has been identical with that of the primary tumor and has usually been trabecular with, in places, solid masses of small polygonal cells dividing actively by mitosis.

In the group of experiments in which animals were allowed to survive up to a period of 12 months (Table IV (1)) adenocystomas in three instances formed metastases. The primary tumors in these animals have been 37 to 55 mm. in diameter, yellowish white in color, and have contained many small cysts. Metastases have repeated accurately the structure of the primary growth, and have consisted in large part of cysts with complex intracystic papillary growths, (Fig. 20).

Cholangiomas in animals that have died or have been killed after 12 months (Table IV) have been accompanied by metastases in 7 instances. The primary tumor has varied in diameter from 13 to 66 mm. and has usually arisen in the upper lappet of the right lobe. The tissue is soft and grayish or yellowish white with low nodules on the surface. Histological examination (Fig. 21) shows adenomatous alveoli like those of the primary tumor together with solid masses of small cells with numerous mitotic figures. In places within these solid masses, as in the parent growth, cells otherwise undifferentiated from those about them arrange themselves to form circular lumina.

DISCUSSION

Butter yellow, doubtless excreted by the liver, induces focal hyperplasia of liver cells which in some instances results in the production of single or multiple hepatomas. It produces tumor-like masses of considerable size, but until metastases are formed it is uncertain when they acquire the potentiality for autonomous growth independent of the causative agent. The appearance of collections of cells with scant differentiation, and the histological evidence of active mitotic division suggest that these tumors are recognizably malignant at an earlier period.

Administration of butter yellow produces multiple foci of focal hyperplasia, cystic ducts, and cholangiofibrosis, and corresponding with these lesions, which are precursors of tumor growth, multiple tumors are formed. Several large tumors of the same or of different types may result, and it is evident that the growth of one does not inhibit the growth of others. On the contrary, occlusion of ducts, such as occurs with cystic ducts or with cholangiofibrosis, seems to stimulate hyperplasia of liver cells and ultimately formation of trabecular hepatomas, for the latter not infrequently occur in association with cyst-adenomas and cholangiomas.

There is no satisfactory explanation of the gland-like or adenomatous arrangement of cells found in some hepatomas. These adenomatous alveoli may be continuous with trabeculae which more closely approximate the normal

columns of liver cells. The occurrence of tubular columns of liver cells in some adenohepatomas, the appearance of lumina in structures that otherwise resemble trabeculae, and the cystic dilatation of some alveoli suggest that obstruction to the escape of normal or abnormal secretion may have a part in the transformation of trabeculae into adenomatous alveoli. The presence of widely dilated duct-like structures at the periphery of the tumor may support this suggestion. The possibility that the cells of newly formed bile ducts that have undergone hyperplasia may assume the characteristics of liver cells cannot be excluded.

The formation of cyst-adenomas from cystic ducts can be traced through stages of less to those of greater complexity of structure, and lesions that are evidently neoplastic retain in places the appearance of cystic ducts. It is possible that the carcinogenic agent, perhaps formed by some chemical transformation of butter yellow, accumulates in the contents of the dilated ducts. Hyperplasia of liver cells in the interstices of groups of cystic ducts supports this possibility.

The formation of cholangiomas from their precursor, cholangiofibrosis, is readily followed, and in most of these tumors the characteristic changes of cholangiofibrosis, namely, duct-like structures lined by cubical epithelium, accumulation of leucocytes, and proliferation of stroma, are recognizable. The ducts contain some agent that causes acute inflammation and fibrosis and the resulting tumor, in contrast to the other tumors that have been described, is a scirrhous carcinoma.

The relation of focal hyperplasia, cystic ducts, or cholangiofibrosis to tumor growth, determines the characteristics of this growth, and produces tumors of distinctive types. In the trabecular hepatoma, the trabecular form of the normal liver cell column persists, and when the tumor becomes malignant, this structure is reproduced in metastases. Cyst-adenomas derived from cystic ducts, acquire a complex cystic structure and may reproduce it in metastases. The cholangioma is characterized by adenomatous alveoli of duct-like form, and by abundant stroma. In metastases from this tumor adenomatous alveoli are reproduced and in solid masses of poorly differentiated cells this tendency to form alveoli is shown by the grouping of cells about circular lumina (Fig. 21).

CONCLUSIONS

A conspicuous feature of the cirrhosis that is produced by butter yellow is the new formation of bile ducts that sprout from ducts of the larger portal spaces and penetrate between "portal units" to anastomose with liver cell columns at the periphery of these units.

Hyperplasia of the liver may occur with butter yellow administration in the absence of cirrhosis or other change associated with widespread destruction of liver tissue; it occurs as diffuse or focal lesions and affects both parenchymal cells and newly formed ducts.

Focal hyperplasia is a precursor of trabecular and adenomatous hepatomas which arise as multiple tumors.

Butter yellow causes localized cystic dilatation of newly formed bile ducts unaccompanied by acute or chronic inflammation and liver cells adjacent to them may undergo hyperplasia.

Cystic ducts by proliferation of their epithelium may form cyst-adenomas.

Butter yellow causes newly formed ducts to undergo dilatation, and with acute inflammation and fibrosis to produce circumscribed macroscopically recognizable lesions (cholangiofibrosis).

Cholangiofibrosis is the precursor of tumors that reproduce the histological characters of small bile ducts (cholangiomas), and often proceed to the formation of scirrhous carcinoma.

Hepatomas, cyst-adenomas, and cholangiomas may become malignant with formation of metastases in which the characteristics of the parent tumor are reproduced.

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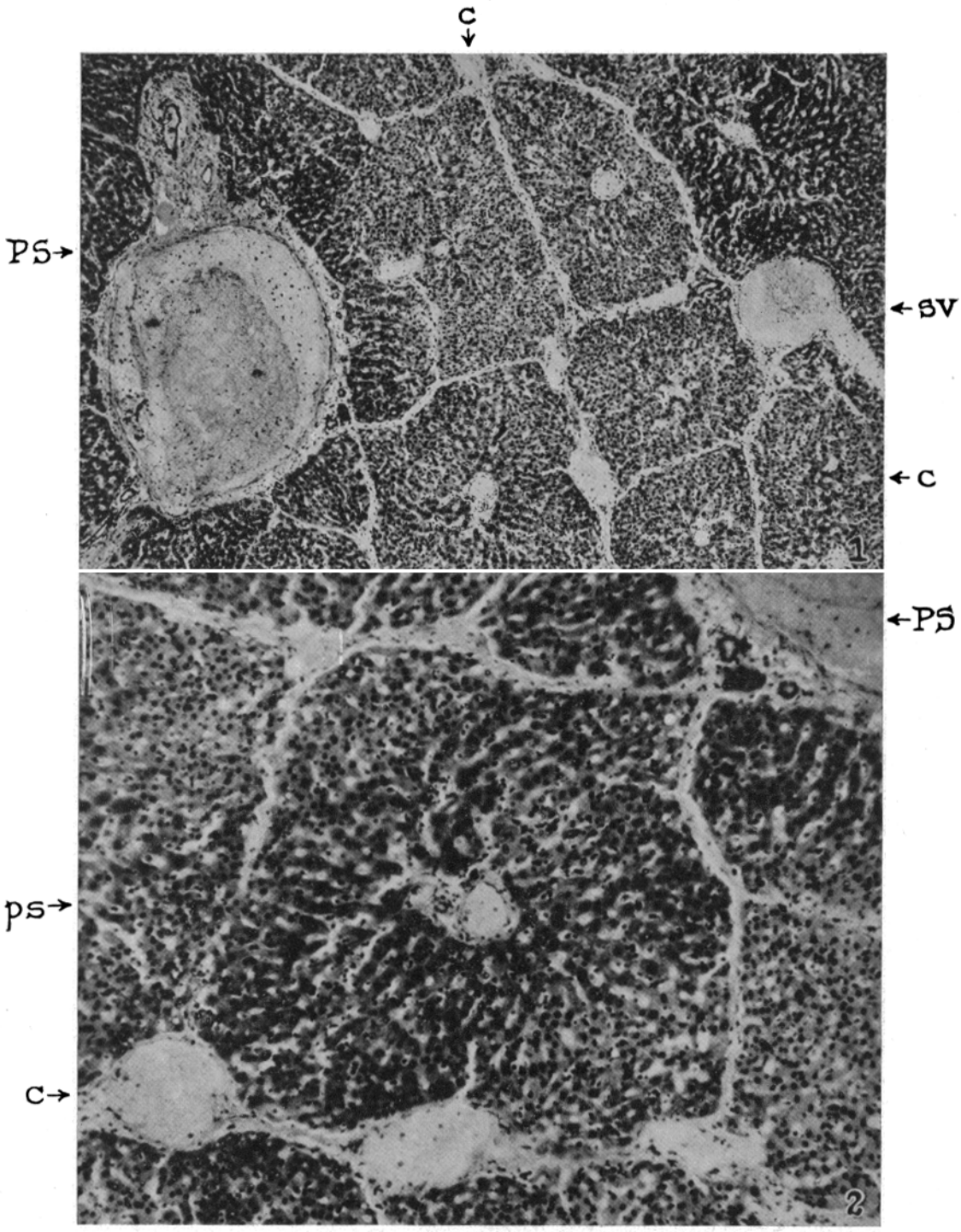
EXPLANATION OF PLATES

PLATE 17

These photographs were made by Mr. Joseph B. Haulenbeek.

FIG. 1. Showing separation of the portal units by interstitial edema. Edematous septa surround the portal units and unite a larger portal space (*PS*) with central (*c*) and sublobular veins (*sv*). Hematoxylin and eosin. × 57.

FIG. 2. Photograph with higher power showing a portal unit isolated by edema. A primary portal space (*ps*) with portal vein, hepatic artery, and terminal bile duct is in the center of the portal unit. Hematoxylin and eosin. × 145.

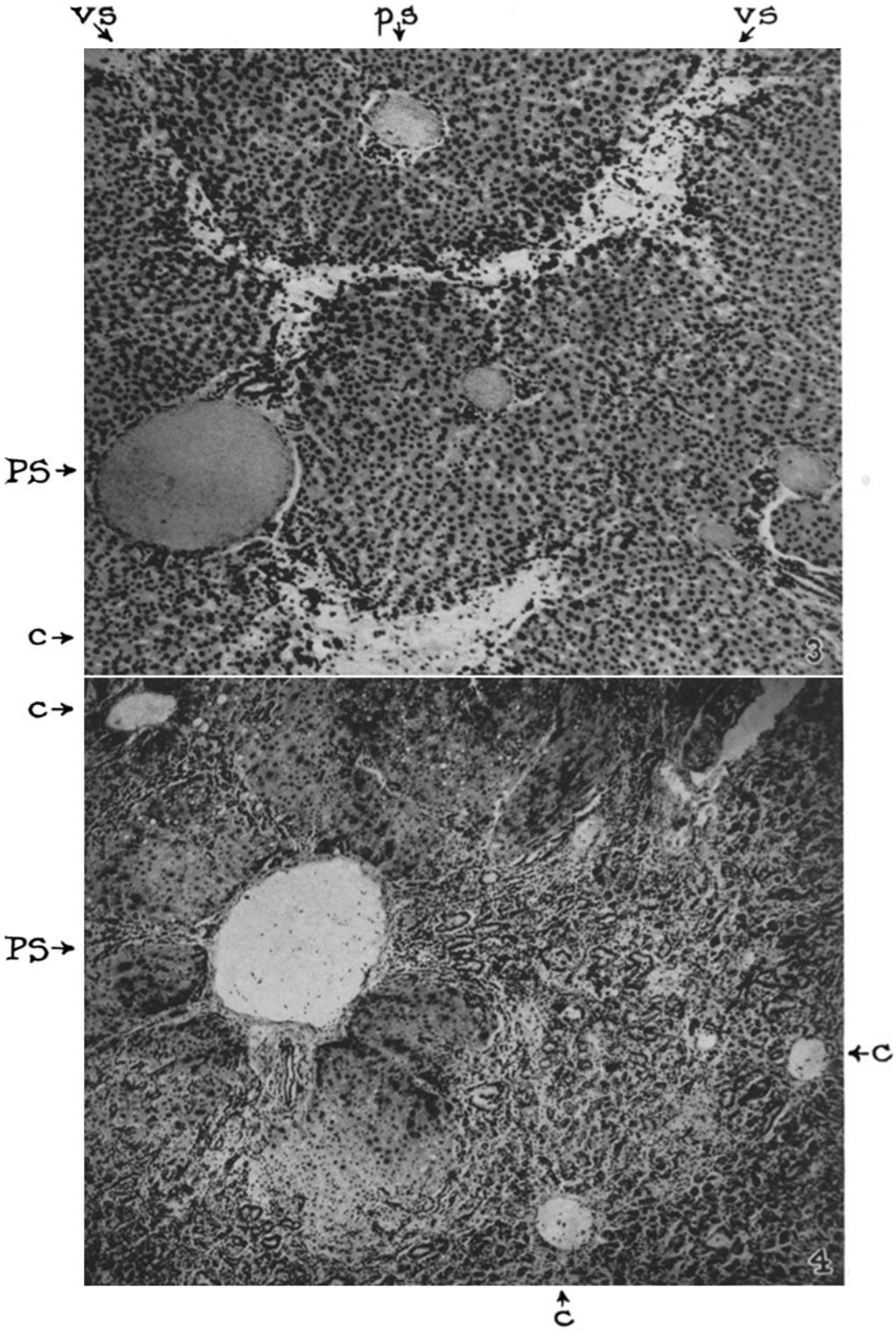


(Opie: Pathogenesis of tumors produced by butter yellow)

PLATE 18

FIG. 3. The liver of a rat which has received butter yellow with diet B during approximately 7 months. There is congestion of capillaries and edema in septa separating portal units at the sites of the vascular septa (*vs*). New bile ducts are sprouting from the larger portal spaces (*PS*) along the vascular septa. Hematoxylin and eosin. × 135.

FIG. 4. Newly formed bile ducts surrounding a large portal space (*PS*) and penetrating to the immediate neighborhood of central veins (*c*). Hematoxylin and eosin. × 68.

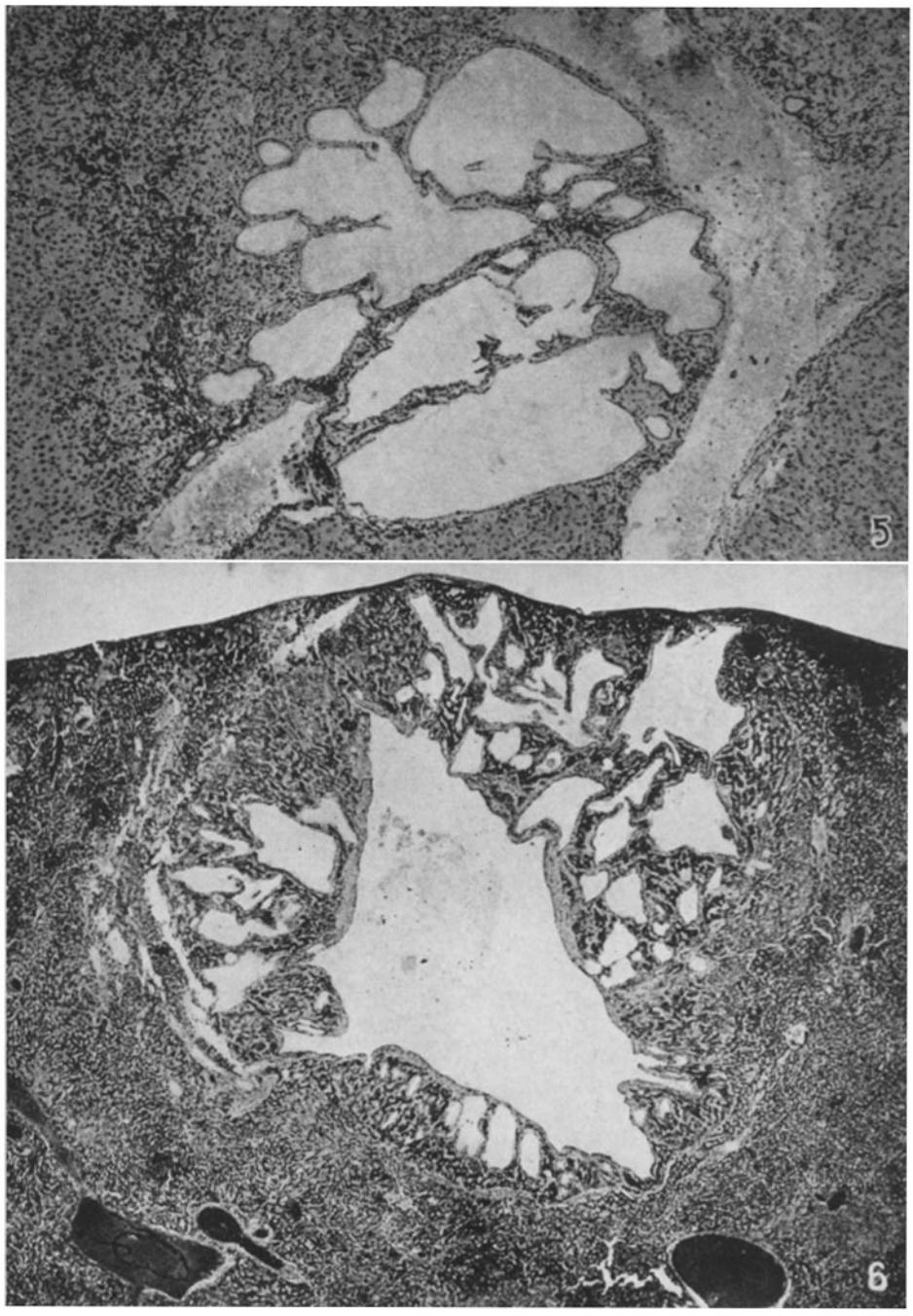


(Opie: Pathogenesis of tumors produced by butter yellow)

PLATE 19

FIG. 5. Cystic ducts in a rat which received butter yellow and diet E during 197 days. Hematoxylin and eosin. × 68.

FIG. 6. Cystic ducts with hyperplasia of immediately adjacent liver parenchyma. Although the small nodule compresses the surrounding tissue it does not show the atypical cellular proliferation of a neoplasm. Methylene blue and phloxine. × 30.



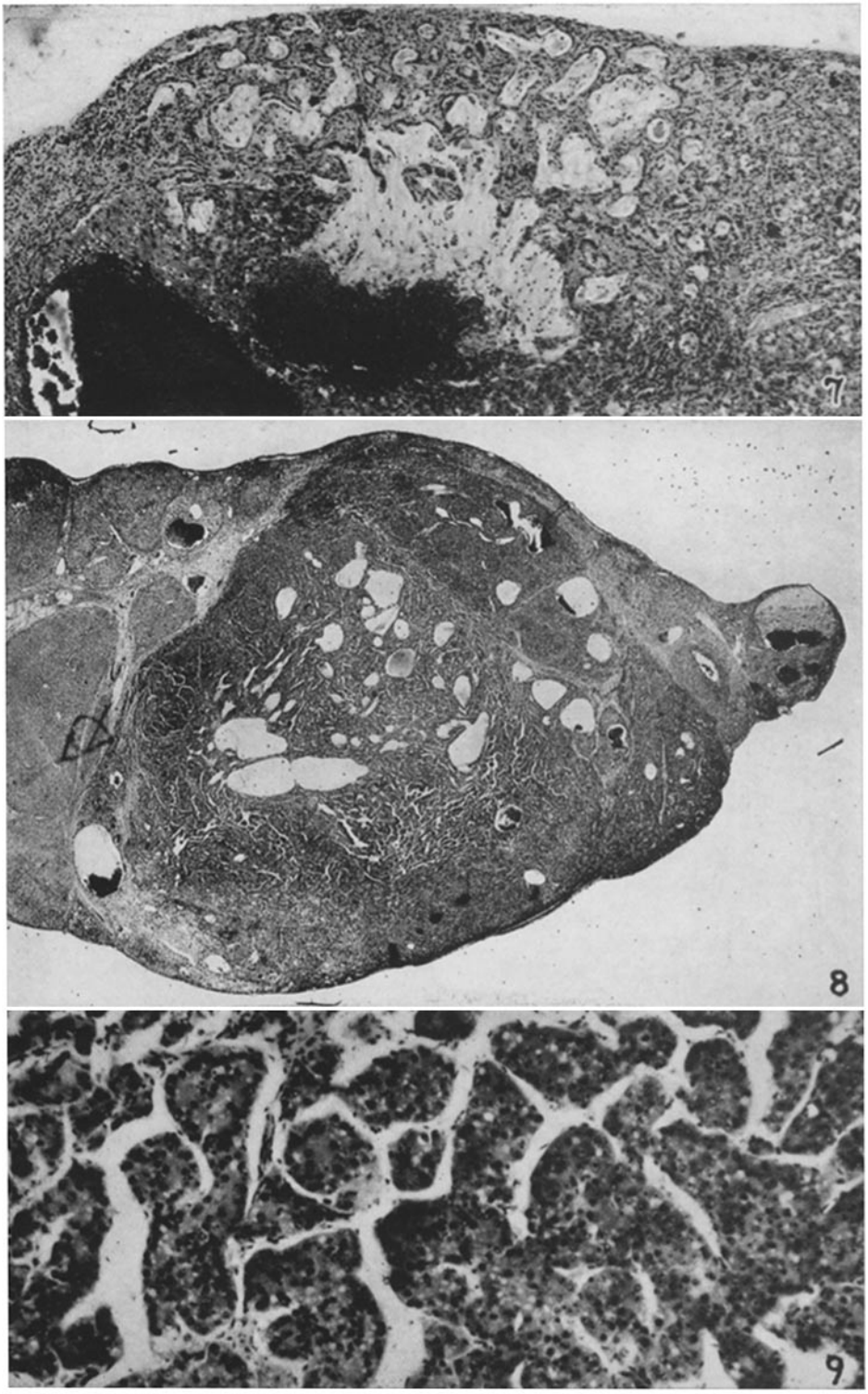
(Opie: Pathogenesis of tumors produced by butter yellow)

PLATE 20

FIG. 7. Cholangiofibrosis in the liver of a rat that has received butter yellow with diet M during 128 days is in the upper part of the figure. A widely dilated space near the center of the figure contains mucus-like material and hemorrhage has occurred into it (black in the photograph). Hematoxylin and eosin. × 75.

FIG. 8. Low power magnification of a small trabecular hepatoma in a rat that has received butter yellow and diet O for 162 days. There is advanced nodular cirrhosis. Giemsa. × 10.5.

FIG. 9. Photograph with high power of the tumor shown in Fig. 8. Giemsa. × 140.



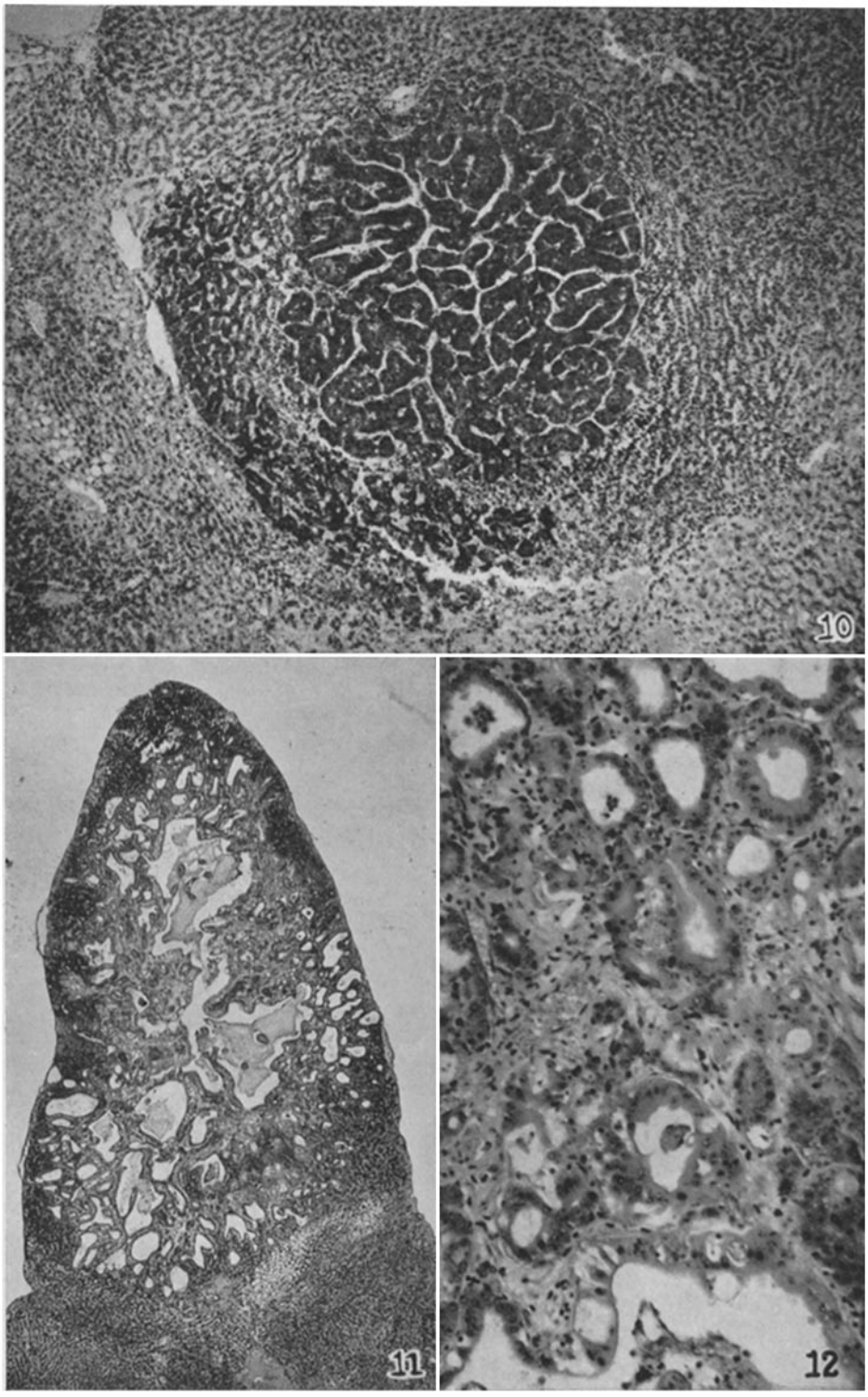
(Opie: Pathogenesis of tumors produced by butter yellow)

PLATE 21

FIG. 10. Trabecular hepatoma with basophile staining of cells in a rat that received butter yellow and diet B for 394 days. Mitoses are numerous in this tumor. Methylene blue and phloxine. × 6.5.

FIG. 11. Low power magnification of small adenohepatoma from rat on butter yellow and diet B for 396 days. Methylene blue and phloxine. × 22.

FIG. 12. High power magnification of the adenohepatoma shown in Fig. 11. Cells forming alveoli are large with abundant acidophile cytoplasm. Methylene blue and phloxine. × 140.



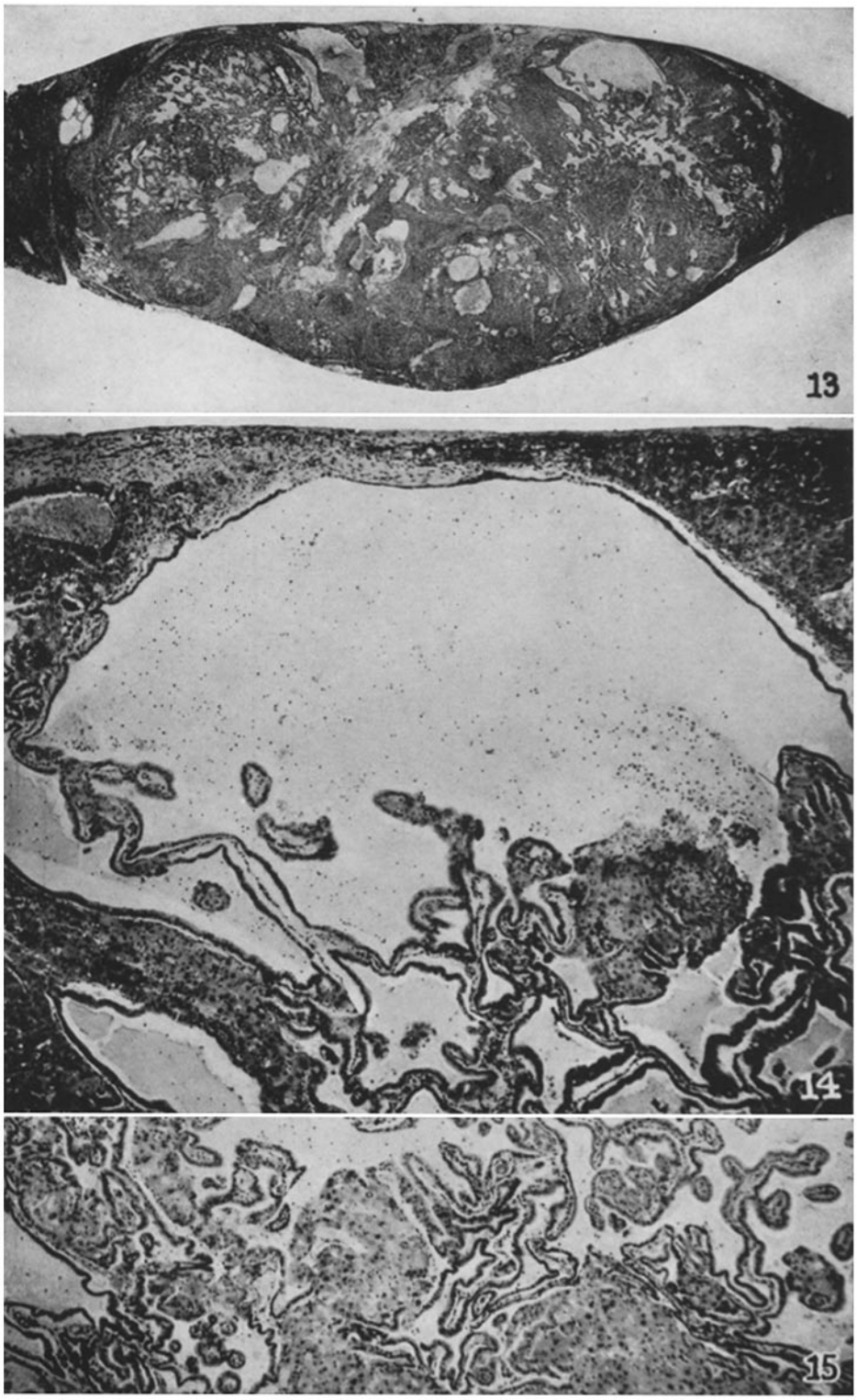
(Opie: Pathogenesis of tumors produced by butter yellow)

PLATE 22

FIG. 13. Photograph with low power magnification of cyst-adenoma from rat on butter yellow and diet D for 313 days. Methylene blue and phloxine. × 7.

FIG. 14. Higher magnification of the cyst-adenoma shown in Fig. 15. On one side of a cyst the epithelial lining is formed by low cubical and on the other side by high cubical or columnar cells. Here active proliferation of cells is shown by intracystic papillary projections. Methylene blue and phloxine. × 55.

FIG. 15. A second section of the same tumor with high magnification showing great variation in size and shape of both cells and tumor alveoli. Methylene blue and phloxine. × 55.



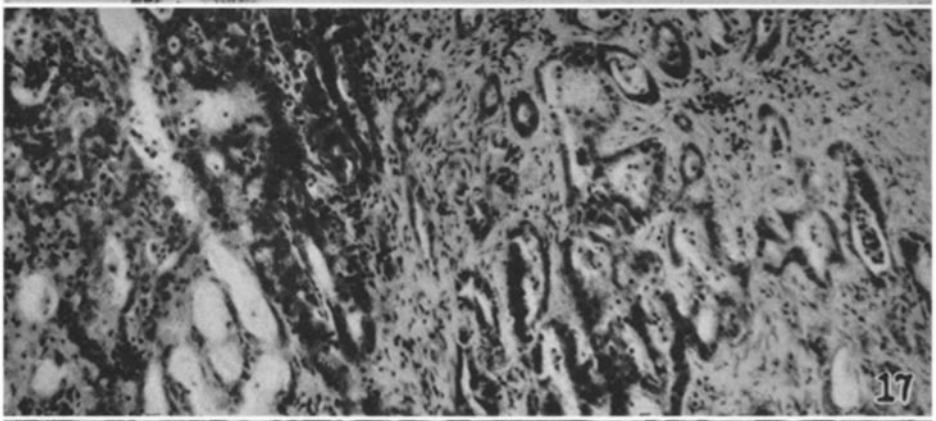
(Opie: Pathogenesis of tumors produced by butter yellow)

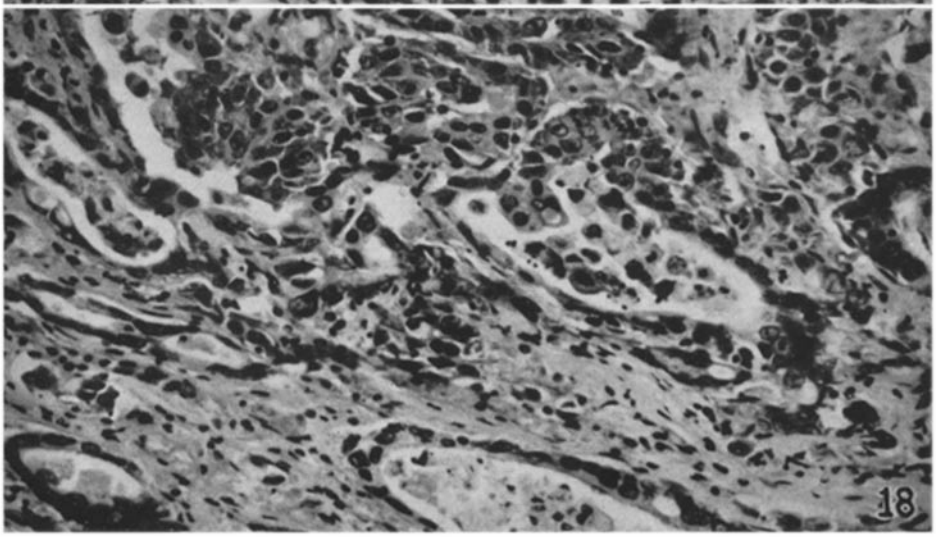
PLATE 23

FIG. 16. Low power magnification of a cholangioma showing umbilication upon its upper surface and dense fibrosis below the site of depression: from rat on butter yellow and diet F for 229 days. Methylene blue and phloxine. × 9

FIG. 17. Higher power magnification of the same tumor showing a part on the right in which the characters of cholangiofibrosis are still well preserved and another on the left in which neoplastic proliferation of cells has occurred. Methylene blue and phloxine. × 145.

FIG. 18. High power magnification of another cholangioma showing downgrowth of epithelial cells into stroma: from rat on butter yellow and diet M for 149 days. Methylene blue and phloxine. × 275.





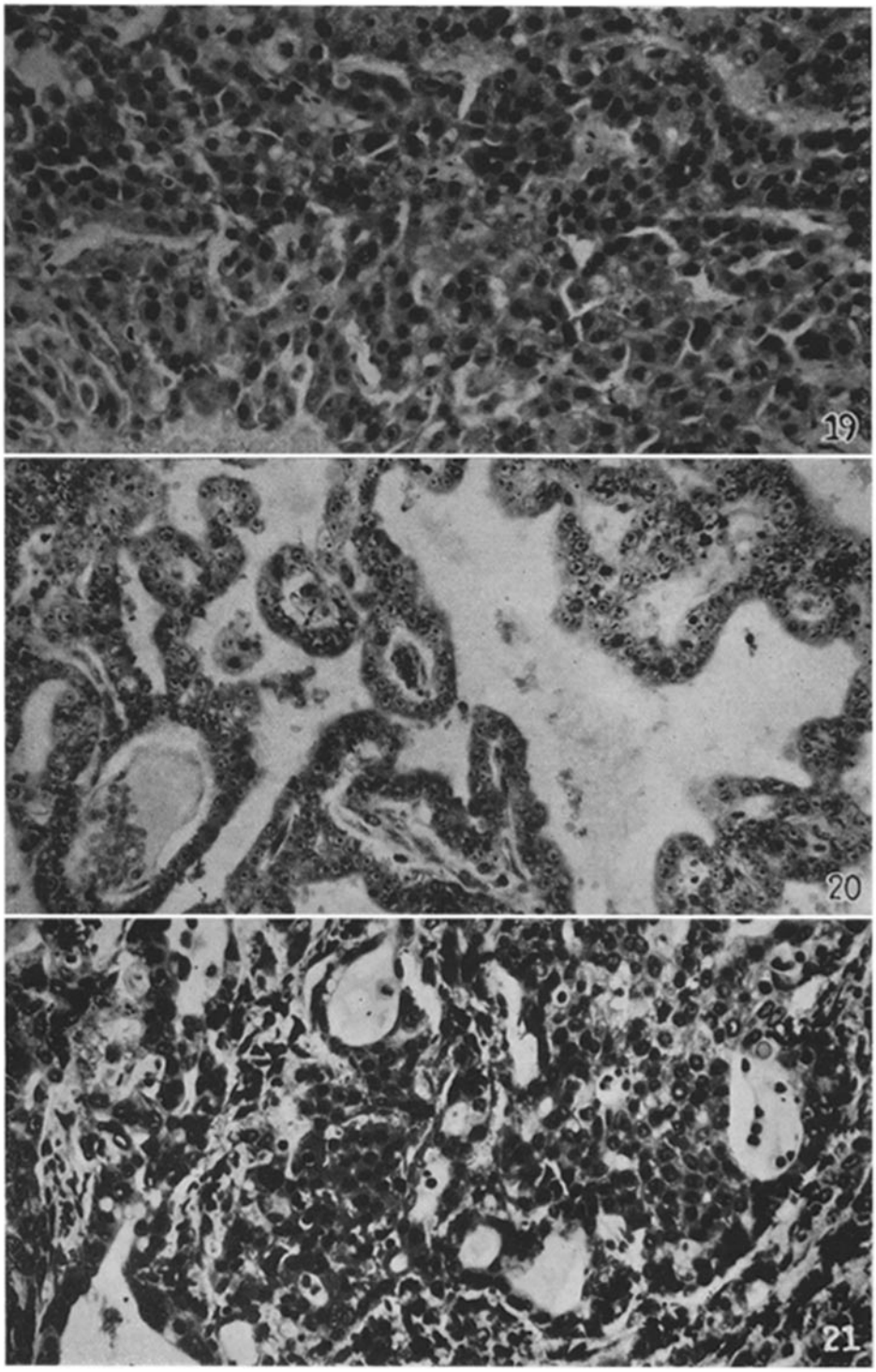
(Opie: Pathogenesis of tumors produced by butter yellow)

PLATE 24

FIG. 19. Metastasis of trabecular hepatoma to mesentery of spleen from rat on butter yellow and diet C for 316 days. Hematoxylin and eosin. × 300.

FIG. 20. Metastasis of cyst-adenoma to retroperitoneal tissue near kidney from rat on butter yellow and diet E for 382 days. Giemsa. × 300.

FIG. 21. Metastasis of cholangioma to surface of diaphragm from rat on butter yellow and diet C for 246 days. Adenomatous alveoli are formed in places, and elsewhere cells which surround a lumen are not differentiated from the cells adjacent to them. Methylene blue and phloxine. × 300.



(Opie: Pathogenesis of tumors produced by butter yellow)