THE HISTOLOGY OF LIVER TISSUE REGENERATION.[1]

By LINDSAY S. MILNE, M.D., M.R.C.P.E., *Assistant to the Professor of Pathology, University of Edinburgh.*

(PLATES XV.–XXVI.)

SINCE the time of Kiernan in 1833, and following his description of the appearances of the structure of the liver as adopted by Johannes Müller, Wepfer, Malpighi, and Ferrein, a more or less uniform terminology has been generally applied to the structural unit of the liver. Thus in references in literature relating to the histology of the liver the nomenclature is based on the idea of the portal space being the periphery of the liver lobule, and that the liver cell trabeculæ radiate round a central vein which leads into the hepatic vein circulation. The thickness of this mantle of liver cells surrounding the central vein is usually reckoned as measuring about 0·6 mm. Sabourin described the liver as being composed of biliary lobules arranged round what he called the portobiliary nodule, and having the terminal bile ducts as their centre. MacCallum described it as a secreting gland having, as in other glands, the excretory duct in the centre and the vascular supply and supporting framework radiating from this point. In spite, however, of this variation, one must agree with Franklin Mall (1906 [54]), who writes in his work on the "Structural Unit of the Liver," that "although probably this latter view is correct, yet the older terminology has become so fixed in literature that it cannot now easily become discarded."

The liver cells appear to radiate out from the central vein as a series of trabeculæ composed of double rows of cells in close apposition, each column being separated by a vascular channel. In the centre of the trabecula runs a minute bile-collecting canaliculus, which is lined by an extremely fine flattened epithelium, and branches of which seem to extend round the individual liver cells. This duct is continued, from the termination of the trabecula, into the portal space, and finds its way into the interlobular bile duct.

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In some lower animals, such as amphibians, this canal, joining the summit of a liver-cell trabecula to an interlobular bile duct, is lined by a cubical epithelium, and its junction with the liver-cell column is sometimes gradual in its transition in type of cell. To some extent a similar appearance may be seen in portions of the liver of some of the higher animals, as, for instance, sometimes in rabbits, but in the healthy liver of man this intermediate portion, as I may call it, is lined only by an extremely delicate flattened epithelium. In children, owing to the growth of the liver, this particular portion of the bile excretory apparatus is much more branched than in the adult, where, the growth of the organ being stationary, these fine channels in the portal spaces tend to pursue a more direct course. This last point, as will be shown later, is of some considerable importance in explaining the much greater frequency in young subjects of the apparently new "bile-duct-like" structures which ramify amongst connective tissue developments in the liver.

The subject of regeneration of the liver parenchyma is one which, if one may judge by the wide extent and extremely conflicting nature of its literature, has occupied a somewhat important position in the sphere of pathological investigation.

Cruveilhier (1833 [17]) and Andral in 1834 probably first introduced the idea that regeneration of the liver tissue was a possibility. The majority of observers since that time have more or less accepted this, but have diverged greatly in their conceptions as to its method of production. Several authorities, such as Albers, Weismann, etc., have been unconvinced of any regenerative capacity in the liver; while others again agree with Aschoff, who considers that exact re-formation of structure is never marked, and least of all in the glandular organs.

Compensatory Regeneration of the Liver Parenchyma.

A demonstration of the fact that regeneration of the liver parenchyma does occur, and also of the methods by which it is accomplished, is to be obtained with perhaps greatest simplicity in those pathological states of the liver where there has been a very widespread rapid destruction of the organ, such as occurs in acute liver atrophy, and especially in those cases which survive into a subacute or chronic stage. In the study of the more acute varieties of the disease the most striking change is the liver-cell destruction, whereas in the later stages repair in the shape of connective tissue formation and liver-cell multiplication are most evident.

Along with Dr. Stuart M'Donald, in the present volume of this Journal (p. 161), I have brought forward in detail several cases of this condition, and have illustrated the features relating to liver-cell regeneration in each, and I need not here make more than passing mention of them. In subacute liver atrophy there are generally irregular nodules, often of a greenish-yellow colour, elevated above the surrounding liver

surface, and also apparent as masses in the substance of the organ. These areas are composed of liver cells which have escaped the destruction of the greater part of the liver, and may be observed to be in very evident multiplication. Some are small and closely packed together as if recently formed, others are very large and sometimes highly multinucleated (Plate XV. Fig. 1).

Indirect division of the liver cells by karyomitosis is occasionally observable, but not altogether common, whilst direct division of the liver cells is of extreme frequency. All stages of this direct division can easily be demonstrated. Some nuclei are extremely large, while others have become constricted in their central zone, and still others can be seen dividing into two distinct nuclei. This division often leads up to the development of a multinucleated cell, yet division of the cytoplasm of the cell is also a distinguishable feature. Even granting that karyomitosis could occur with great rapidity in this process, there is no evidence that these two apparently distinct methods of liver-cell division are combined as different stages in the same process. As a result of the proliferation of liver cells which occurs in this disease, the liver lobules in the preserved liver-cell areas have largely lost their trabecular structure and have become greatly enlarged and somewhat irregular. These cases, then, would illustrate a strenuous effort on the part of the spared liver tissue to reconstruct, by means of a hypertrophy and hyperplasia of the surviving liver cells, a sufficiency of functioning liver parenchyma for the economy of the individual.

Compensatory growth of the liver parenchyma is also to be noted in a variety of other diseases. Several authors have drawn attention to the gross modification of the shape of the liver which has become evident when a part has been destroyed by echinococcus cysts. Kahn ([43]) described such a condition, and drew attention to a diagram in Marchiafava's atlas which showed the left lobe of the liver atrophied, the right somewhat reduced in size, and the Spigelian lobe enormous. Josias, Reboul, and Vaquez ([71]) described almost similar cases as a result of suppurative hydatid cysts. Schorr ([81]), Frerichs ([29]), Hollefeld ([40]), Kretz ([48]), Reinecke ([72]), and others have shown that syphilitic obliteration of the vessels going to one lobe of the liver leads to atrophy of the corresponding lobe, and that a corresponding compensatory enlargement of the other occurs. Beneke ([8]), in a case of diffuse syphilis of the liver in a child $2\frac{1}{2}$ years of age, found an enormous enlargement of the Spigelian lobe, but was not able to decide whether he was dealing with local giant growth or with a compensatory hypertrophy following on atrophy of other areas.

Yamagiva ([91]) noted a great enlargement of the Spigelian lobe in a case of chronic venous congestion. I have seen several cases of this sort. One occurred in a woman, æt. 56 years, who had died suddenly from cerebral hæmorrhage, and with no obtainable previous history. The liver was very small, oval in shape, and with its left lobe atrophied into a fibrous band. In this case a syphilitic affection of the vessels at the root of the left lobe had led to their obliteration and

the consequent atrophy of the lobe they supplied. The microscopical examination of the right lobe showed the individual lobules to be larger than normal, but retaining a definite trabecular arrangement of the liver cells. Another case of much the same character occurred in a woman, æt. 74 years, who had died from mitral stenosis with heart failure. All the organs, including the liver, showed very evident chronic venous congestion. The liver lay completely to the right of the middle line of the abdomen, and reached down to the level of the umbilicus, measuring 11 in. in its vertical diameter. Its lower border was thick and rounded and about 2 in. thick. The Spigelian lobe was also very large. The left lobe had atrophied into a fibrous mass, again probably as a result of syphilitic process obstructing the branches of the hepatic artery and portal vein at the root of the left lobe (Plate XXV. Fig. 34). On microscopic examination of this case the liver lobules looked enormous,—there was, however, a high degree of chronic venous congestion. In connection with the production of such cases as these it is necessary, if one reasons from animal experiments, that both portal vein and hepatic artery should be obstructed before similar appearances of complete atrophy are produced. In the animals I used for the purpose of experiment—cats and rabbits—ligature of the branch of the hepatic artery going to one lobe of the liver had no effect at all, and ligature of the same branch of the portal vein caused necrosis of the corresponding portion of the liver, sparing, however, in almost every lobule an irregular ring of liver cells round the periphery. The lobe affected shrunk considerably, but never underwent total necrosis, as occurred when both vessels were obstructed.

In connection with irregularities in the shape of the liver resulting from compensatory development of new liver tissue, one has also to point to some of the fantastic shapes assumed in some cases of cirrhosis of the liver where certain less damaged portions of the organ become greatly enlarged (Plate XXV. Fig. 31).

Regeneration from Liver Cells directly.

With the aid of the microscope, areas of multiplication of the liver cells have been recognised by many observers.

Kelsch and Kiener (1876) noted them in a case of malarial cirrhosis, and Sabourin (1880) very clearly pointed out hyperplastic foci of liver cells in a tubercular case. More recently similar hyperplastic areas have been remarked in subacute liver atrophy (Meder, M'Phedran and M'Callum, Adler Steinhaus, Barbacci, Stroebe, W. G. MacCallum, Yamasaki, Marchand, Ali Bey Ibrahim, Schlichthorst, von Kahlden, etc.), also in cases of cancer of the liver, and particularly in relation to the question of hyperplasia initiating cancer (Kretz, Wegelin, Witwicky, Paltauf, Travis, Fabris, Fabyan, Rolleston, Muir, Simmonds, Sabourin, Necker, von Hansemann, Perls, Schüppel, Lubarsch, Orth, Löhlein, Lehne, Heukelom, Weigert, Oertel, Rohwetter, etc., and especially Schmieden).

There are many affections of the liver, particularly where the volume of highly functioning tissue has become reduced, in which, microscopically, evidences of regeneration of new liver cells can be demonstrated. One of the commonest of these is chronic venous congestion, in which disease there tends to be considerable destruction of the liver cells in the neighbourhood of the hepatic vein circulation. In the outer parts of the liver lobule hyperplastic masses of liver cells are frequently observed. These areas are generally composed of large or somewhat irregularly sized liver cells closely packed together, and often presenting double nuclei (Plate XXIII. Fig. 24). I have frequently seen similar hyperplastic nodules in livers which have been extensively infiltrated with cancer, and also where there has been a widespread development of tubercles, cysts, and abscesses. They can generally be extremely well seen in cases of subacute liver atrophy and in common cirrhosis, and I found them particularly evident in two cases of primary liver-cell cancer (Plate XXIII. Fig. 25).

Before the time karyomitosis was described, liver cells had been considered to proliferate by amitotic, direct division.

Tillmanns (1875) saw karyokinetic figures in liver cells near scars, and considered them to be in a process of regeneration. Ziegler and Obolonsky (1887), in experiments with phosphorus poisoning, noticed a slight attempt at formation of new liver cells, and several times observed karyokinetic figures in liver cells. Bizzozero and Vassale (1887) noted occasional mitotic figures in liver cells up to adult age in animals, and the general opinion of such authors as have observed this is, that it is a feature in the process of replacement of physiologically destroyed liver cells. Reineke and Holm (1898) believe that although karyokinesis is the usual method of proliferation of a liver cell, yet in many pathological conditions amitosis is also in evidence. Balbiani and Henneguy (1898) have described multiplication of liver cells by karyokinesis and amitosis in the same specimen. Adler (1904) has also admitted the occurrence of amitotic division as well as karyokinesis of the liver cells in cases of eclampsia and phosphorus poisoning. Flemming (1893) remarked that amitosis is an attempt at regeneration, which in mammals at any rate did not lead to a partition of cells, but he admits that this point remains to be settled.

In the careful examination of many livers which have been the seat of acute degenerative processes, evidences of new formation of liver cells are to be found frequently. Occasionally karyomitosis is to be observed in the liver cells, but appearances of direct division are extremely common. The nucleus first seems to become extremely large, and then to become constricted laterally into a dumb-bell shape, and eventually to divide into two. Division of the cytoplasm is not quite so easy to observe; it is, however, a distinguishable feature.

In a case of lymphatic leukæmia I have noted very extensive regenerative changes in the liver. Many of the liver cells were in process of destruction, yet a considerable number also showed

karyokinetic figures (Plate XXIII. Fig. 23). Appearances of direct division of the liver cells were very frequent (Plate XXIII. Fig. 22). A liver which was the seat of numerous lymphadenoma nodules showed abundant evidence of liver-cell multiplication. Round the lymphadenoma foci the liver cells collected in hyperplastic masses which contained some very large hypertrophic cells, commonly with two nuclei and also clumps of smaller darker-staining single nucleated liver cells. Besides very numerous evidences of direct cellular division there were a very considerable number of liver cells in karyomitosis. As many as six liver cells in a state of karyomitosis could be observed in one instance in one high-power microscopic field (Plate XXIII. Fig. 21). I have also observed karyokinesis in liver cells, occurring with some frequency in a case of subacute liver atrophy, and also in the hyperplastic nodules of liver cells which were commonly to be observed in a case of primary liver-cell cancer, with no accompanying cirrhosis, in a man, æt. 68 years.

Although karyokinesis is then a method of liver-cell multiplication, direct division of the nucleus apparently would appear to be the usual procedure. Thus it would seem that in these above-mentioned cases the development of new liver cells could be traced as a multiplication directly from pre-existing liver cells, and as, presumably, being developed by a compensatory process following on reduction in the volume of the liver parenchyma.

The Origin and Function of the "Bile Ducts" which appear in Pathological Developments of Fibrous Tissue in the Liver.

Besides the origin of new liver cells which has already been discussed, another source has been very largely advocated in the literature of liver regeneration. In interstitial-tissue developments in the liver small "bile duct-like structures" are often to be observed ramifying amongst the new tissue. These "ducts" have been credited with considerable importance, as they have been looked on as the means by which the damaged liver reconstructs new tissue, and the question of their origin, function, and future has led to a most perplexing amount of controversy.

In subacute liver atrophy the islets of hyperplastic liver cells are separated by fibrous tissue which has replaced the necrotic liver parenchyma. In these areas the liver cells have disappeared, and their place is at first taken by distended capillaries. Soon, however, the lobule collapses, and fibrous tissue extends in from the region of the portal spaces. This new fibrous tissue is permeated by ramifying ducts with a very definite and regular structure. These can readily be distinguished from the old interlobular bile ducts, which often can still be recognised by their smaller size, and by the fact that their lining cells stain more deeply and give a somewhat more marked eosinophil

reaction in their cytoplasm. If the lobular structure of the liver is still observable in these areas one can notice that these apparently new ducts are most evident some little distance out from the old interlobular duct, and often occupy positions which were previously the site of liver cells in the periphery of a lobule (Plate XXVII. Fig. 2). Sometimes the lumen of these tubes is continuous with that of the interlobular bile duct, although frequently they have only a comparatively attenuated type of epithelium before they join it (Plate XVIII. Fig. 4). This appearance is more easily observed when serial sections are examined. Sometimes, particularly at their free ends, the lining cells of these ducts multiply locally into small nodes (Plate XVIII. Fig. 4). These "ducts" can also sometimes be observed to join on to a liver-cell column at the edge of a liver-cell islet, and the lumen is noticed to be continuous with the bile canaliculus in the interior of the trabecula. In none of the cases of subacute liver atrophy I have studied was there any evidence that these ducts were atrophic or degenerate liver cells, nor were there any appearances to suggest that either the liver cells or bile ducts had proliferated to form them. Also, although their lining cells did occasionally multiply into little nodes there never was reproduced any cell with any resemblance to a liver cell.

The diversity of opinion as to the origin of these ducts is somewhat interesting.

Wagner (1862) first described them, and he also noted that they often communicated with columns of atrophied liver cells, but not with interlobular bile ducts. He looked upon them as belonging to ramifications of the hepatic artery, but as he could not inject them from this source he admitted the possibility of their being atrophied liver cells or branches of the portal vein. Liebermeister (1864) identified them as pre-existing formations derived chiefly from vessels, and also to some extent from a proliferation of connective-tissue cells. With Rokitansky ([75]), however, he believed them to be chiefly formed from branches of the portal vein. Eppinger (1875) viewed them as proliferations of connective tissue, as they were especially obvious near the interlobular veins. Cohnheim (1876) decided that the white blood corpuscles were their progenitors. Schmidt (1880 [80]) expressed still another view, that they arose from the lymphatics. This is a mistake which is quite easily understood, especially if the idea of their origin is based on injection experiments; as it is now well known what extremely intimate relationships the small branches of the portal vein, the bile ducts, and the lymphatics have in the portal spaces [Herring and Simpson (1906 [38]) and Franklin Mall (1906)]. Schaper and Cohen (1905) advance as their view, that the possibilities of regeneration in glandular organs depends on certain cell complexes which, even in the adult, remain undifferentiated. It is these which are responsible, when the necessity arises, for the formation in the liver of the delicate new ducts which are capable of metamorphosis into functioning liver cells.

The majority of authors have described these "ducts" as arising from the interlobular bile ducts or from the liver cell columns, either in consequence of atrophy or of an actual sprouting process.

That they were simply due to the collapse of the liver structure and a

resulting crowding together of interlobular bile ducts has been advocated by Hlava (1882), M'Phedran and M'Callum (1884), Kronig (1887), van Haren Noman, etc. The suggestion that they were developed as sprouts from the bile duct was supported by Zenker (1872), Lewitsky and Brodowsky (1877), Dinkler (1887), Manglesdorf (1882), Hedenius (1884), Hirschberg (1886), Orth (1887), Schaper (1889), Meder (1895), Janowski (1892), Yamasaki (1903), Marchand (1905), Millar (1908), etc. Thierfelder (1874) and several others have recognised them as bile ducts both physically aggregated together and actually newly formed.

Cornil (1871 [15]), Friedländer (1877), and Ackermann (1880) all arrived at much the same view, that the liver cells atrophy, and the duct becoming embedded in embryonal tissue gets stretched and lined by epithelial cells which grow down from the adjacent interlobular bile ducts. Hyami (1906 [41]) gave some support to this conclusion, as his idea was that the sprout from the bile duct grew down along the line of the atrophied liver column. This latter view has received considerable confirmation in the work of a large number of authors. MacCallum was able to observe the widening out of the basilar membrane of the bile duct, and through it came the newly forming bud. Kretz (1894, 1900, 1902 [4]), Hyami (1897), Marchand (1892,) Janowski (1892 [42]), Charcot and Gombault (1876), etc., have all observed these "ducts" closely connected with both liver-cell trabeculæ and interlobular bile ducts.

Another school of observers has decided that the "ducts" are simply atrophic liver cells. Perls, Brieger, Heukelom, Aschoff, Orth, Posner, Klebs, Goodhardt, Janson, Rolleston and Marchand have endorsed this.

Finally, the ducts have been considered to be the result of an active proliferation of the liver cells. Kelsch and Kiener (1876), Schmidt (1880), Wannebroucq and Kelsch (1880), Barbacci (1901), Ribbert (1904), and Melchior (1907) noted this in their observations. Hanot and Gastou considered that where bile was not being excreted the usual antiseptic action was in abeyance, and poisonous and irritating toxins from the intestine initiated the proliferation of liver cells, such proliferation appearing in the form of "ducts." Hess (1900), discussing the appearance of "ducts" in rupture of the liver, concludes that they grow mainly from the liver cells, but also to some extent from the bile ducts. Muir (1908 [60]), in cases of rupture of the liver, also states that they arise from a proliferation of liver cells at the edge of the wound.

These "ducts," in whatever way they have been produced, have also been credited with the ability to develop into liver cells. Waldeyer (1868) was the first to suggest that these ducts, which he observed in a case of acute yellow atrophy, were developed as an attempt at liver-cell regeneration through the medium of bile-duct epithelium proliferation. In the same year also, Klebs ([46]) described peculiar cell tubes in a case of "rothen Atrophie," which he regarded as derived chiefly from included liver cells, although they looked very like gall ducts. He admitted that a re-formation of liver tissue was accomplished both from an activity of liver cells and through the medium of the gall ducts. He observed basket cells (Korbzellen) at the termination of some of the "ducts," and considered them as regenerated liver cells. They were probably, however, only old fatty liver cells. Zenker (1872), Hedenius (1884), Hirschberg (1886), Orth (1887 [64]), Mangelsdorf (1882), Schaper (1889), Schlichthorst (1897), Stroebe (1897), Rolleston (1905), Dreschfeld (1881), Hanot (1895), Melchior (1907), all considered the "ducts" as being potentially able to reproduce liver cells. Meder (1905) concludes by stating that in regeneration of liver cells the bile ducts are of much greater importance than the liver cells directly. Steinhaus (1891), Hyami, and a few others have denied that these apparently new ducts can re-form liver cells.

To conclude this historical part, and to illustrate the uncertainty that is commonly entertained with regard to these "ducts," I might quote a few conclusions from the work of W. G. MacCallum (1904 [53]), who states that "while the ducts may of course be atrophied liver cells, yet the majority are not to be accounted for in this way as the cells lack the usual appearances of liver cells in atrophy. Also one cannot be sure that they are all derived from the bile ducts, although the main weight of evidence is that they are the result of an intercalary proliferation by mitosis in the bile ducts. Some can also be seen to grow from the peripheral portions of liver-cell trabeculæ. Where the liver cells are destroyed these strands proliferate inwards and attempt to establish a connection with the liver cells, and if they do not succeed they end blindly. The liver cells and bile ducts, then, can produce an equivalent type of cell so far as regeneration is concerned."

In attempting the elucidation of the nature and function of these "apparently new ducts" I shall first bring forward a case of what may be styled a monolobular or biliary cirrhosis. This occurred in a child four months old, who had died in a state of severe jaundice which had been progressive since the day after birth. At the autopsy this condition was found to be due to a congenital obliteration of the bile ducts. The liver was greatly enlarged, somewhat increased in consistence, and of a bright green colour from bile congestion. Microscopically, the bile ducts, bile canaliculi, and liver cells were congested with bile. Round the periphery of each lobule was a narrow zone of young fibrous tissue. The lobules were apparently smaller than normal, as the liver cells in their peripheral parts had become replaced by this connective tissue (Plate XXII. Fig. 14). The central vein appeared in its normal position in the interior of the lobules, and the liver-cell trabeculæ radiated out from this in the usual way and showed practically no evidence of hyperplasia. This histological picture of monolobular cirrhosis had apparently been produced as the result of a necrosis of the liver cells in the periphery of the lobule, this area being replaced by connective tissue. In these granulation tissue tracts there ramified an enormous number of epithelial tubes resembling bile ducts. These could readily be distinguished from the old interlobular bile ducts, which still retained their normal positions, by the shape and staining reactions of the lining cells. These "ducts" were often joined on to columns of liver cells, and the lumen was directly continuous with the fine bile canaliculus in the interior of the liver-cell trabecula (Plate XXII. Fig. 15). With almost equal frequency they were united to the interlobular bile ducts, and their lumen was perfectly continuous. Occasionally in one field of the microscope, and easily in serial section, these "ducts" could be seen to be in connection with both liver cells and interlobular bile ducts. It could thus be shown that the bile channels connecting the liver cells with the exterior were complete (Plate XVI. Fig. 2). They were congested with bile, and it was easy to understand in this case that the bile was not in any way a secretion of these ducts, but was merely retained above

the obstruction in the common bile duct, which was quite absent in its lower part. The "ducts" were lined by a cubical epithelium, generally thickest in the intermediate portion of its course between the liver cells and the interlobular bile duct. It often presented lateral branches along its course. Sometimes it joined the liver-cell column or interlobular bile duct by a gradual transition in type of cell, but frequently its epithelium became very attenuated before the junction, and occasionally was simply a capillary-like tube. There was no evidence whatever of multiplication of the cells of such tubes, nor was there any sign of proliferation of the liver cells or interlobular bile duct to form them. Had there been any sign of proliferative activity on the part of either of these structures one might have been tempted to adopt the idea that they had extended their cells along the lines of the old connecting channel, as often occurs in cancerous invasion of the liver along the lines of the capillaries. The view which best accounts for the very regular appearance of these "ducts," their definite relationships, and the absence of proliferative changes seems to be, that they are simply the normal bile canaliculi, which connect the liver cells with the interlobular bile duct, becoming evident. These canaliculi are more resistant to the necrotic processes which cause the disappearance of the liver cells, and, being altered in their relationships and becoming embedded in granulation tissue, their fine flattened epithelium swells up and gives rise to a cubically lined duct. A somewhat analogous condition of affairs takes place in the lung in interstitial pneumonia, where the lining cells of the alveoli become greatly swollen. This idea will be further substantiated and the future of these ducts more definitely studied in relation to cirrhosis of the liver, and from results based on experimental work.

The appearances in this case were almost reproduced in a cat, which I accidentally discovered to have a liver in a state of cirrhosis of a more or less definite monolobular type (Plate XXIV. Fig. 26). The common bile duct in this case, however, was normally patent, and there was no bile congestion. Greenfield has also described cirrhosis in the cat's liver, and other observers have noted it in other animals.

Another very instructive case in relation to the study of the "ducts" occurred in a child, 6 months old, who had died after an attack of summer diarrhœa of three days' duration. The intestines were somewhat catarrhal, but the rest of the internal organs showed no special change except the liver, which contained a round tumour mass about 3 in. in diameter, of a whitish colour and containing large blood spaces. Microscopically, it appeared to be of the nature of a primary liver-cell adenoma; the rest of the liver was quite unchanged save for an area around the tumour corresponding to the very definite capsule which could be seen to surround it. In this region there were apparently a very considerable number of "ducts" possessing the usual definite structure, and lined by brightly

staining cubical cells. These areas were interspersed with almost equal sized areas of attenuated liver cells (Plate XXII. Fig. 19). The liver cells, which appeared in rows, did not in their atrophic state stain nearly so clearly as the lining cells of the "ducts." At the margins of these areas the two types of cells communicated closely, and a column of liver cells seemed to join on directly to a "duct" and to have an apparently continuous lumen. In the centre of the "duct" areas the old interlobular bile duct could be observed, and it could also be noticed that the apparently new "ducts" were also in connection with it. Unlike what occurred in the liver-cell areas, the apparently new "ducts" ramified amongst young connective tissue. At first it looked as if these "ducts" were only atrophic liver cells, although their better staining reactions and their more definite shape practically disproved this. These appearances, which have been described, were apparently due to the collapse of the lobule by the pressure of the tumour growth. There had occurred as the result of liver-cell destruction some fibrous tissue formation from the portal spaces in the compressed area. The fine bile canaliculi in the neighbourhood of the portal spaces, which had withstood the destruction removing the liver cells, had in their new surroundings assumed this new, more "duct-like," appearance.

Common Cirrhosis of the Liver.

A study of common cirrhosis of the liver, although difficult, is of considerable importance in elucidating some of the problems connected with liver regeneration. Owing to the extreme variety in type of this disease the conceptions of it as regards etiology, pathology, and classification have given rise to almost boundless discussions of a very diverse nature.

The hardened condition of the liver was perhaps first described by Vesalius (in 1514). Laennec (1819) gave the disease its familar name, but mistook the yellow parenchymatous masses for malignant new growth. Johannes Müller (1843) declared that cirrhosis of the liver consisted chiefly of a hypertrophy of connective tissue extending intralobularly at the expense of the gland structures, and was irresistibly progressive. Diehl (1860) made a distinct advance when he noted, "Sed hepar granulosum non semper diminutum videmus hypertrophia secundaria parenchymatis quod restat, hepatis volumen naturale conservatur." Ponfick (1891) remarked that the new formations which are regenerated have such a low functioning value as to be very little use in replacing the destroyed tissue. This pessimistic view has, however, not been altogether sustained, as Hanot and Gilbert (1890), Fraenkel (1890), Beale (1889), and Dieulafoy (1881 [19]) all state that some cases of cirrhosis of the liver may, even after definite clinical symptoms have appeared, be recovered from completely, so far as the health of the patient is concerned. Van Heukelom (1894) expressed his opinion that cirrhosis of the liver depended on a necrotic process followed by fibrous tissue production ("progressiv Degeneration und sklerogenen Schädigung der Leber"). Kretz (1900) goes a stage further, and adds to

this a regeneration of liver parenchyma to complete the picture. His definition of cirrhosis of the liver is that of a "herdweise, lokalizierten, rezidivierenden, chronischen, Degenerations-prozess mit eingeschiebenen Regeneration des Parenchyms."

In the study of cirrhosis of the liver it is unfortunate that such a multiplicity of nomenclature for the different types of the disease has been introduced.

The term "monolobular" or "fine" cirrhosis indicates appearances in the liver such as have already been noted in a case of congenital obliteration of the bile ducts. There is a destruction of the liver cells at the periphery of the lobule, and this area becomes replaced by fibrous tissue. This damage to the liver cells at the periphery of a lobule may be due to a great variety of causes, such as some general toxic disease or intestinal poisoning. The damaging agent may also be extended from the bile ducts by bile duct obstruction, and the accumulation of bile acting as a destructive and irritative agent. It has been fairly definitely shown that bile, even in its normal state, has a very decidedly toxic effect on liver cells.

"Biliary" cirrhosis has been accounted a condition which has its causal factor related in some way to the bile passages, but also some cases have been classed under this name on account of a large number of "bile ducts" being evident in the new fibrous tissue. Owing to bile congestion and young inflammatory productions the liver in these cases is often enlarged, and has been designated as hypertrophic.

Such terms as "portal," "coarse," "multilobular," "atrophic" have been applied to the "ordinary," "common" cirrhosis so frequently to be observed in "alcoholic" subjects. It would appear to be due to a very frequently occurring damage to small areas of the liver, as might be produced by the action of intestinal toxic agents. These numerous and repeatedly occurring necroses, soon after they are produced, become overgrown by fibrous tissue. After this has continued for some time, and the functioning capacity of the liver has begun to be reduced, a corresponding compensatory reconstruction of liver cells takes place. The result of this continuing degeneration and regeneration will naturally cause an extremely varied picture, and according to the degree of these processes and the extent of time over which they have lasted will the very varied appearances in different cases and in parts of the same liver be determined. For a time the regenerative capacity of the liver cells may supply a sufficiency of tissue to ensure the liver carrying out its functions satisfactorily, but after a time this reconstructive process may become so taxed that fibrous tissue repair and consequent atrophy of the organ becomes the prevailing feature.

If at any stage in the disease the factors leading up to it should remain in abeyance the liver assumes the appearances of a "quiescent" cirrhosis.

As illustrative of some of the processes which determine the diverse appearances of the liver in cirrhosis, I might briefly point out a few facts in relation to several examples of different types of the disease which I have been able to study. The first case occurred in the liver of a very alcoholic man, æt. 60 years, who had died as the result of very extensive burns. There was no previous history which could be related to the liver. The organ was slightly reduced in size, pale, and somewhat yellowish in colour, and of firm consistence. Its surface had the appearance of very fine morocco leather, and the substance of the organ, on section, presented a somewhat similar appearance. Microscopically, the liver lobules could be moderately easily defined. The liver cells, particularly in the peripheral parts of the lobules, had become very degenerated and atrophic, and their place was largely occupied by dilated blood vessels and connective tissue, which was spreading in from the portal tracts. This fibrous tissue also contained a considerable admixture of elastic elements, but very few "duct structures." In practically every lobule, especially near the more peripheral parts of the preserved parenchyma, clumps of liver cells could be observed in a state of hyperplasia. They were aggregated in densely packed masses of cells, some of which were greatly swollen, whilst others were small and darker in their staining reactions. The larger cells very frequently contained two nuclei, which sometimes could be observed to be formed from a single large dark nucleus by direct division. No mitotic figures could be found. The nuclei in these hyperplastic areas, as indeed also to some extent in the normal liver, stained very diversely when such a stain as eosin-methylene-blue was employed—some showing a clear bright blue pale nucleus with a red nucleolus, whilst others were dark blue, and others again of a pinkish-red colour. It might be suggested that these appearances indicated different phases in the life of the cell, and also were related to the occurrence of the bright and dark liver cells so frequently seen in hyperplastic areas. Yet they do not seem easy to define in this way, and sometimes different types of staining reactions could be made out in two nuclei in the same cell. Almost exactly similar appearances were seen in the liver of a very alcoholic woman, æt. 40 years, who had died as the result of chronic nephritis and a ruptured aortic valve. Such hyperplastic and hypertrophic areas are quite similar in appearance to the proliferative nodules, which are to be observed as the result of an apparently compensatory development in a variety of diseases, where there has been a great reduction in the volume of the functioning parenchyma of the liver, such as occurs in chronic venous congestion, cancer, cysts, etc.

Another type is represented in the liver of a man, æt. 47 years, who gave no history of alcoholism, dyspepsia, or of any illness that could account for the condition found in the liver. He died after a two months' illness, associated with a gradually developing ascites, and

for the last month jaundice had become progressively intense. At the autopsy the liver was slightly reduced in size, and was extremely firm. It was finely but irregularly granular on the surface, and in internal structure showed small yellowish, rounded, parenchymatous masses, mainly about an eighth of an inch in diameter, situated amidst a fibrous stroma. Microscopically, the fibrous tissue was present in great amount, and contained in most parts a very large proportion of elastic tissue. The liver-cell islets were very irregular, and in only a few could any resemblance to a lobular or regular trabecular structure be recognised. There was considerable evidence of degeneration of the liver-cell areas in patches (Plate XXII. Fig. 16), and there were also many areas where the liver cells were hypertrophic and hyperplastic (Plate XXII. Fig. 17). The intervening stroma of fibrous tissue was obviously of varying age, as some was dense and of comparatively old standing, whilst other portions presented the features of young granulation tissue. Amongst this interstitial tissue, particularly in the denser parts, very few "bile duct structures" were observable, but there were a comparatively large number of apparently isolated little clumps of liver cells, or even single liver cells. These cells looked, for the most part, fresh and not in the least as if pressed on by fibrous tissue overgrowth, and they very frequently were in very intimate relationship with small dark cubical cells, which were generally arranged in duct form (Plate XVII. Fig. 3). Where two liver cells were in apposition, these small dark cubical cells were not interposed between them, but were situated only on the free side of the liver cell (Plate XVII. Fig. 3*a*). These ducts could be easily distinguished from the interlobular bile ducts by their smaller size and the darker staining of their lining elements. A common appearance was that of a definite large liver cell lying on the side of, in the direct course, or at the termination of, one of these apparently new ducts, which otherwise were lined by a definite cubical epithelium (Plate XXII. Fig. 18). Several problems in relation to the appearances in this case present themselves for explanation. Are these isolated liver cells old or new? Are the small dark cells, arranged in duct form and often in close relationship to definite liver cells, some phase in the process of decay or compression of liver cells? Are these ducts simply aggregated interlobular bile ducts, altered in their staining reactions by compression? Are they a phase in the production of new liver cells by multiplication of the original liver cells? Are they a transformation of bile duct sprouts effloresceing into liver cells? Are the liver cells not simply forming a channel to remove their excretory products, or *vice versa* are the ducts a purposive sprouting from the bile ducts towards the liver cells?

To decide, first of all, if the theory which has been advanced by many authors, that atrophying liver cells can come to resemble these "bile-duct-like" structures, one can, I think, arrive at fairly definite

ideas on this point from a study of the atrophy which takes place in the liver cells in congenital syphilis, waxy or chronic venous livers, around tumours, etc. In such conditions, atrophying or degenerate liver-cell columns have perhaps a few superficial resemblances to the definite, clear-celled, regular, cubical-lined ducts, but they can very readily be distinguished (Plate XXI. Fig. 10).

To supplement the evidences derived from the microscopic appearances seen in the liver in this case, an attempt was made to throw light on the problems under investigation by studying the phenomena occurring in transplanted liver tissue. In this connection I performed a series of transplantation experiments with liver tissue, in order to study the fate of liver cells which had become displaced from their normal surroundings. At first fairly small pieces of liver tissue were implanted into the subcutaneous tissue, on to the surface of the spleen, and in folds of the omentum in rats and rabbits. The liver tissue to be implanted was removed from its original site and transplanted with as great rapidity and aseptic precaution as possible. The host for the implantation was sometimes the same animal or one of the same species of the same or different ages, or of another species altogether (*e.g.* implanting cat's liver into a rat). The inevitable result was that the included portion of liver tissue simply became rapidly necrotic. Some of these experiments were repeated, very small pieces of liver tissue being used, but these also, in a very few days, were lost sight of altogether. In two old rats, however, where pieces of rat's liver had been implanted amongst the muscular tissue of the abdomen, the liver tissue was found to be persistent in both, three and a half and four months respectively, after the operation. The liver tissue was easily recognisable, although considerably infiltrated by lymphocytes and plasma cells. The liver cells were largely pigmented and somewhat degenerated, but showed no signs of formation of new tissue (Plate XXI. Fig. 9). With a series of implantations of fœtal liver tissue the results were still more disappointing, as in every case, and with great rapidity, the foreign tissue was removed. In none of these experiments was there reproduced any structures with any resemblance whatever to the "bile-duct structures" seen in fibrous tissue formations in the liver.

The explanation of the appearances in the case of cirrhotic liver last described may be considerably elucidated by the aid of serial sections. By this means it can be shown that many of the small clumps of liver cells are only the outlying spurs of larger, irregular, liver-cell masses, and also one can often trace back the ducts with which these apparently isolated liver cells are in contact for long distances into larger ducts, and into definite interlobular bile ducts. Sometimes, of course, they only end blindly, or are only connected with a bile duct. Very often these tubes, which are lined by a definite cubical epithelium, become very attenuated in their lining cells before joining either the liver-cell trabecula or the interlobular bile duct. It could therefore be noticed that the isolated liver-cell clumps, which were in such close relationships with these "ducts" lined by small darkly staining cubical cells, were only retaining their normal connections with their excretory bile channels. It might also be supposed that, as a result of the change in environment of these tubes, their epithelial lining cells became swollen and assumed a cubical

appearance. I was never able to observe any definite evidence of these "ducts" having resulted from the proliferation of any collection of liver cells; indeed it would be hard to imagine how a liver-cell collection could give rise to structures with such very definite shapes and relationships. Similarly, I have never found definite evidences of these apparently new ducts being sprouted from the interlobular bile ducts. Mitotic division and multiplication of the lining cells of the interlobular bile ducts is sometimes seen, yet that fact is no evidence that they give rise to the apparently new ducts. These new ducts do often show their epithelium multiplying locally into clumps of small dark cells, but although this may be a step in the direction of formation of new liver parenchyma, the clumps never come to resemble the appearances presented by liver cells in any phase of their regenerative existence. I also think that even if the epithelium of the interlobular bile ducts shows evidences of regenerative reconstruction, this does not necessarily indicate that new ducts are about to be formed.

In this connection I might point to the work of Serafini (1907), who studied the regenerative capacity of the gall-bladder epithelium of the guinea-pig after its removal with a pad of cotton wool. The new cylinder epithelium was practically made good by the eighth day. Indeed, one can see very marked evidences of regeneration of the bile-duct epithelium in the liver in cases of infection of the bile ducts by *Distomum hepaticum* in pigs and cattle. In the later stages of this disease the inflammatory changes induced in the ducts often lead to their obliteration by fibrous tissue, in which can be seen the atrophic remnants of the original lining of the duct (Plate XXI. Fig. 13).

Plate XXI. Fig. 12 shows the interior of a small duct in the liver of a cat eight days after the common bile duct had been ligatured and injected with a strong solution of pancreatin. Throughout the liver even the small bile ducts were very catarrhal, and showed evidences of multiplication of their lining cells. In coccidiosis of the liver in rabbits it is also well known that there is an enormous new formation of bile-duct epithelial cells (Plate XXI. Fig. 11).

To trace the fate of the "ducts" further I might have recourse to the case of a very alcoholic man, æt. 48 years, who seven months before death had developed ascites, which, along with a slight trace of jaundice, had persisted up till the time of his death. The liver in this case was extremely reduced in size, particularly on the right side. The surface of the organ was somewhat coarsely granular, and on section irregular parenchmatous masses were separated by a dense stroma of fibrous tissue. The Spigelian lobe measured 4 in. in diameter by 1½ in. in thickness, and was not nearly so fibrous (Plate XXV. Fig. 32). It was apparently enlarged as the result of a compensatory process developing from some less damaged portion of the organ, and following the widespread destruction of the rest of the liver tissue. Microscopically, in this case the fibrous tissue was very dense and of old standing, and very regular in outline. On high-power examination it could be seen to contain a considerable number of very fine

"ducts," which, like those with a more definitely cubical lining in more acute types of cirrhosis, could on serial section be observed to extend between liver-cell trabecula and interlobular bile duct. There would appear then to be an atrophic stage of these ducts, owing to the compression of the surrounding fibrous tissue.

The description of the next two cases would, I think, illustrate the behaviour of the liver cells in the advanced stages of the disease, as far as regeneration is concerned. One is that of a girl, æt. 13 years, who had died after an operation of trephining for cerebral tumour. Three years previous to this she had developed severe rickets, and had become somewhat mentally defective; shortly afterwards choreiform movements became evident, and had been intermittently present up till death. There had been no symptoms which could be related to the liver. At the post-mortem examination the liver was found to be slightly reduced in size and slightly granular, with a fairly regular size of parenchyme nodule, about three-eighth's of an inch in diameter, embedded in a thin fibrous stroma. Microscopically, the liver-cell islets were more or less regular in outline, and enclosed in strands of moderately dense fibrous tissue containing a fair proportion of slightly atrophic looking "duct structures" (Plate XXIV. Fig. 27). In some areas the trabecular arrangement of the liver cells was preserved, yet even in these same islets, in certain places, and more generally in other parts, the liver cells were collected into hyperplastic clumps. The portal spaces and hepatic venules seemed very much disarranged in their relationships. Some central veins lay quite close to the portal spaces, while others were much further than normal from each other. Some of these central veins lay quite close up to the fibrous tissue stroma, or were even embedded in it. These liver-cell islets were very varied in outline, yet had none of the appearances of compression by fibrous tissue overgrowth, and in each liver-cell mass there usually appeared fewer portal spaces and central veins than normal, a large area having perhaps no central vein at all, or only one, and that irregularly situated.

This case, then, would appear to illustrate that cirrhosis of the liver is the result of a widespread repeated destruction of small areas of the liver tissue, and that these destroyed areas are replaced by fibrous tissue. When this degeneration has reduced the volume of the liver to a sufficient extent the remaining liver cells proliferate and form the irregular masses the occurrence of which is the rule in cirrhosis of the liver. This process may go on till the capacity for regeneration is worn out and atrophy becomes the predominant feature. Yet at any stage the disease may become quiescent, and a sufficiency of liver tissue may be left or be regenerated to fulfil the ordinary functions of the organ.

An example of the quiescent stage of liver cirrhosis is given in a man, æt. 37 years, who presented no alcoholic or syphilitic history. He died after a laparotomy had been performed on account

of a swelling which could be felt in the upper part of the abdomen. At the autopsy the liver was found to be greatly reduced in size, and to be situated mainly on the right side of the abdomen, as the left lobe had shrunk into a fibrous looking mass. The right lobe was quite round, and showed on its under surface three large nodules, one of which had been felt during life (Plate XXV. Fig. 33). The surface of the organ was slightly irregular, and on section a dense fibrous stroma pervaded the organ. These nodules, again, seemed to be evidence of compensatory local hyperplasia of relatively undamaged portions of the liver substance. A somewhat similar appearance of local hypertrophy is given in Plate XXV. Fig. 31. On microscopic examination the fibrous reticulum here was very dense and sharply defined from the liver tissue. It contained no evident bile ducts, save some few definitely old-standing interlobular bile ducts (Plate XXIV. Fig 29). The liver-cell masses were irregular in contour and size, but had mainly a trabecular arrangement, though these trabeculæ were often very undulating. The relationships of the central veins and portal spaces were also irregular (Plate XXIV. Fig. 28). These appearances would show how very much disarranged the circulation through the liver may become, and how the vessels going through the regenerated tissue may often be long and tortuous. This would account for a very large amount of the increased blood pressure which occurs in the portal system in old-standing liver cirrhosis.

In cirrhosis of the liver, then, the multiplication of the liver cells which occurs is derived, altogether, from a proliferation of pre-existing liver cells, and the "ducts" which are very obvious in some, especially more acute cases, are derived from a swelling up of the epithelium of the pre-existing fine bile canaliculi which have escaped the necrotic process. The lining cells of these often do multiply locally, but never develop into liver cells. In old-standing cases of cirrhosis they may be observed to have become fine and attenuated and ultimately almost invisible.

Experimental Work.

A very interesting advance can be made in the study of liver regeneration, when the exciting factor is known and when a definite age can thus be assigned to the resulting formations.

Hess described three cases of rupture of the liver of some standing, and noted epithelial buds growing from the liver cells, and also in certain places from the gall ducts, but observed no mitoses. Muir also described three cases, one of five days, one of seventeen days, and one of thirty-one days' duration. The case at five days showed only very slight new formation, while that of seventeen days showed a very marked formation of duct-like structures amongst the fibrous tissue of the wound. In the case of thirty-one days' duration these ducts were not quite so evident. He figures "duct-like" structures growing from the ends of liver-cell trabeculæ exposed at the margin of the wound, and considers them as proliferated from the liver cells,

depending on a breach of continuity. The histological picture in these cases is almost identical to what may be obtained experimentally in wounds in the liver of similar duration.

Italian observers were first in the field in the experimental study of repair of the liver tissue after damage to the continuity of the organ. Colucci (1883) described the formation of new liver cells as arising from white blood corpuscles by a division of their nuclei. Corona and Tizzoni (1883) noticed a proliferation of the liver cells, which they considered to be due to a direct mechanical action causing a division of the liver cells. Griffini (1884 [32]), as a result of his experimental work, states that "cette transformation des cellules des cordons epitheliaux en petites cellules hepatiques donne lieu au systeme trabeculaire de nouveau parenchyme." These observers only removed minute portions of the liver, as did also Rubasci, Clemenci, and Ughatti. Canalis (1886) was more ambitious, and removed larger pieces (2 to 3 cms.) in dogs and guinea-pigs. He recognised a new proliferation of liver cells, and also a new formation of bile ducts, but could not agree with Griffini that the latter were formed from old bile ducts. These observations paved the way for the studies of Podwyssozki ([67]), whose work must be considered as the first really valuable experimental research, and his results, although published in 1886, carry us very nearly up to our present-day knowledge of liver-cell regeneration. He removed small wedge-shaped areas from the livers of rats and rabbits, and observed what he thought to be reactive proliferative changes in the adjacent liver cells in fifteen to twenty hours. In two and a half days, especially in rats, mitotic figures were evident in many of the liver cells near the wound, and often some considerable distance away from it. Some of these mitotic figures depicted in his diagrams are only the results of nuclear degeneration, yet some are undoubted karyomitoses. From four to six days onwards the bile ducts were noted to have sprouted out into the scar tissue of the wound, and by the tenth to twelfth day they had become liver cells. Prus (1887) confirmed this work.

Later observers have removed larger quantities of the liver tissue. Ponfick (1890) first attempted this, using in his experiments an enormous number of rabbits, and having a very large mortality, which increased in proportion to the amount of liver tissue removed. In the animals which survived there was a very rapid increase in size in the remaining portion of the liver, and the individual liver lobules became very large and irregular, like a clover leaf. Franklin Mall, criticising Ponfick's work, thinks that the liver lobules in hypertrophy do not enlarge in size, but sprout as do the embryonic liver lobules. Floeck (1895), von Meister (1894), and Yamagiva (1901) all repeated this work and arrived at somewhat similar conclusions. Von Meister also noted that there was no evidence in his experiments that the newly formed bile ducts were transformed into liver cells. Yet de Bary (1897), in his cases of excision of parts of the liver, notes that a metamorphosis of the liver-cell trabeculæ takes place into small dark "duct-like" structures.

Various other methods have been adopted by recent observers to elucidate this question. Lapayre used carbolic acid injection, and others have used turpentine. Hyami (1906) employed an injection of 10 per cent. aleuronat in an emulsion with 6 per cent. sodium chloride. This was introduced into the liver, and produced a mild productive inflammatory reaction, and he states that only after three or four days were there any signs of regeneration of the liver cells, and in five to twelve days the bile ducts commence proliferating. Only once was he able to observe a liver cell in mitosis, and yet there were abundant evidences of liver-cell multiplication.

After trying a series of experiments, consisting of injection of various toxic agents into the liver and obtaining unsatisfactory results,

so far as the study of regeneration was concerned, I followed the method of making small wounds in, and cauterisations of, the liver substance of rats and rabbits, but the main change which was thus induced was simply the formation of a fibrous scar in the injured part. There certainly were occasionally slight attempts at liver-cell multiplication and some slight formation of "bile ducts" at the edge of the scar tissue. To obtain a reaction of any magnitude I had to follow Ponfick and von Meister's methods of removing large pieces of the liver substance. This removal of large portions of the liver did not produce anything like the mortality which Ponfick observed in his work, although it certainly was in direct proportion to the amount removed. The method I found best and associated with a very low death-rate was to ligature the branch of the portal vein and the accompanying hepatic artery supplying the left lobe of the liver. The greater part of the lobe may then be removed, as is desired, with practically no hæmorrhage. The portion supplied by these vessels rapidly undergoes necrosis: in twenty-four hours appears white, and in forty-eight hours shows a red ring demarcating it from the surrounding healthy liver tissue, and after three days the necrotic portion has begun to be separated off by fibrous tissue formation. To accomplish this result both hepatic artery and portal vein branches must be obstructed. Ligature of the right or left branch of the hepatic artery in cats and rabbits produces no apparent histological change in the liver, and ligature of one branch of the portal vein causes necrosis of the greater part of the interior of the liver lobule, but leaves an irregular layer of unaffected liver cells at the periphery of each lobule. The corresponding portion of the liver, although it soon shrinks, never assumes the white and wholly necrotic appearance which results when both vessels are obstructed. Following this necrosis of nearly half the liver, the remaining healthy portion begins to increase in size with great rapidity, and to a large extent independently of the possibly increased amount of blood traversing it. This increase in volume has generally attained its maximum by the end of three weeks, and very little growth is to be observed after this. By the end of this time the necrotic portion, or what was left of it at the time of operation, has shrunk into a small rounded mass, often cystic in its interior, and surrounded by a definite capsule of fibrous tissue. This mass, owing to the increase in volume of the healthy remaining liver tissue, generally becomes almost buried in the substance of the organ. At the end of three weeks the liver tissue is of a rounded shape, and when the left lobe has been removed the gall bladder may have come to lie far over on the left side of the abdomen. These experiments were carried out to some extent in cats, but more fully in rabbits. In rats comparatively large portions of the liver tissue may be removed directly with practically no danger or inconvenience to the animal. The results in all these animals were practi-

cally the same, but owing to the type of liver cell in the rabbit the histological changes in this animal were much more easily studied. Histologically, the process of compensatory repair begins early, and seems to depend for rapidity of occurrence and amount of development directly on the proportion of liver tissue thrown out of function at the time of operation.

Throughout the entire remaining portion of the liver, when nearly half the organ has been destroyed, there are obvious active regenerative processes instituted. In twelve hours the liver cells at the periphery of each liver lobule have become enlarged and somewhat pale, but with a brightly staining nucleus. The trabeculæ of liver cells near the portal space region have become somewhat more undulating than normal. In thirty-six hours this is very marked, but as yet no new liver cells have appeared. In three to five days the liver cells at the periphery of the lobule are still large, pale, and hypertrophic, and they, slightly more often than is the case with the liver cells in the interior, have two nuclei. Several small dark cells can sometimes be seen amongst the large hypertrophic cells. With the appearance of new liver cells the lobule begins to enlarge. The most marked proliferative changes seem to occur in the outer intermediate portion of the lobule. In from five to ten days the normal double row of liver cells constituting a trabecula seems to have split into a mass of cells four or more deep. As a rule mitotic figures in these multiplying liver cells are of comparatively rare occurrence. Yet evidences of direct division are plentiful. It is somewhat difficult to explain why, in cases which have been subjected to seemingly identical conditions, there should be such variations as occur in the frequency of karyokinetic liver cells.

As a whole, in rabbits, karyokinesis in the liver cells is extraordinarily rare, but in rats this is rather more frequently found, but not with, so far as I could make out, any degree of certainty as to time of appearance. In one specimen of a rat's liver, from which a large amount of liver tissue had been removed four days previously, I found a considerable number of liver cells in karyomitosis scattered all through the remaining liver tissue. Yet in other rats of similar size, which had been subjected to a similar operation and examined at the fourth day, karyomitoses were very rare. Generally these mitotic liver cells may be found from the third to the tenth day in rats, but I have never been able to observe them outside this period. In rats, then, as well as in rabbits and cats, the multiplication of the liver cells tends to be by a simpler type of cell division. In the rat also the appearances of the intralobular proliferation are rather more like what occurs in man, in that there are, more often than in rabbits, irregular masses of hyperplastic liver cells to be observed in the lobules.

In three to four weeks the lobule is very much enlarged, often having a radius from the central vein to the portal space of about

twice the normal of 0·6 millimetres. The outline of the lobule may also appear slightly irregular, but I have never seen it so irregular as Ponfick described it in his cases. The cells in the outlying parts of the portal spaces are still greatly increased in size and somewhat irregular in shape, and very frequently contain two nuclei. In the intermediate zone it is very hard to make out any trabecular structure at all, as the liver cells have split into large numbers of small darkly staining closely packed uninucleated cells. The portion of the lobule immediately round the central vein often shows very little variation from normal (Plate XX. Fig. 7).

After six weeks the appearances begin to assume a more normal type as the majority of the newly formed cells have assumed the character of fully formed liver cells, and an irregular trabecular arrangement can now be observed such as is found in the more resting types of cirrhotic livers. These evidences of liver-cell multiplication are then extremely like what occurs in the liver-cell islets in subacute liver atrophy, where there is a similar compensatory demand for new liver parenchyma.

These experimental cases were also of use in the attempt to elucidate the production of the "duct-like" structures which are so commonly seen ramifying amongst fibrous developments in the liver. In my description I shall make use of a series of injuries to the liver as uniform as possible, but with regard to these ducts it makes very little difference in their production whether a large or a small amount of the liver has been destroyed. In some wounds in the liver practically no ducts appear to be formed in the marginal fibrous tissue, and in others they are very abundant.

In the series of cases in rabbits where branches of the hepatic artery and portal vein had been obstructed, and the corresponding area of liver tissue necrosed, a common appearance near the margin of the healthy tissue in the first two days was the existence amongst the necrotic liver tissue of preserved portal spaces. These more resistant interlobular bile ducts in a portal space sometimes showed a thin ring of undamaged liver cells round them (Plate XXVI. Fig. 35). By the fifth or sixth day these isolated foci have become completely altered in appearance, as they are foci from which great inflammatory reaction proceeds. Newly developed capillaries and connective-tissue cells extend into the surrounding tissue, and amongst this granulation tissue are seen numerous "duct-like" structures, with very definite outlines, and particularly evident in the outermost part of the portal space (Plate XXVII. Figs. 36 and 37). They are often joined on to such liver cells lying round the portal space as have been spared the necrosis. They are, however, just as much in evidence if no liver cells are in the immediate neighbourhood. These apparently new ducts can also, sometimes, be seen with the lumen directly continuous with that of the old interlobular bile duct in the centre of the portal space.

From this duct they are readily distinguished by their finer lining epithelium, and by the fact that the cytoplasm of the component cells stains somewhat differently. Also they do not possess an elastic lamina, as do most interlobular bile ducts. This fact was also noted by Flexner (1900 [24]). At this stage on the fifth day they are generally lined by a fine attenuated epithelium, almost like that of a blood capillary, and they are occupying the situations of the fine, almost invisible, bile canaliculi which normally conduct the bile from the liver cells to the interlobular bile duct.

Portions of this canalicular system, particularly near the portal space region, have escaped the general liver-cell destruction, and the epithelium is now becoming swollen up to give rise to the "duct-like" structure lined by a cubical epithelium so commonly described. (An almost similar process can be seen to occur in the lining cells of the air spaces in the lung in interstitial pneumonia.) Along the line of the wound these ducts also frequently appear, and they are generally in connection with the exposed ends of liver-cell trabeculæ and with a lumen directly continuous with that of the bile canaliculus inside a liver-cell trabecula. On the third or fourth day these are fine and hair-like, and lined by a very flattened epithelium; by the fifth or sixth day they have become lined by a larger type of cell, which is always most cubical at its free end. On the tenth day the tubes are quite like the ducts that may commonly be seen in the fibrous tissue of cirrhotic livers, and they are lined by a definitely cubical epithelium (Plate XIX. Figs. 6 and 6*a*). I could observe no sign that either the liver cells or the interlobular bile ducts formed these structures by a proliferative process. At the edge of the wound, liver-cell proliferation may occur, but this never gives rise to duct-like structures resembling at all closely these apparently new tubes. As has already been noted, some wounds, particularly those which have been made by very clean cuts, show no "ducts" in the newly laid down fibrous tissue at the margin of the wound. In areas of fibrous tissue in many diseases of the liver sometimes only a few or no "ducts" may be seen, and in other parts of the specimen the ducts are very numerous. This variation depends on the irregular exposure of the bile canaliculi, should they have survived the destruction of the liver cells, and in their new environment have become altered in appearance. These tubes get greatly modified in their relationships by inflammatory formations distorting and carrying them into the surrounding tissues. Their lining cells do to some extent multiply locally, but there is never any creation of cells with any marked resemblance to liver cells. At the third or fourth and up to the sixth week the margin of the wound is composed of fibrous tissue, in which, often, are islets composed of masses of these "ducts" round old preserved portal spaces which had been spared in the edge of the necrosis (Plate XXVI. Fig. 38). All along the margin of the wound are numerous ducts, which are in direct

connection with the exposed liver-cell trabeculæ (Plate XVIII. Fig. 5). After six weeks up to two months the fibrous tissue environment of the "ducts" becomes denser and the latter begin to atrophy, and the lining epithelial cells shrink and become attenuated (Plate XXVI. Fig. 39).

At two to three months, and afterwards, the ducts, although they may sometimes still be observed, have become extremely attenuated, and may not be more readily manifest than are "ducts" in the fibrous tissue of an old-standing quiescent cirrhotic liver (Plate XXVI. Fig. 40). These "ducts," then, would seem to atrophy without ever attaining to the production of any new functioning liver tissue. There are never to be observed groups or columns of newly developed liver cells amongst the fibrous tissue in the situations where earlier there were numerous "bile ducts," as one would expect to see had any transformation of the lining cells of these "ducts" into liver cells taken place.

Conclusions.

1. The liver is capable of a great degree of regeneration when a sufficient reduction in the volume of its parenchyma has taken place.

2. That this is chiefly a compensatory change is illustrated by the extensive regeneration which occurs in cases of subacute atrophy, cirrhosis, chronic venous congestion, cancers, cysts, atrophy of one lobe, acute destructive diseases, etc., and in experimental removal of portions of liver tissue. In all these diseases the essential factor initiating the regeneration has been extensive liver-cell destruction.

3. This regeneration results from a proliferation of liver cells directly, and with no transition in type of cell.

4. The chief regenerative capacity of the liver cells is manifested in those situated in the outer half of the liver lobule.

5. Liver-cell multiplication takes place usually by a direct division of the cell. Karyomitosis is also fairly often seen in the liver cells, but it is not the general method of cell division.

6. Cirrhosis of the liver depends on a primary necrosis of tracts of liver cells, and on these areas being replaced by fibrous tissue which is developed from adjacent portal spaces. When a definite ring of liver cells is destroyed in the periphery of the liver lobule a condition of monolobular cirrhosis is induced.

Subacute liver atrophy results when the destruction is extremely widespread and rapidly completed, and the patient survives some time. The remaining liver cells take on hyperplastic growth.

Ordinary atrophic cirrhosis is the result of very repeated damage to the liver parenchyma, with a corresponding development of fibrous tissue in place of the necrotic areas. There also occurs a compensatory liver-cell hyperplasia which accounts for the extremely varied histological and macroscopic picture of this disease.

7. In practically all destructive conditions in the liver where fibrous tissue is being laid down, numerous "bile-duct-like" structures can be observed ramifying. They are often in connection with either the old interlobular bile duct or a liver-cell trabecula. In some pathological conditions these ducts are lined by a fine somewhat flattened epithelium, and in others they are lined by a very definite cubical type of cell. Experimentally, they become conspicuously evident about the fourth or fifth day as tubes lined by an attenuated epithelium; by the tenth day they are definitely cubical in type, and from two months onwards they atrophy, and although their lining cells sometimes multiply locally, they never reproduce any cells with any close resemblance to liver cells.

These "ducts," then, seem to be formed from a becoming evident of the delicate normal bile-conducting channels which extend between the liver cells and the interlobular bile ducts. When these structures, which are apparently more resistant to destructive agents than the liver cells, become exposed in granulation tissue, their lining cells swell up in the same way as do the lining cells of the air alveoli in the lung in interstitial pneumonia. By this means the cubically lined ducts are formed which are so commonly to be observed in pathological developments of fibrous tissue in the liver.

8. The entire regeneration of the liver cells is accomplished directly from previously existing liver cells, and the amount of and capacity for this reconstruction of liver tissue would seem to indicate the high degree of importance of a sufficiency of liver parenchyma to maintain the normal economy of the individual.

I must express my very sincere thanks to Professor Greenfield, in whose laboratory this work was conducted, for his many kindnesses in connection with it. To Mr. Richard Muir I also owe a very special debt of gratitude, both for his untiring help and for the illustrations which he has prepared with his customary great skill and accuracy.

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Note.—For the references concerning the regeneration of liver tissue which occurs in *Acute* and *Subacute Liver Atrophy*, see paper on "Subacute Liver Atrophy" in this volume of the Journal (p. 161).

DESCRIPTION OF PLATES XV.-XXVI.

PLATE XV.

FIG. 1.—Section from the interior of a "yellow area" of a case of subacute liver atrophy in a boy 6 years of age, who had died in the eighth week of an attack of jaundice. The liver cells composing this area can be seen to be in a state of active multiplication. Some of the cells are small, uninucleated, and darkly staining, while others are large, pale, and sometimes contain two or more nuclei. One of the liver cells can be seen in karyokinetic division. The trabecular arrangement has become very irregular, and in parts is no longer evident.

PLATE XVI.

FIG. 2.—Section of the liver of a child, four months old, who had died from chronic jaundice, since the day after birth, as the result of congenital obliteration of the bile ducts. The liver was in a state of monolobular cirrhosis, and in the young fibrous tissue at the outer parts of the lobules there were a large number of "ducts" lined by cubical epithelium. The illustration shows one of these "ducts" communicating with an interlobular bile duct, and also having its lumen directly continuous with the bile canaliculus in the interior of a liver cell trabecula.

PLATE XVII.

FIG. 3.—A small clump of liver cells isolated in the fibrous tissue of a cirrhotic liver. The liver cells are considerably separated up by fibrous tissue, and can be seen to have very close relationships with ducts lined by darkly staining, generally cubical-shaped, cells.

FIG. 3A.—A few liver cells isolated amongst the fibrous tissue of a cirrhotic liver, showing their relationships with duct-like structures.

PLATE XVIII.

FIG. 4.—One of the ducts which extended outwards amongst the new fibrous tissue in the region of the portal tracts in the "red areas" of a case of subacute liver

atrophy of about two months' duration. It can be seen to be connected with an interlobular bile duct by a rather narrow neck. Some proliferation has occurred in the lining cells of the outer end of the "duct."

FIG. 5.—Section from the margin of a necrosis in the liver of a rabbit of three and a half weeks' duration. It shows one of the numerous "ducts" which extend outwards amongst the new fibrous tissue, and are often in connection with the exposed ends of the liver-cell trabeculæ. Its lumen can be seen to be continuous with the bile canaliculus between the liver cells.

PLATE XIX.

FIG. 6.—The margin of a necrosis in the liver of a rabbit of thirteen days' duration. It shows the development of "ducts" in direct continuity with liver-cell trabeculæ. Near the liver cells their lining epithelium is often very attenuated, but in their more outlying portions it has assumed a more cubical type.

FIG. 6A.—Shows the very delicate connections of the "ducts" with the liver-cell trabeculæ. From the same case.

PLATE XX.

FIG. 7.—A lobule of a liver from which a large portion had been removed three and a half weeks previously. The lobule has become very much enlarged. Its peripheral cells are large and pale. In the intermediate zone a great proliferation of small darkly staining liver cells can be observed.

PLATE XXI.

FIG. 8.—A portal space of a liver in a state of acute liver atrophy of sixteen days' duration, showing numerous "ducts" extending outwards from the portal space in the newly developing granulation tissue. (× 60.)

FIG. 9.—Liver cells of a rat persisting in the subcutaneous tissues, three and a half months after the transplantation. The liver tissue is considerably invaded by inflammatory cells. (× 250.)

FIG. 10.—Liver cells atrophied by the compression of fibrous tissue in congenital syphilis. (× 300.)

FIG. 11.—Section of the liver of a rabbit affected with coccidiosis. It shows the enormous proliferation of bile-duct epithelial cells which occurs in the disease. (× 100.)

FIG. 12.—Liver of a cat into whose bile duct a strong solution of pancreatin had been injected eight days previously. A small bile duct can be seen in a state of intense catarrh. (× 110.)

FIG. 13.—A bile duct in the liver of an ox, which has become obliterated by inflammatory changes following on infection by liver flukes. Remains of the old bile duct, still lined by a columnar epithelium, are to be found amongst the fibrous tissue. (× 60.)

PLATE XXII.

FIG. 14.—The liver from a case of monolobular or biliary cirrhosis occurring as the result of congenital obliteration of the bile ducts. It shows a great number of "ducts," which ramify amongst the fibrous tissue at the periphery of the lobule. (× 60.)

FIG. 15.—A higher power view of the same case, showing the close connections which often occur between the "ducts" and liver-cell trabeculæ. The "ducts" are very regular in shape, and are lined by a smaller type of cells than the liver cells, and are also darker in their staining reactions. (× 270.)

FIG. 16.—A section of a cirrhotic liver showing extensive degenerative changes. Numerous small clumps of liver cells also appear isolated amongst the fibrous tissue. (× 60.)

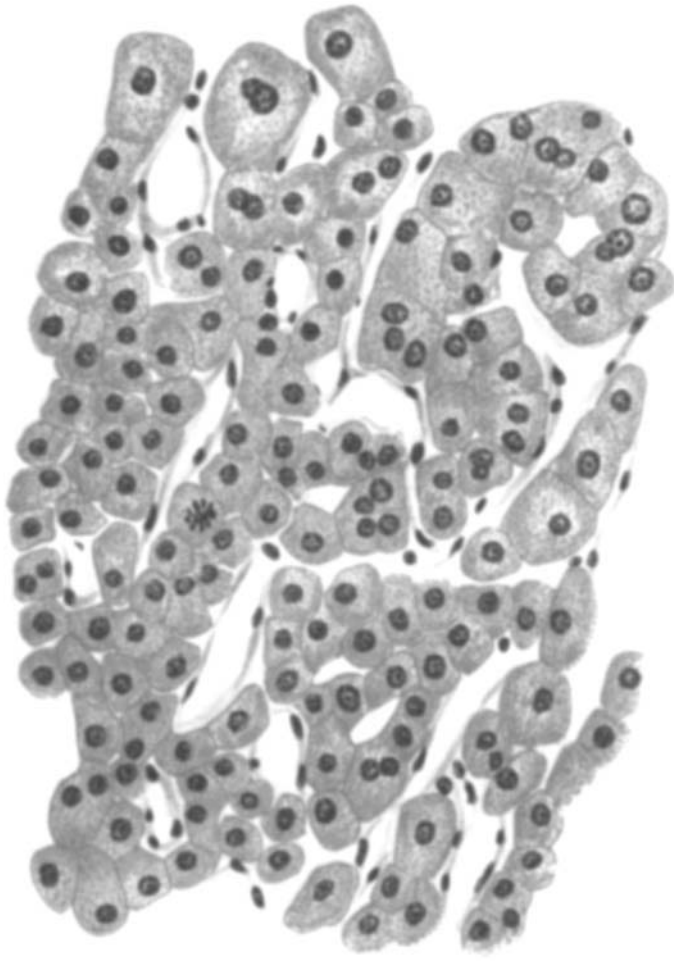


Fig. 1.

Fig. 2

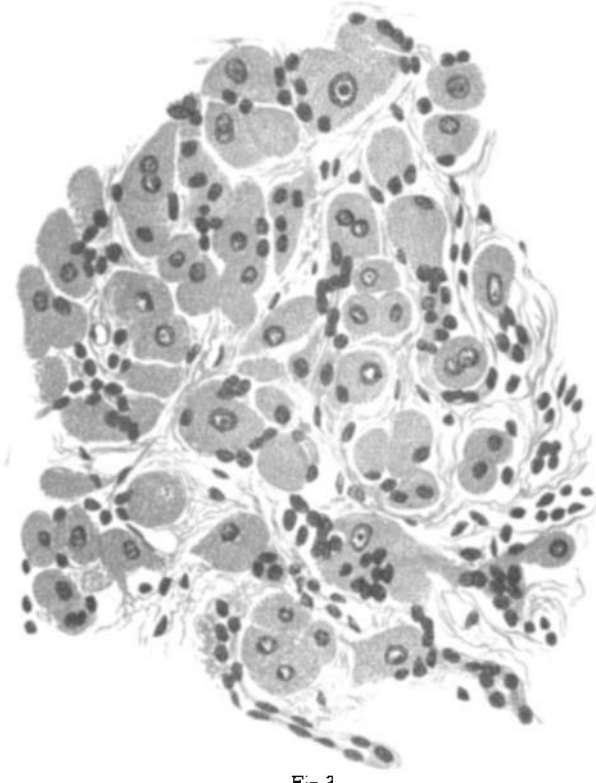


Fig 3.

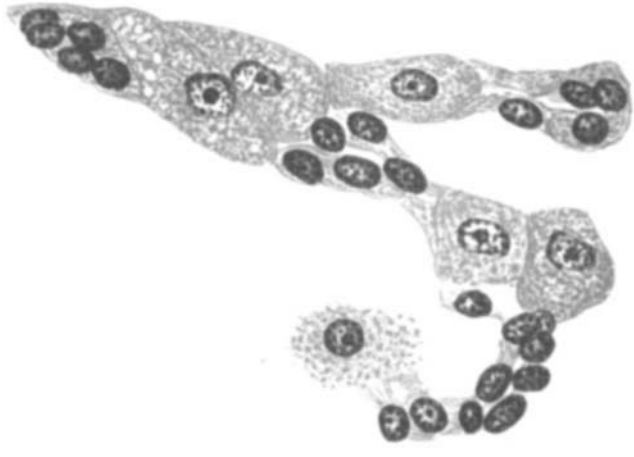


Fig. 3 a

Fig. 5

Fig. 6.

Fig. 6 a.

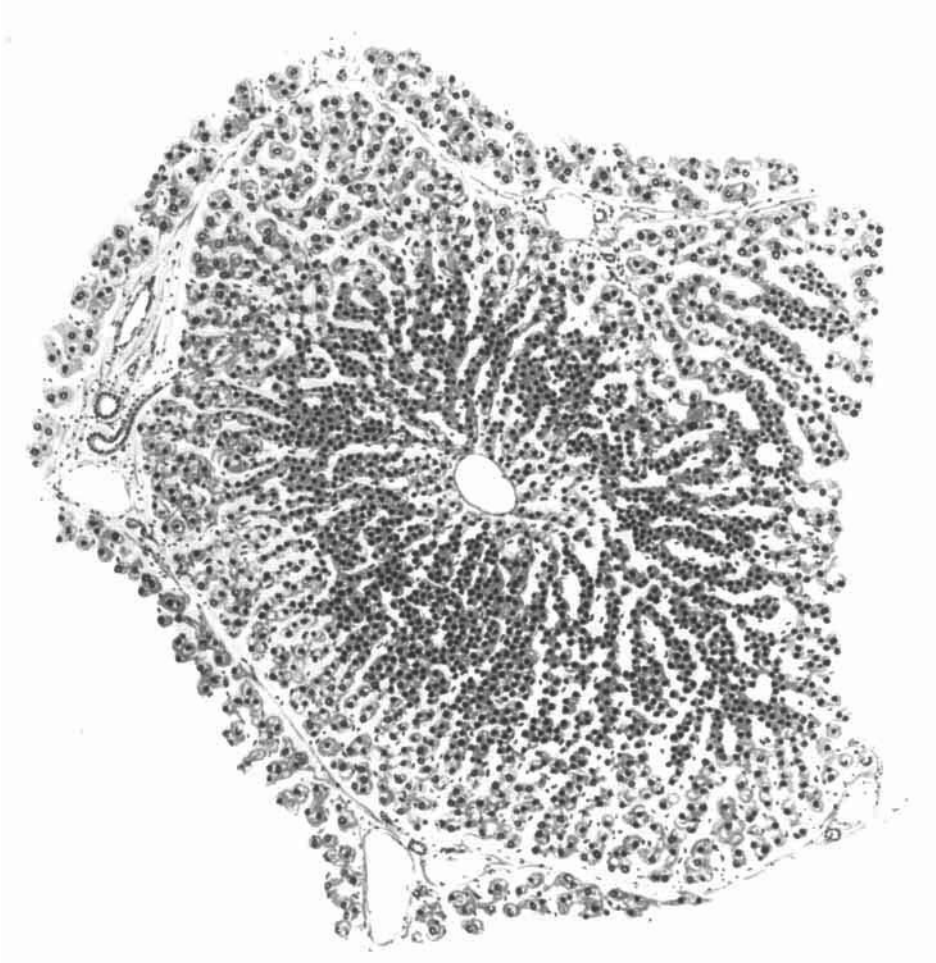


Fig 7

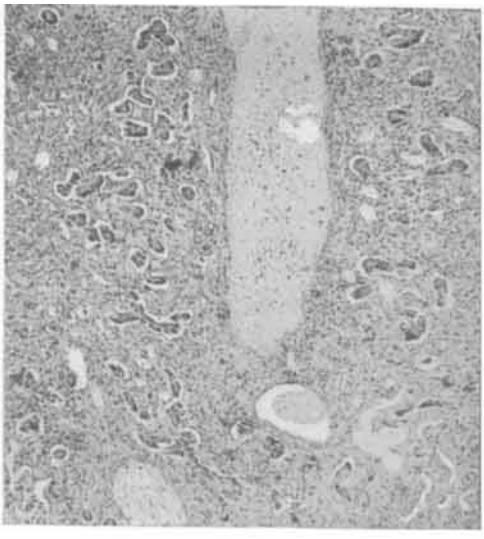


Fig. 8. (× 60.)

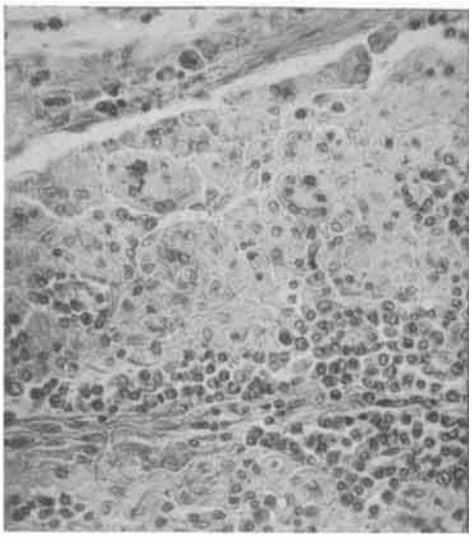


Fig. 9. (× 250.)

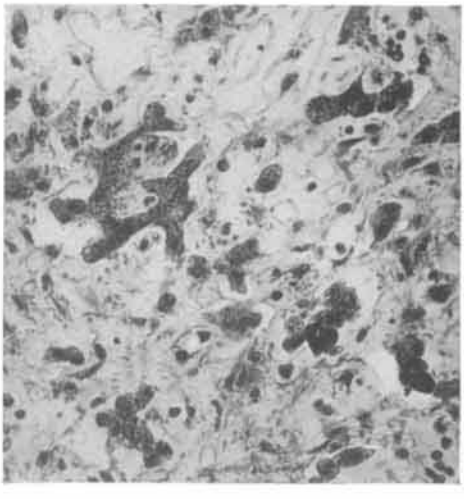


Fig. 10. (× 300.)

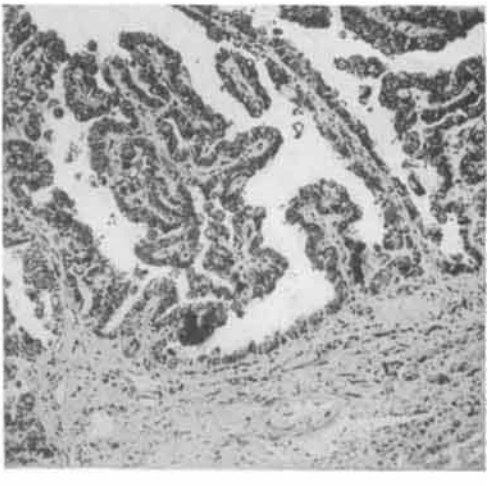
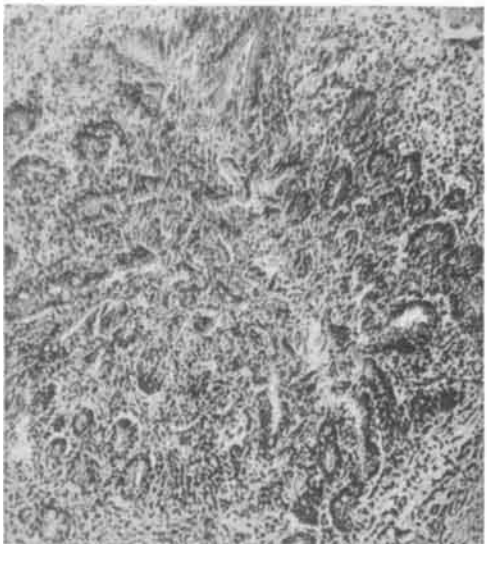


Fig. 11. (× 100.)



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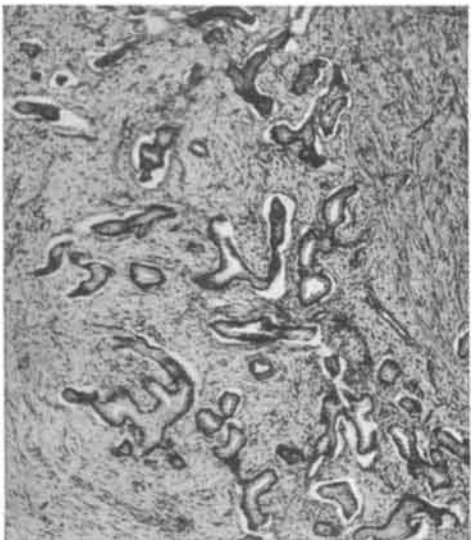

Fig. 13. (× 60.)

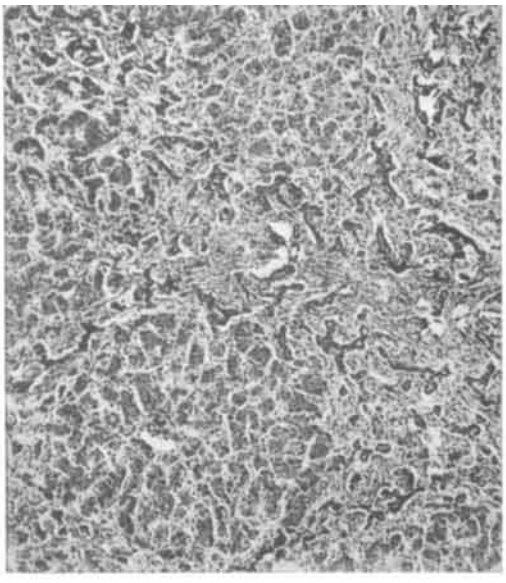

Fig. 14. (× 60.)

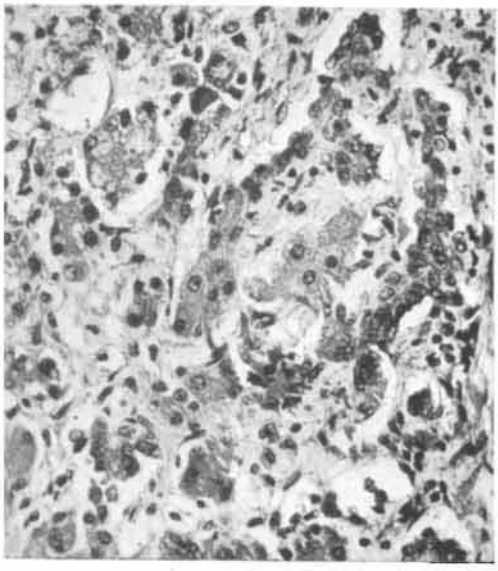

Fig. 15. (× 270.)

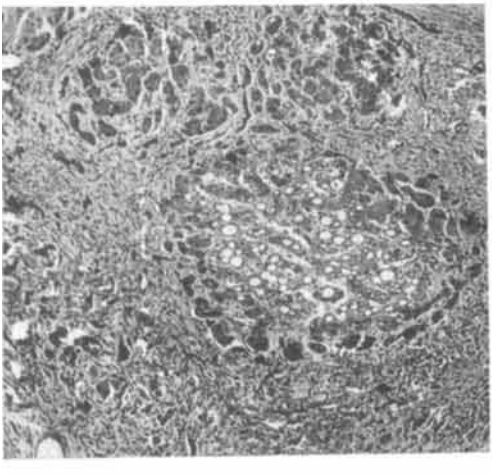

Fig. 16. (× 60.)

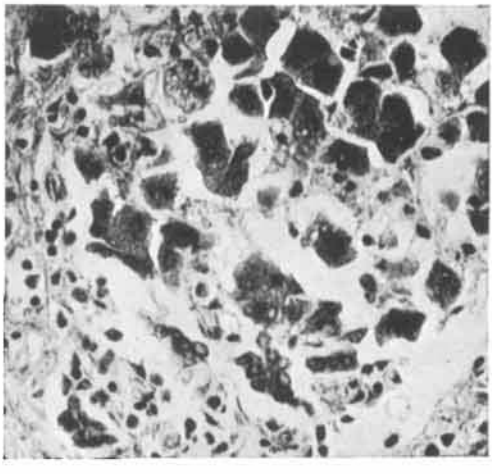

Fig. 17. (× 300.)

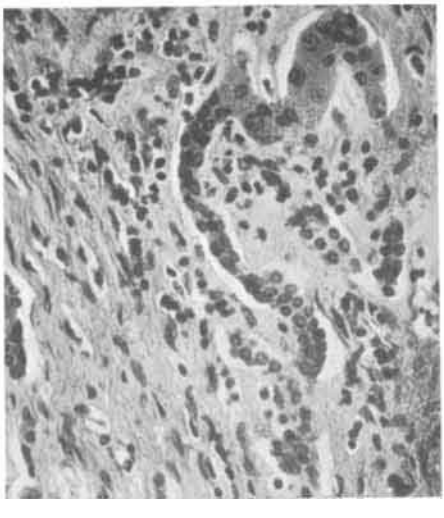

Fig. 18. (× 300.)

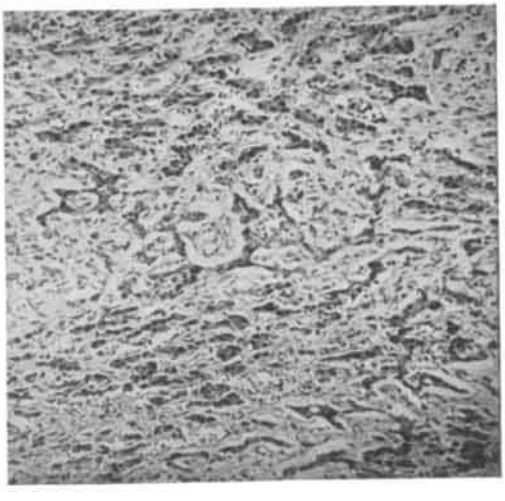

Fig. 19. (× 120.)

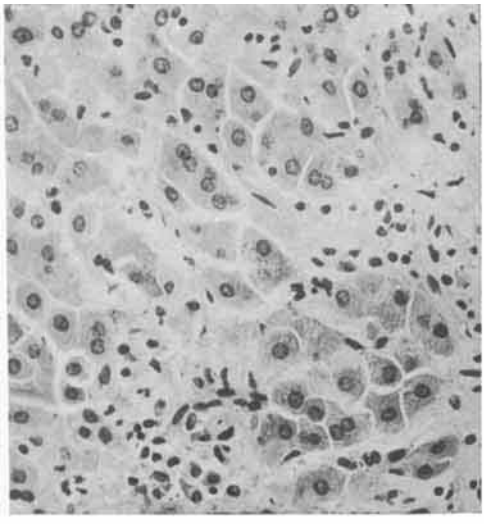

Fig. 20. (× 300.)

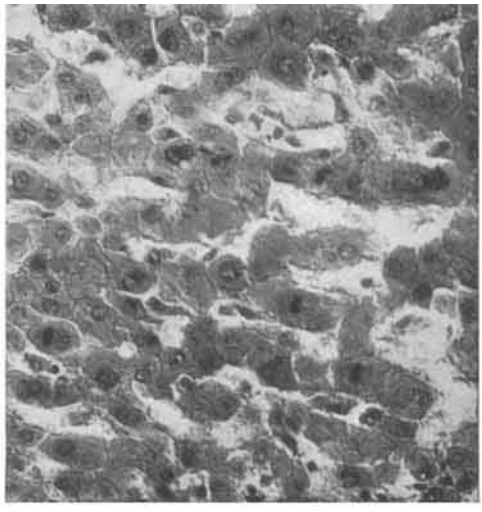

Fig. 21. (× 300.)

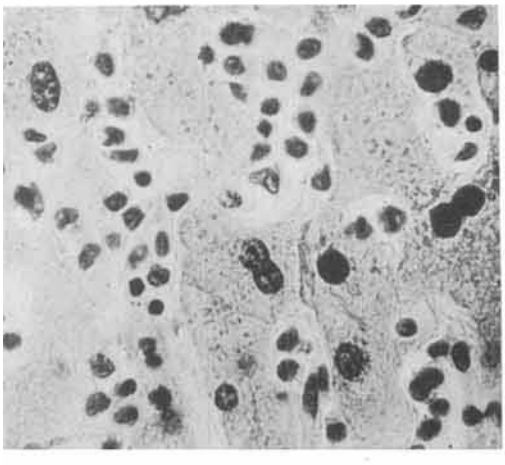

Fig. 22. (× 500.)

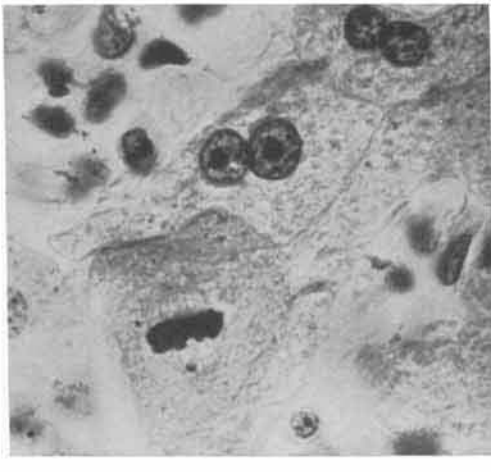

Fig. 23. (× 1000.)

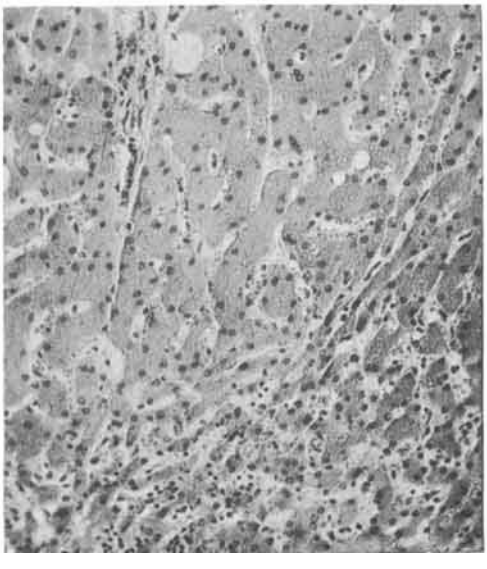

Fig. 24. (× 100.)

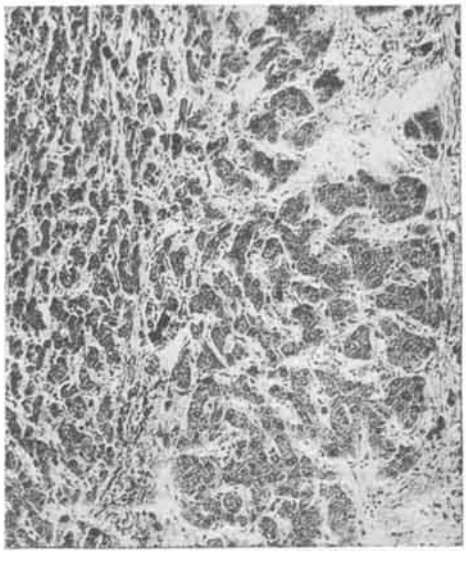

Fig. 25. (× 160.)

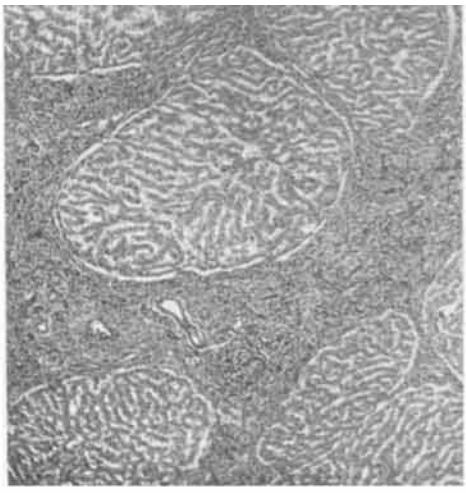

Fig. 26. (× 60.)

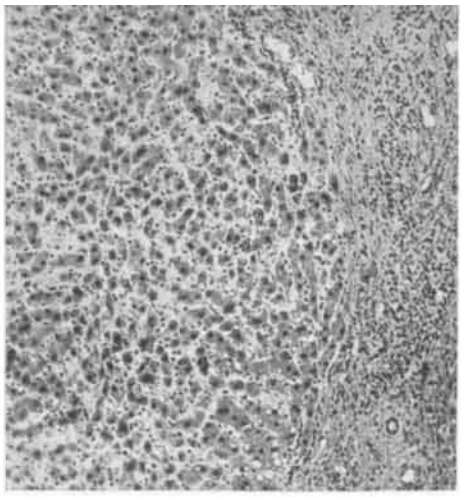

Fig. 27. (× 90.)

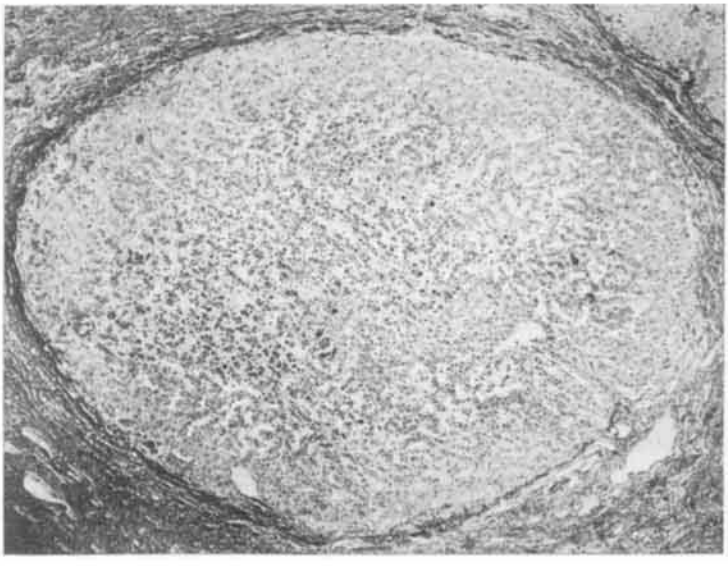

Fig. 28. (× 60.)

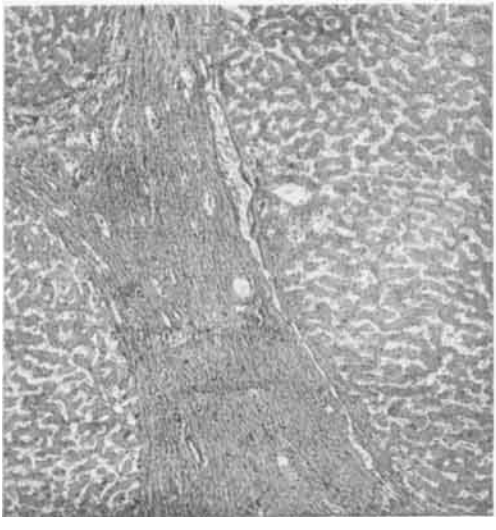

Fig. 29. (× 60.)

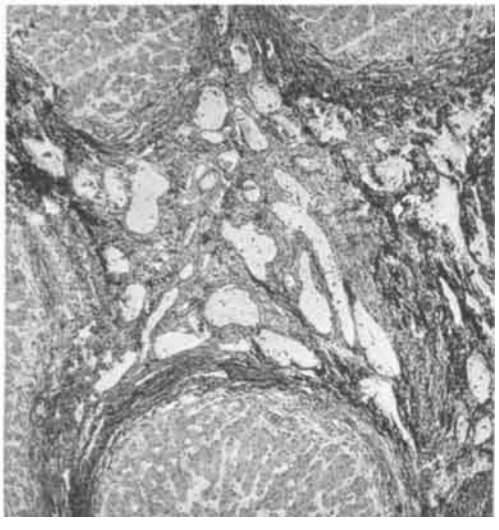

Fig. 30. (× 50.)

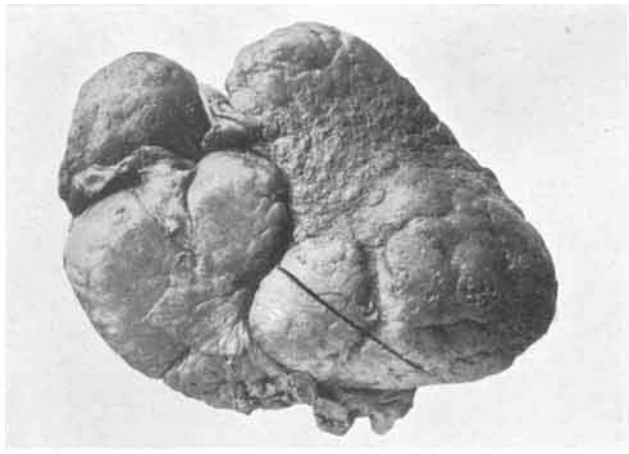

Fig. 31.

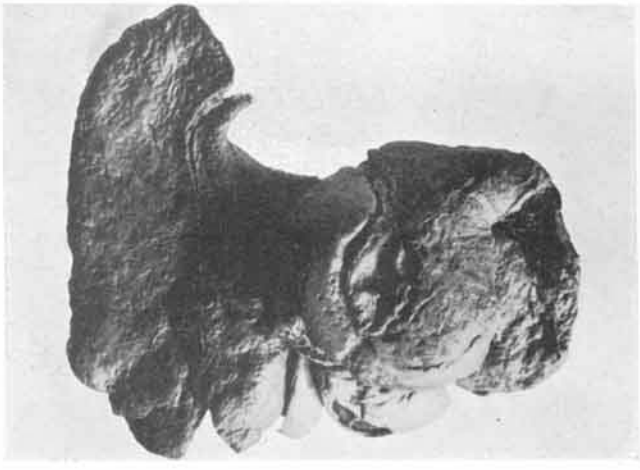

Fig. 32.

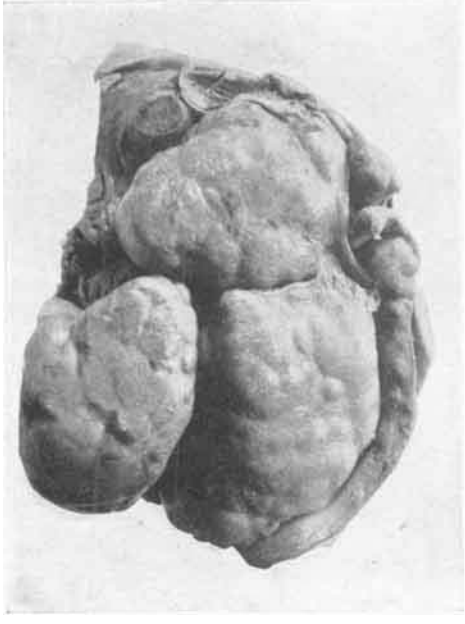

Fig. 33.

Fig. 34.

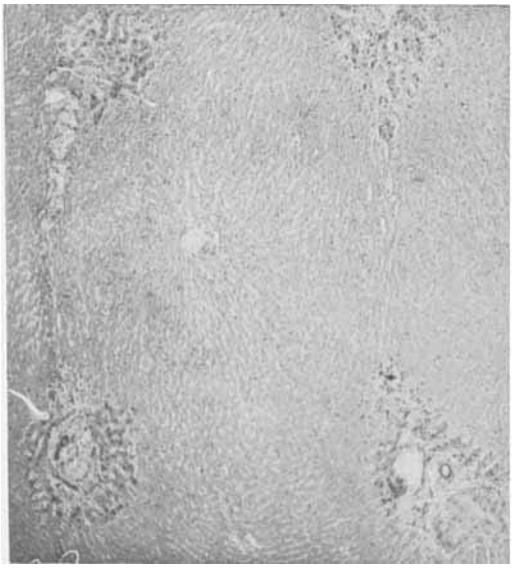

Fig. 35. (× 75.)

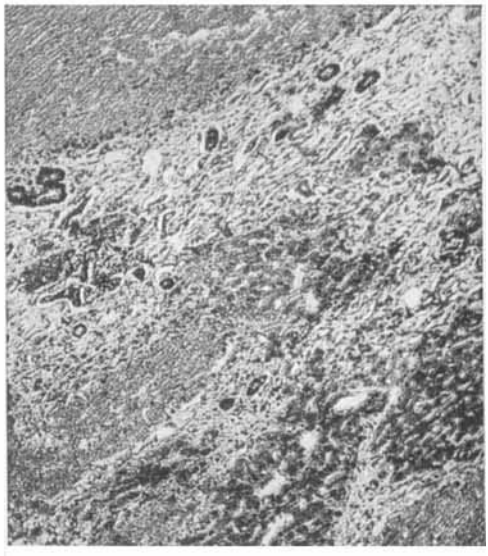

Fig. 36. (× 75.)

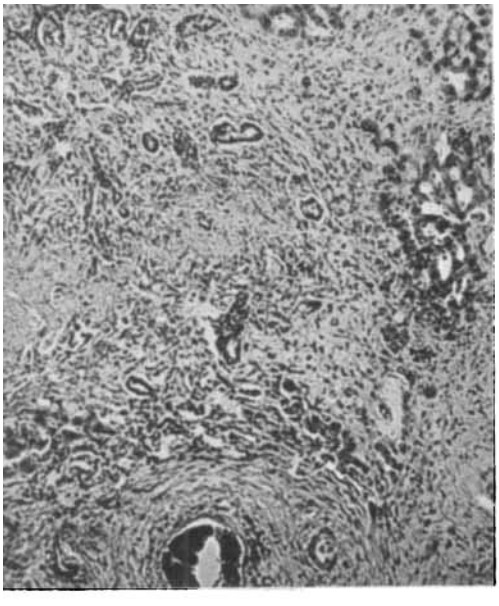

Fig. 37. (× 75.)

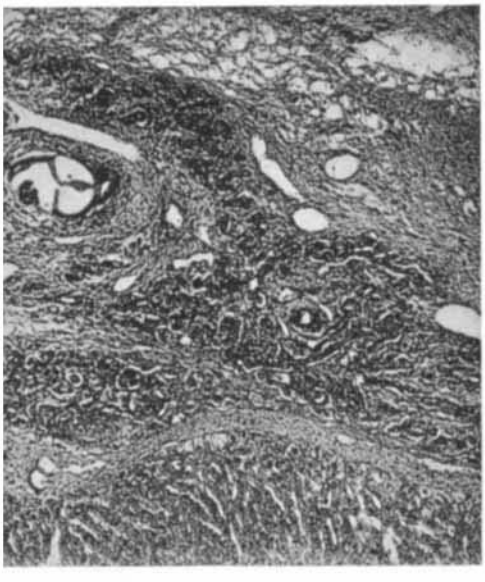

Fig. 38. (× 75.)

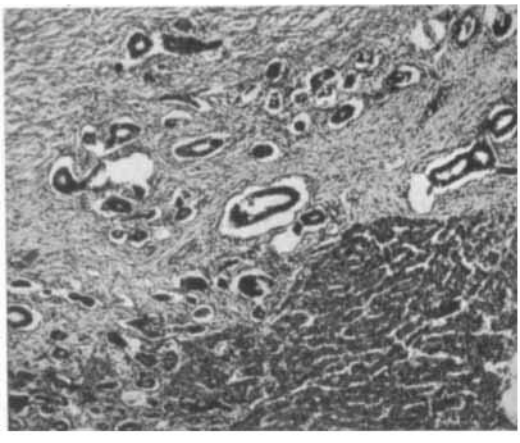

Fig. 39. (× 75.)

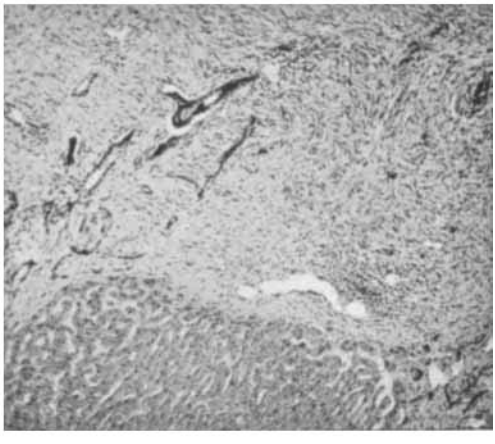

Fig. 40. (× 60.)

FIG. 17.—An isolated liver-cell clump, from the same case, showing the close relationships of the liver cells with small darkly staining cubical cells often arranged in duct shape. (× 300.)

FIG. 18.—An isolated column of liver cells, from the same case, showing its connection with a long "bile duct." This duct, by the aid of serial sections, could be traced back to an interlobular bile duct. (× 300.)

FIG. 19.—Section from the apparent capsule round a primary tumour in the liver of a child 6 months old. This area is composed of atrophic liver cells which alternate with fibrous areas, in which ramify numerous "bile ducts" lined by a cubical epithelium. These ducts may be seen in the centre of the illustration, and they frequently communicated with liver-cell columns and also with interlobular bile ducts. (× 120.)

PLATE XXIII.

FIG. 20.—A clump of hyperplastic liver cells isolated amongst fibrous tissue in a cirrhotic liver. (× 300.)

FIG. 21.—A section of the liver from a case where there had been extensive infiltration of lymphadenomatous nodules. Several liver cells may be seen in mitotic division. (× 300.)

FIG. 22.—A section of the liver from a case of leukæmia. It shows evidences of cellular multiplication by amitosis. Some of the liver cells have very large nuclei, other nuclei are somewhat dumb-bell shaped, and frequently two nuclei can be seen in a cell. (× 500.)

FIG. 23.—A liver cell showing mitotic nuclear division. From the same case. (× 1000.)

FIG. 24.—A group of hypertrophic liver cells from a case of chronic venous congestion. (× 100.)

FIG. 25.—An area of hypertrophic liver cells from a case of old-standing cirrhosis of the liver. (× 160.)

PLATE XXIV.

FIG. 26.—Cirrhosis in the liver of a cat. (× 60.)

FIG. 27.—Cirrhosis of the liver in a girl 13 years of age. The liver cells are very irregularly arranged, and vary in size and staining reactions. (× 90.)

FIG. 28.—A nodule of liver cells in a cirrhotic liver showing no definite lobular structure. Only two hepatic venules appear in this mass, and they are very irregularly situated. (× 60.)

FIG. 29.—A quiescent cirrhosis. The fibrous tissue is dense, sharply defined, and contains practically no "ducts" lined by a cubical epithelium. The liver cells have a trabecular arrangement. A central vein may be observed close to the fibrous tissue. (× 60.)

FIG. 30.—A quiescent cirrhosis showing congestion of the fibrous tissue. It is stained by Weigert's resorcin-fuchsin method, and shows the large amount of elastic tissue formation which is generally to be noted in these cases. (× 50.)

PLATE XXV.

FIG. 31.—Cirrhotic liver showing hyperplastic nodules projecting on its surface. (Breadth of liver, 6¾ in.)

FIG. 32.—Cirrhotic liver showing great enlargement of the Spigelian lobe. (Breadth of liver, 7½ in.)

FIG. 33.—Cirrhotic liver showing almost complete atrophy of the left lobe. Three large nodules can be seen projecting from the under surface of the organ. (Vertical diameter of the liver, 5¼ in.)

FIG. 34.—Liver showing almost complete atrophy of the left lobe. The Spigelian lobe and right lobe are greatly enlarged. (Vertical diameter of the liver, 11 in.)

PLATE XXVI.

FIG. 35.—The margin of a necrosis, in the liver of a rabbit, of forty-eight hours' duration. Four portal spaces with a narrow ring of liver cells round each can be seen to have escaped the necrotic process. (× 75.)

FIG. 36.—The margin of a necrosis of six days' duration. Numerous "ducts" may now be seen to be developing. (× 75.)

FIG. 37.—A necrosis of eight days' duration, showing numerous "ducts" developing along the margin of the liver tissue, and also amongst the new fibrous tissue from the regions of the portal spaces which have been spared the destructive process. (× 75.) Many of the "ducts" are also becoming displaced by the development of inflammatory tissue.

FIG. 38.—The margin of a necrosis of three and a half weeks' duration, showing very numerous "ducts" amongst the newly developed fibrous tissue. (× 75.)

FIG. 39.—The margin of a necrosis of six weeks' duration, showing the atrophy of the "ducts" which is beginning to take place. (× 75.)

FIG. 40.—The margin of a necrosis of two months' duration. The "ducts" are now embedded in dense fibrous tissue, and have become very much atrophied. (× 75.)