On the Relation of the Liver Cells to the Blood-vessels and Lymphatics.

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(Communicated by Professor E. A. Schäfer, F.R.S. Received May 31,—Read June 14, 1906.)

(From the Physiological Laboratory of the University of Edinburgh.)

[Plates 22 and 23.]

The description by Schäfer (55) of a network of fine channels in the cells of the liver of the rabbit and cat which can be filled with injection material from the blood-vessels, and the confirmation of his observations in the livers of other animals as the result of our own experiments (26), have opened up several important questions concerning the minute anatomical structure of the liver. The presence of intracellular channels in the liver cells communicating with the blood-vessels is difficult to reconcile with the generally accepted views on the relations of the blood-vessels and lymphatics to the liver cells. Of late years several observers (Browicz (8), Schäfer (55)), have cast doubt on the presence of perivascular lymphatics in the liver lobules, and have suggested a direct supply of blood plasma from the vessels to the interior of the liver cells without interposition of lymph spaces. That the walls of the capillary blood-vessels of the liver possess a peculiar form of endothelial lining has been long recognised (Kupffer (37), Ranvier (50), and others). More recently Minot (45), from a study of the development of the liver vessels, has concluded that they are not true capillaries which have grown into the organ, but "sinusoids" which have been formed by a growth of the liver blastema into a large blood sinus, which, although having the appearance of capillaries, are actually spaces between the columns of liver cells lined by cells of an embryonic character.

To resolve the question of the relationship of the blood and lymph to the liver cells, we have in many kinds of animals injected the blood-vessels with carmine gelatine, and have, in dogs and cats, injected the large lymphatics of the liver with the same material. We have also injected the bile ducts in a number of animals and have further examined sections of liver stained by special methods. The results of our observations are recorded in this paper.

We are indebted to Professor Schäfer for help and advice in our work, and to Mr. Richard Muir for the care with which he has executed the accompanying drawings. The expenses of the research have been defrayed

by a grant from the Moray fund for the prosecution of research in the University of Edinburgh.

The Injection of the Liver Cells from the Blood-vessels: Method employed.

The injection material we have used in the majority of our experiments was carmine gelatine made up according to Carter's formula. The solutions of gelatine and ammoniacal carmine were filtered separately and very carefully, then mixed and rendered slightly, but distinctly, acid with acetic acid. With such an injection mass there is no staining of tissues, and no diffusion through the walls of ordinary capillaries. On some occasions we used Prussian blue gelatine and also watery Prussian blue, but we found both of these too diffusible; nor do they allow of such good fixation of the tissues as can be got by putting carmine gelatine injected preparations into cold formalin. We also tried thick suspensions of Chinese ink, and in one case hog's lard melted and filtered. Of all these, the carmine gelatine mass has given by far the best results.

The apparatus used for injection consisted of a large pressure bottle with rubber pump attached and another tube leading to a bottle containing the injection mass. A T-shaped junction on its course was led off to a mercury manometer, which indicated the pressure employed. The bottle containing the injection mass was immersed in a large bath of warm water; the mass flowed from it along a rubber tube to the cannula.

The injection was made as soon after death as possible. The animals were killed either by an overdose of chloroform or by coal gas. The thorax or abdomen was opened and the cannula tied to the aorta or the portal vein. The rubber tube and cannula were carefully filled with injection mass, to the complete exclusion of air bubbles, a side tube being adapted to the cannula to facilitate this. This side tube being then closed and the animal immersed in the water bath, the pressure was gradually raised by pumping air into the pressure bottle, the inferior vena cava having first been opened above the diaphragm to allow the free escape of blood. When the injection was made by the portal vein we ligatured the inferior vena cava below the liver to prevent any backward flow through the large veins to other parts of the abdomen. We did not, as a general rule, wash out the blood-vessels by the previous injection of salt solution, but when this was done we had a second bottle containing the saline solution attached by T-tubes to the same system; by opening and closing clips on the tubing we could inject either with carmine gelatine or with salt solution. The fluids injected, bottles, tubing, and animal were kept at body temperature by complete immersion in the water bath. The washing out of the blood-vessels by salt solution is

unnecessary in the case of the liver, and as a rule we found that we got better injection preparations by using the carmine gelatine to drive out the blood, but the cells of the liver can be injected quite well after a preliminary injection of salt solution, and even after the injection of saline saturated with chloroform.

When the injection material was flowing freely from the opened inferior vena cava the portal vein was ligatured. In some experiments we allowed the injection material to fill up and distend the liver by ligaturing the inferior vena cava above and below the diaphragm before tying the portal vein, in others we left the inferior vena cava unligatured and allowed free escape of the fluid all the time, so as to avoid any excess of pressure. In one animal, a dog, the portal vein and hepatic artery were ligatured, and the cannula was put into the aorta close to the heart, a pressure of 120 mm. Hg. being used. There was a free escape all the time from the inferior vena cava above the diaphragm, but, nevertheless, some of the injection mass passed backwards along the hepatic veins and reached the central parts of the lobules of the liver. Even under these circumstances the liver cells near the central vein of each lobule were copiously injected with the carmine gelatine. The pressures employed varied from 60 to 160 mm. of Hg when the injection was made from the aorta, and rarely exceeded 20 mm. of Hg when made from the portal vein.

We have also injected several frogs with carmine gelatine. In order to get a free flow of the gelatine through the vessels, each frog was pithed and then placed for half an hour in the warm bath (37° C.), at the end of which time it was sufficiently warmed to prevent solidification of the gelatine.

In all cases, after the injection was completed, the liver was removed if the animals were large; if small, the abdomen and thorax were freely opened, and the liver or the whole animal placed at once in 10-per-cent. solution of formalin with some ice added. When the gelatine had set the liver was removed, cut into pieces and put back into 10-per-cent. formalin. When thoroughly fixed, pieces from different parts of the organ were dehydrated with alcohol, cut in paraffin, and lightly stained with hæmatoxylin. If weak hæmatoxylin be used the carmine retains its colour while the nuclei of the liver cells give a good blue contrast. Deep staining must be avoided as it stains the gelatine and masks the carmine.

The use of two injection masses of different colour we found to be unsatisfactory. We tried Prussian blue mass followed by carmine gelatine in one dog, keeping up the first injection for some time so as to fill the lymphatics of the liver. Carmine gelatine was then run through to wash away the Prussian blue from the blood-vessels, and to fill them with red injection.

After fixation with alcohol, sections of the liver showed a great deal of staining with Prussian blue; the blood-vessels were imperfectly injected; some contained the first injection, some nothing but carmine mass; but in most the two were mixed. The first injection tends to adhere to the walls of the blood-vessels, and is taken up by Kupffer's cells; this also occurs frequently in poor injections where blood and injection mass mingle, and it is the walls of the vessels which are chiefly coloured by the pigment. The liver cells in the above experiment contained Prussian blue mass in some situations, and carmine gelatine in others. We tried two different injection masses in several other animals, but the results were unsatisfactory, partly on account of the manner in which the first injection mass clings to the walls of the vessels, and partly because the Prussian blue (which we used for a colour contrast) is diffusible.

In several experiments, after injection of carmine gelatine, we washed out the vessels with a large quantity of salt solution. Sections of such livers showed the vessels for the most part free from colour, but the liver cells containing carmine gelatine, which was also present in Kupffer's cells.

Previous Observations.

That it is possible to inject liver cells from the portal vein was shown by Asp (3) in 1873. Asp employed a solution of alcannin in turpentine oil, and injected the portal vein with a pressure not exceeding 30 mm. Hg. The alcannin finds its way into the liver cells, which present appearances similar to those produced in them by injecting the bile ducts. Asp specially noted that though the alcannin penetrates into the liver cell, both from bile ducts and capillaries, it does not pass through the liver cell, so that capillaries cannot be injected from the bile ducts, nor bile ducts from the capillaries. No particular notice appears to have been taken of this discovery, nor, apparently, did Asp himself attach importance to it.

It was noticed by Hüttenbrenner (31), and later by Rutimeyer (54), that cinnabar particles, when introduced into the circulating blood by the jugular vein, are soon found in the liver cells (among other places). Rutimeyer found them in the cells of the peripheral parts of the lobules an hour after injection in the dog, and more uniformly distributed if a longer time had elapsed between the injection and death. The same is true of the fat globules after intravenous injection of milk.

In 1895 J. W. and E. Hewat Fraser (20) drew attention to the presence of inter- and intra-cellular passages in the liver of the frog. They used both Hoyer's lead chromate gelatine and Carter's carmine gelatine, and injected from the bulbus aortæ at a pressure of from 1 to 4 inches of mercury.

They stated that the injection material penetrates between the liver cells and finds its way into them by one or more fine channels, reaching even into the nucleus and ending in knob-shaped dilations. Increase of injection pressure causes an accumulation of the injection mass in reservoirs or vacuoles in the cell protoplasm, but does not distend to any appreciable extent the fine passages leading to them from the blood-vessels. The brothers Fraser concluded that in the frog at least a system of fine plasmatic channels exists in the liver, too small to admit the corpuscles of the blood, but affording a direct means of communication between the blood plasma and the interior of the liver cells.

Nauwerck (46), after reading Fraser's paper, looked carefully over a series of preparations of injected human livers, and found the injection present in the liver cells, often in ring-shaped canals round the nucleus, but never penetrating into it. Nauwerck described two intra-cellular networks of fine channels, one system belonging to the bile ducts and revealed by special stains, the other pertaining to the blood-vessels.

Browicz (9), from a study of normal and pathological human livers, and from the result of the intravenous injection of solutions of hæmoglobin in the dog, came to the conclusion that there must exist in the liver cells special afferent nutritive channels or canaliculi. These channels convey plasma and even red blood corpuscles directly to the nuclei of the liver cells. The red blood corpuscles are broken down in the cells, and the hæmoglobin is stored in the nuclei and converted into bile pigment; a second set of fine intra-cellular channels conveys the bile as it is formed into the bile canaliculi. Browicz (8) doubted the presence of perivascular lymphatics round the liver capillaries, and believed in a communication between the blood capillaries and the cytoplasm and even the nuclei of the liver cells. He found red blood corpuscles in various stages of disintegration in the liver cells. If in the dog's liver hæmoglobin had been injected intravenously some hours before death, free hæmoglobin and crystals of hæmoglobin were present both in the cytoplasm and in the nuclei. Browicz did not see the intracellular canaliculi, but argued that they must be present, though exceedingly fine.

In 1901 Schäfer (55) described specimens of livers of rabbit and cat in which the liver cells everywhere contained networks of injection material—carmine gelatine—which had entered them from the blood-vessels. The livers had been injected from the portal vein, but no record of the pressure employed had been kept. Schäfer described the injection within the cells as "confined within sharply-defined, somewhat varicose, intercommunicating canaliculi, many of which are in the immediate neighbourhood of the nucleus or nuclei," but he did not observe any injection actually within the nuclei.

He described the injection as having the same intensity of colour within the cells as in the blood-vessels, and hence inferred that it had not passed into the cells by way of lymph spaces, or it would have become diluted with lymph. Nor had the injection passed into the cells after having been extravasated into the intercellular biliary canals, for the latter contain no trace of injection and were, indeed, completely invisible. This was also the case with the perivascular lymphatics of the lobules (if such vessels exist). In the substance of the lobules the injection was confined to the blood-vessels and to the intracellular channels. There was no diffusion of carmine, and the cell nuclei were wholly unstained. The injection material was also apparent, but of a fainter colour, *i.e.*, in a diluted condition, in the lymphatics accompanying the branches of the portal and hepatic veins. The connective tissue around these vessels and extending a short distance into the lobules was stained by carmine. Schäfer was unable to detect the existence of perivascular lymphatics in the liver lobules. His description of the injection appearances agrees very closely with what Browicz assumed might be found. Browicz, to whom Schäfer sent preparations, states in a later paper that "the injection appearances are nothing less than ideal."

In 1902 E. Holmgren (28) described channels in the liver cells of the hedgehog. Within these channels are what he terms "Trophospongien," viz., the intracellular processes of certain multipolar cells. Functional or metabolic changes inside the processes lead them to become more or less fluid in character, and give the cell in which they are embedded the appearance of possessing a network of canals. In a later paper Holmgren (29) criticises the views of Browicz and Schäfer. He states his belief that the plasmatic channels described by them are the same as his "Trophospongien," and as such are not in direct connection with the blood-vessels, but open into perivascular lymphatics. The "Trophospongien" are, according to his view, processes of Kupffer's cells which extend into the protoplasm of the neighbouring liver cells, and he considers this view the more probable because of the property Kupffer's cells possess of destroying red blood corpuscles, thereby constituting a kind of trophic element in connection with the liver cells. In yet another paper Holmgren (30), after repeating the statements he had already made, expresses the opinion that the star-shaped connective-tissue cells, described by Reinke in the liver, may with great probability be regarded as the origin of the "Trophospongien" of the liver cells.

With regard to the injected preparations described by Schäfer, one of which he had the opportunity of examining, Holmgren enunciates the confident opinion that the intracellular injection described is an artefact produced by too high pressure having been employed in making the

injection, and believes that he can show that the injection material in these preparations has passed from the blood-vessels into the liver cells by way of the lymphatics.

Schäfer (56), in replying to Holmgren's criticisms, points out that although the pressures employed in injecting the preparations described are not known, the appearances cannot be artefacts, for the injection is uniformly present in the cells throughout the liver, except in the immediate neighbourhood of extravasation, where it is absent or imperfect. The appearances which Holmgren considered to indicate the injection of perivascular lymph spaces are merely clefts in the gelatine produced by shrinkage during the process of hardening by alcohol, a portion of the gelatine tending, under these circumstances, to adhere to the wall of the capillary. It is this layer which Holmgren has taken to represent the injection of a perivascular lymphatic.

Description of our Experiments: Results Obtained.

It was to settle the question as to what amount of pressure is required to inject the liver cells from the blood-vessels that we began our work, a preliminary note of which was published last year. We made a commencement by injecting rats with carmine gelatine from the aorta in the manner already described. The rat was selected partly because of its small size, and partly because Dr. H. K. Anderson, of Cambridge, had sent to Professor Schäfer last summer specimens of rat's liver in which the cells were beautifully injected with carmine gelatine.*

In all, 20 rats were injected from the aorta close to the heart, at pressures varying from 80 to 120 mm. Hg. The inferior vena cava was opened above the diaphragm, and in most cases remained open so as to allow a free escape of blood and injection mass. In others the inferior vena cava was ligatured after the injection mass had begun to flow out, and the injection pressure was maintained some minutes longer before tying the portal vein. In all these rats the liver cells contain carmine gelatine. Some of the best specimens were obtained with a pressure of 80 mm. Hg in the aorta, and a free escape from the inferior vena cava during the whole period of injection, which occupied about five minutes. This pressure cannot be called excessive. The flow from the inferior vena cava was quite free all the time, and the pressure in the vessels of the liver must have been very low. Fig. 4 is from a specimen injected at this low pressure. Increase of pressure

* Dr. Anderson informs us that the rat was injected from the aorta, and that during the injection there was a free escape from cut intercostal arteries; therefore the pressure in the portal vein could not have been excessive.

distends the blood-vessels and increases the amount of injection mass in the liver cells—not by the appearance of more canals, but by the widening of vacuole-like spaces on their course. The intracellular channels of the rat's liver appear, therefore, to be very easily injected; in fact, it would seem difficult to avoid injecting them.

We are able to confirm the observations of the brothers Fraser on the frog; a pressure of 20 mm. Hg at the bulbus aortæ injects the cells of the liver so as to show fine channels ending in vacuole-like dilatations. We are unable to find any injection in the nuclei. Carmine gelatine is not, however, a very suitable injection for cold-blooded animals.

Four rabbits were injected; all show the fine intracellular channels described by Schäfer. Three were injected from the aorta with pressures varying from 100 to 120 mm. Hg; one from the portal vein with 30 mm. pressure. But in this latter experiment there was considerable leakage from rupture of one of the large branches of the portal vein, and the pressure in the liver must therefore have been less than that recorded by the manometer.

Two guinea-pigs were injected with aortic pressures of 100 mm. and 120 mm. Hg respectively. The injection mass did not run well in the first experiment, but the livers of both animals show intracellular injection.

One hedgehog, injected from the aorta at a pressure of 120 mm. Hg, shows the intracellular injection appearances; there was a free flow of injection mass from the inferior vena cava the whole time. In the only other hedgehog tried the injection fluid did not flow properly, and the injection failed, the vessels of the liver being incompletely filled. The injection mass employed in this case had been made up a considerable time, and we have found by experience that the best results are obtained with freshly prepared material.

In the dog, every injection shows the intracellular channels. In one animal, a young one, a pressure of 100 mm. in the aorta gave injection appearances throughout the cells of the liver. In another the injection was made by the portal vein, the inferior vena cava having been opened in the thorax and ligatured in the abdomen below the liver. Three hundred cubic centimetres of Ringer's solution, saturated with chloroform, was first perfused at a pressure of 20 mm. Hg; the carmine gelatine was then allowed to run in, the inferior vena cava next ligatured, and a pressure of 20 mm. Hg maintained during 10 minutes. Many of the liver cells contain carmine gelatine, which has the appearance of being accumulated chiefly in rounded masses as if in distended vacuoles.

In one of the injections from the aorta, as already mentioned, the portal

vein and hepatic artery had been tied prior to the experiment, and the injection mass passed backwards from the inferior vena cava along the hepatic and sub-lobular veins, and reached the inner parts of the lobules, filling the cells copiously with injection. In this case the pressure in the hepatic veins must have been very low, because there was a free escape all the time from the inferior vena cava in the thorax.

The portal vein of another dog was injected for one hour with Prussian blue gelatine at a pressure of 40 mm. Hg, the object of the experiment being to fill the lymphatics of the liver with blue injection mass. Carmine gelatine was next run through until it escaped from the inferior vena cava, then the large vessels were ligatured and the liver placed in cold alcohol. Sections show Prussian blue gelatine in some cells and carmine gelatine in others. The Prussian blue is, however, very faint and diffuse in those lobules which are well injected with the carmine mass, but of a deep colour in the connective tissue of Glisson's capsule.

Another dog was injected with melted and filtered hog's lard through the portal vein at a pressure of 40 mm. Hg, a piece of liver having been first removed as a control. The cells of the injected liver are full of fat globules, as shown by staining with Sudan III and osmic acid.

In the cat a pressure of 100 mm. Hg in the aorta with free flow from the inferior vena cava gives a plentiful injection of the cells throughout the liver. We injected seven cats, with positive results in every case. A pressure of 20 mm. Hg in the portal vein is sufficient to give very good injection of the cells. In one cat 600 c.c. of Ringer solution, saturated with chloroform, was perfused through the liver from the portal vein. Carmine gelatine was then injected at a pressure of 40 mm. Hg; the injection appears in the liver cells. Chinese ink rubbed up with salt solution was also employed in one animal. Some of the smaller particles were found in the liver cells, but most of the particles were too coarse.

One ferret was injected from the aorta with a pressure of 80 mm. Hg, the inferior vena cava being open the whole time. The liver cells show intracellular injection to a marked degree.

The monkey presented unexpected difficulty. One only (*Macacus rhesus*) was successfully injected from the portal vein with a pressure of 60 mm. Hg, the inferior vena cava being open. The liver of this monkey shows intracellular injection, but not uniformly. The intracellular channels seem to be much finer than those of other animals. In some cells there are rounded accumulations of injection material, as though lying in distended vacuoles (fig. 6). Two other monkeys injected from the aorta, at pressures of 120 and 130 mm. of Hg respectively, show intracellular injection, but not well

marked. Others failed to show the intracellular channels. The cells of the monkey's liver appear, therefore, to be injected less readily than those of the other animals investigated, and for their injection comparatively high pressures were necessary. But whether this result is due to the fine calibre of the channels, or to some unknown accidental circumstance, we are unable to say.

Three birds, a common fowl and two pigeons, were injected from the right aortic arch at pressures of 100 and 130 mm. Hg. Many of the liver cells contain fine threads of carmine gelatine, with occasional rounded or irregular accumulations. In these birds, as in the monkey, the intracellular passages are very fine (fig. 9).

From what has been stated about the pressures of the injections employed, it will be seen that they are not excessive, and many of them might quite well be exceeded by that of the blood during life. When the inferior vena cava was ligatured before the injection pressure was cut off, the pressure in the liver vessels must have risen considerably, but even in these cases, when the injection was made from the aorta, the mass, with the exception of that entering by the hepatic artery, has already traversed one set of capillaries before it reaches the liver. As has already been insisted on, some of the best injections were made with the inferior vena cava open. In the rat an aortic pressure of 80 mm. Hg, with free escape of the injection mass from the inferior vena cava, yielded one of the best of our preparations (fig. 4), the cells being injected uniformly throughout the organ. In one cat a pressure of 20 mm. Hg in the portal vein, with free escape from the inferior vena cava, also yielded a typical injection of the cells. In a dog, with hepatic artery and portal vein tied, there was sufficient backward flow to inject the cells at the central parts of the lobules, although the inferior vena cava was open in the thorax.

The appearance of injection material in the liver cells cannot therefore be ascribed to excess of pressure in the blood-vessels. The character of the injection in the cells, too, as was pointed out by Schäfer, is against any such supposition. In many places there are definite fine channels continuous with the lumen of the blood-vessel, forming a network within the cell. Dilatations on the network are often seen. These are probably vacuoles in the cytoplasm, and may be more distended with increase of pressure, but the typical network is best seen when a moderate pressure only has been employed.

The presence of injection within the cells is not the result of vital activity of the cell protoplasm. The circumstances of its occurrence are against this supposition, as well as the fact that the cells can be injected a considerable time after the death of the animal. Perfusion of large quantities of chloro-

form saline does not prevent the subsequent injection of the cells; and in one cat, perfusion of 300 c.c. of a 2-per-cent. solution of cyanide of potassium was followed by a successful result, although in this experiment the liver cells were considerably altered and the nuclei shrunken.

The appearances are against simple filtration from the blood-vessels into the cells. The colour of the carmine mass is of the same tint within the cells as in the blood spaces, and in many places a direct connection between the two can be made out. There is, besides, no diffusion of carmine and no staining of nuclei by it.

The question of the relation of the lymphatics to the injection is an important one. The injection certainly passes readily into the lymphatics and appears in the large trunks of the portal spaces. In these situations the injection mass contained in the large trunks is of a lighter colour and is obviously diluted with lymph, but the injection mass within the lobules shows no such appearance of dilution, nor, indeed, are any spaces visible but the blood spaces occupied by the injection. The mass in these is usually in close contact with the liver cells, and where spaces exist between the two they are clearly the result of shrinkage of the gelatine. If perivascular lymphatics exist in the lobules they must be filled with the injection mass, if it is through them that the liver cells become injected. We can make out no appearances suggestive of such a path for the injection. The injection is inside the blood-vessels and inside the liver cells; nowhere else within the lobule.

The relation of Kupffer's cells to the injection is an interesting one and will be dealt with more fully later. In a complete injection these cells are more or less hidden by the carmine gelatine, but in an imperfect injection, where comparatively little colouring matter is mixed with the blood, it is seen that Kupffer's cells are frequently coloured as though they had picked up the carmine from the vessel contents. (The presence of colouring matter on either side of the nucleus of a Kupffer's cell gives in such cases an appearance which might be mistaken for carmine gelatine on either side of the vessel wall.) When the blood-vessels are washed out after an injection of carmine gelatine, the injection mass often adheres to the vessel wall closely applied to the liver cells, and is especially noticeable at the Kupffer's cells. The cytoplasm of these cells is usually injected, but not the nucleus. The injection within their cytoplasm seems to be uniform and not confined to channels as within the liver cells, but the cytoplasm of Kupffer's cells is so small in amount that it is difficult to determine what has really happened, whether injection, infiltration, or absorption. The latter is possible and seems probable where the injection mass is small in amount and mixed with the blood.

We have seen no evidence in support of Holmgren's theory of Kupffer's cells sending processes into the liver cells to form "Trophospongien." The injection in the liver cells bears no apparent relation to Kupffer's cells; moreover, the latter are placed at comparatively wide intervals from one another, and it is inconceivable that they should send processes to all the intermediately situated liver cells.

A perfectly satisfactory conclusion with respect to the lymphatics can only be arrived at by the actual injection of the lymphatics of the liver. We shall revert to the subject again when dealing with the injection of the lymphatics.

In none of the specimens is there any indication of the injection having burst into the bile ducts or capillaries and so entered the liver cells. The characteristic network of the bile capillaries is not seen in any of our injected specimens, and there is no injection inside any of the issuing bile ducts, nor are the epithelial cells lining the ducts injected. After the injection mass has entered the liver cells it is difficult to wash it out by perfusion of the vessels with salt solution.

We have little to add to the description of the intracellular channels already given by Schäfer in the rabbit and cat liver. They form an irregular network in the liver cell. This network may be in direct communication at more than one point with the blood spaces. As a rule, the channels leading into the cell are fine—far too fine to admit red blood corpuscles; sometimes, however, a comparatively wide opening is seen. A ring-shaped channel is sometimes seen round the nucleus, as described by the Frasers in the frog, and by Nauwerck in the human liver. The injection frequently has the appearance of rounded or irregularly shaped accumulations, as if in vacuoles of the cytoplasm; a cell may contain many such clumps, and connections between them are not always apparent.

The injection is often seen in close contact with the nucleus, or both nuclei where two are present. As a rule it does not penetrate into the nuclei, but in the rat we have found it inside the nuclear membrane; when this is the case, it appears diffused throughout, and not lying in special intranuclear channels. The intracellular channels vary to some extent in the livers of animals of different species, but their general features are the same. The network arrangement is best seen in the rat and the rabbit. They are larger and more moniliform in the cat, still more so in the dog. In the monkey, as already intimated, they are very fine and less readily injected; and in the few birds we have examined they are also very fine.

We have not extended our observations to reptiles and fishes.

Our attention has been drawn by Mr. Richard Muir, of the Pathological

Department of the University, to a preparation of human liver from a case of so-called chloroform poisoning. There is extreme fatty degeneration (?) of the liver cells, and fat emboli in the blood-vessels throughout the body. In the liver, many of the vessels are filled with fat in continuous lines, and here and there are distinct connections between the fat in the cells and the fat in the vessels. The appearance is, indeed, very similar to the injection appearances recorded. The preparation was stained with osmic acid (fig. 10).

In examining sections of the dog's liver (uninjected) stained with eosine and methylene blue we have frequently seen crystals in the nuclei of the liver cells. The crystals are prismatic in shape and vary in length. Some are short and cause no distension of the nuclear membrane, while others are as much as three times the diameter of an average liver cell nucleus, and the nuclear membrane has the appearance of being stretched by the crystal (figs. 1 and 2). We have never seen these crystals in any other situation than inside the nuclei of liver cells, and they seem to occur with equal frequency in the lightly staining and in the darkly staining nuclei. We have seen them in the dog's liver in specimens from five different animals. Most of the preparations were fixed in 10 per cent. formol, but one specimen was fixed several years ago in corrosive sublimate for class purposes; sections of it show numerous crystals. Not more than one crystal is found in a nucleus. There is little nuclear network in the liver cells of the dog, but a nucleolus is present and is in the crystal-holding nuclei always situated close to the nuclear membrane on one side of and immediately opposite the middle of the crystal. The crystals stain with eosine rather more deeply than the nucleoli and red blood corpuscles. They are of prismatic form, with sharp edges, and closely resemble crystals of hæmoglobin. In some nuclei, irregular or rounded masses similarly staining appear. In the cytoplasm of some cells red blood corpuscles are present, some of which are unaltered and others more or less disintegrated. The crystals are probably composed of hæmoglobin or, at any rate, of some derivative of the blood pigment. That they are formed during life is obvious from the enlarged size of the nuclei which contain them and the adaptation of the shape of the nuclei to the size and shape of the contained crystal.

Similar crystals were described by Browicz in 1899. Browicz found them in the dog's liver after the intravenous injection of a solution of Merck's hæmoglobin. He also described the breaking down of red blood corpuscles in the liver cells and storage of hæmoglobin in the nuclei. The presence of hæmoglobin in the liver cell nuclei was one of the chief arguments advanced by Browicz in favour of a very intimate relationship between the circulating blood and the interior of the liver cells.

In the dog's liver there is, then, as Browicz originally stated, good evidence that red blood corpuscles pass into the liver cells and are broken down there. Hæmoglobin readily crystallises in the dog, which may account for its presence in a crystalline form in the liver cells of this animal. Further search may show similar crystals in the liver cells of other animals, although we have ourselves failed to find them, nor have we succeeded in finding them in the dog in unfixed and unstained sections.

The Lymphatics of the Liver. (*Observations of Previous Inquirers.*)

The lymphatics of the liver were investigated by Mascagni, Cruickshank, Lauth, Arnold and Sappey. As a result of the work of these and other anatomists arose the classical division of the liver lymphatics into a superficial and a deep set. The method employed by the older investigators was that of injection with quicksilver, whereby the vessels were distended and made clearly visible.

Kiernan (33), in 1833, stated that when the bile ducts are injected with fluid the injection material frequently passes into the lymphatics, and that even the injection of the portal vein or hepatic artery may be followed by a like result. Beale (4), in 1859, made use of this fact to inject the lymphatics of the ox's liver. He perfused water through the portal vein at a moderately high pressure until the main lymphatic trunks were distended. A cannula was then tied into one trunk and the liver subjected to pressure to squeeze out the fluid. When the lymphatics were thus emptied an injection of coloured material was made through the cannula; sections of the liver were subsequently cut and examined. Beale described lymph vessels on the surface of the liver and in the portal spaces, and thought they might occur inside the lobules, but he had not sufficient evidence to make a positive statement on this point. Teichmann (59), in 1861, described the lymphatics of the human liver. A superficial set forming an irregular network below the peritoneal coat passes by the ligamentum suspensorium to the diaphragm, and through it to join the thoracic duct. On the concave surface of the liver the superficial vessels unite near the gall bladder, some pass to the convex surface of the liver, some to the portal vein, and others sink into the substance of the liver to join the deep set. The latter vessels accompany and surround the branches of the portal vein, hepatic artery and bile ducts, and run between the lobules, but Teichmann could not follow them into the lobules. The injection mass frequently passed through the lobules as far as the central vein. Teichmann could not satisfy himself that it was in lymphatics. The deep set runs to lymphatic glands along the portal vein and then into the thoracic duct.

In 1864, Carter (13), employing carmine gelatine as an injection mass, came to the conclusion that there exists in the liver "a direct communication between the lymph vessels and those of the blood, and their distal as well as their proximal extremities, and in the former position through tubes of dimensions so small as to preclude the possibility of the blood corpuscles entering them." Carter also noted that injection of the portal vein of the liver with injection mass of one colour followed by injection mass of another colour results in a mixture of the two in the lymphatics. He found lymph vessels passing into the lobules and ending in nucleated fusiform cells, and the injection, he said, passes into these cells, which he regarded as the origin of the lymphatics.

In the same year, 1864, MacGillavry (42) published the results of his researches on the lymphatics of the liver. His observations have been widely accepted, and his paper is perhaps the one on the subject which is best known, although much of his work had been anticipated by Beale and by Carter. MacGillavry ligatured the lymphatic trunks of the liver in the portal fissure of a living dog. The lymph in such an experiment soon distends the vessels and brings about a natural injection. MacGillavry could not find the surface lymphatics described by Teichmann and earlier workers, and assumed that they are not a feature of the liver in the dog (and rabbit), but he noticed a large trunk on the gall bladder with numerous branches coming to it from the parenchyma of the liver. The main vessel on the gall bladder accompanies the cystic duct, and ends in a lymphatic gland near the duodenum. The deep lymphatics issue from every lobe of the liver, and follow the portal vein, appearing like strings of pearls, because of the numerous valves on their course.

In another series of experiments the lymphatics were injected with a cold watery solution of Prussian blue. To enable the injected fluid to overcome the resistance offered by the valves, MacGillavry steeped the liver for several hours in weak spirit prior to making the injection. (The alcohol causes the valves to shrink and so renders them inefficient.) A cannula was then inserted into one of the main trunks near the portal fissure, and injection fluid forced in. In some experiments the blood-vessels were subsequently injected from the portal vein with material of a different colour. The livers were hardened and sections cut. MacGillavry described the origin of the deep lymphatics from three different sources: (1) A tubular network surrounding the blood capillaries and stretching from the borders of each lobule to its central vein, and looking very like a network of injected capillaries. In transverse section of the blood capillaries of a lobule, the lymphatics surround each vessel in a ring-shaped manner, and the walls of the lymphatics are

composed of fine connective-tissue fibrils on the one hand and liver cells and bile capillaries on the other; (2) lymph lacunæ lying in the interlobular connective tissue; (3) narrow anastomosing tubes of spindle-shaped appearance with much sharper outlines than are possessed by the ordinary lacunæ; these also lie in the connective tissue.

No lymphatics occur where there are no blood-vessels, and the latter are everywhere accompanied by perivascular lymphatics.

MacGillavry's results were not accepted by Hering (25), who also injected the lymphatics. Hering could find no evidence of a perivascular injection in the lobules of the rabbit's liver, and although he succeeded in producing in the human liver and in the dog's liver the appearances shown by MacGillavry, he expressed the belief that they are artificial spaces resulting from imperfect methods of injection and preparation. He pointed out that the previous soaking of the liver in spirit must produce shrinkage and alteration in other parts of the liver besides the valves of the lymphatics, and that the injection passes into clefts which do not exist during life, but are the result of *post-mortem* changes. In the rabbit's liver Hering found no trace of perivascular spaces in the lobules; the cells of the capillary wall are in direct contact with the liver cells, and bile capillaries do not come in contact with blood capillaries at any place as stated by MacGillavry. Hering thought it probable that MacGillavry had produced extravasation and rupture into the blood capillaries, and that subsequent injection of these with material of a different colour gave rise to the appearance of one injection surrounding the other in a ring-like manner. Hering also criticised the method of filling the lymphatics by injection of the bile ducts. He found that by careful injection of the bile ducts the injected material can be made to pass into the bile capillaries at the periphery of the lobules and enter the liver cells there, at first in small amount, but later in sufficient quantity to fill the cells entirely; rupture then takes place into the blood-vessels. If the blood-vessels be now injected with a differently coloured mass, sections of the liver will show the first injection material surrounding the second, and giving appearances which MacGillavry said could only be produced by the distension of perivascular lymphatics.

Irminger and Frey (32) injected the bile ducts with watery Prussian blue and described extravasations into the lymphatics, which they said looked like blood-vessels. They were able to obtain these results only in the rabbit's liver; they agreed with MacGillavry that there are perivascular lymphatics in the lobules.

Biesiadecki (7), in 1867, described spaces between the vessel walls and liver cells in human livers which had been the subjects of chronic venous

congestion ; he considered these to be lymphatics. He made several attempts to inject them, but could not succeed in forcing injection mass past the valves of the main lymphatic trunks, and when he employed the method of injection by puncture the injection material flowed equally into blood-vessels and the spaces round them.

Kölliker (35) emphasised Hering's opinion that in the rabbit's liver, at all events, extravasation from the bile capillaries passes not into lymphatics, but into the blood-vessels.

Kisselew (34), in 1869, injected the liver of the dog and pig, using injection mass of one colour for the lymphatics and of another colour for the blood-vessels. He described perivascular lymphatics within the lobules with walls consisting of fine fibrillar material and endothelial cells. He also found lymphoid nodules in the substance of the pig's liver. He agreed with MacGillavry that the capillaries inside the lobules are surrounded by lymphatics.

Asp (3), in 1873, injected the bile ducts of the rabbit's liver with a solution of alcannin in turpentine oil and found that it passed from the bile capillaries into the liver cells. He also injected watery Prussian blue, and found it throughout the lobules outside the blood-vessels occupying spaces which he considered lymphatics.

Fleischl (19), in the following year, laid stress on the fact noted by Ludwig that after ligature of the common bile duct in the living dog the lymph issuing from the liver is tinged with bile, showing that the obstructed bile channels at some part of their course come into communication with the lymphatics. He tried injection of the bile ducts in the dog with alcannin dissolved in turpentine oil, but the experiments were not successful. The rabbit gave him better results, and Fleischl supported MacGillavry in believing in the existence of lymphatics within the liver lobules.

Wittich (62) came to the same conclusion after employing the method of *intra vitam* injection of sulphindigodate of soda in rabbits.

In 1875 Budge (12) described the results of a large number of injections he had made of the lymphatics of the liver in different animals. He states that asphalt dissolved in chloroform makes the best injection fluid for the lymphatics, and that it is advisable to inject the blood-vessels in addition with coloured gelatine. He also injected the lymphatics with a solution of nitrate of silver to determine whether they are lined with endothelial cells. Some of the injections were made by tying a cannula into one of the large lymphatic trunks in the portal fissure, but his best results were obtained by the employment of a method suggested by Fleischl, viz., the puncture by the fine nozzle of the injecting syringe of the wall of one of the hepatic veins. By

this procedure the injection mass is forced into large lymphatic vessels which lie deeply in the wall of the vein, and it can readily pass from them through the liver to issue by the efferent lymphatics in the portal fissure. Budge was the first to describe the lymphatics of the hepatic veins, and to show that they are very numerous in this situation. He stated that the trunks are large, and have no valves; in the walls of the large veins there may be from 60 to 70 trunks, in the medium sized veins 15 to 20, and from 3 to 5 in the small, and they are lined with endothelial cells. Budge found that the injection passed through the walls of the lobules of the liver in spaces lying between the walls of the blood-vessels and the liver cells, and opened into the large vessels accompanying the branches of the portal vein. The spaces, he argued, must be lymphatics, because they afford the only means of communication between the hepatic and portal lymphatic trunks.

In 1876 Kupffer (37) described in the liver the star-shaped cells which have since borne his name. He believed them to be connective-tissue cells lying outside the blood-vessels of the lobules, and related in all probability to the origin of the lymphatics.

Heidenhain (22), in 1881, spoke of Kupffer's cells as taking a probable part in the formation of perivascular lymphatics.

Disse (15), in 1890, published the results of a number of experiments in which the liver lymphatics were injected by Fleischl's method. Disse removed the liver immediately after death, opened the hepatic veins and introduced the nozzle of the syringe through the wall of one of the veins from the inside. Watery solutions of Prussian blue, and in one case a 0·75-per-cent. solution of silver nitrate were injected, and in many of the experiments the blood-vessels were subsequently filled with carmine gelatine from the portal vein. After the injection was completed the liver was fixed and hardened in alcohol. Disse reviewed the work of previous observers, and raised the objection to most of it that, although spaces had been injected, it had not been shown that they had definite walls bounding them. He held that it was neccessary to prove that the spaces have demonstrable walls which can be shown without filling them with injection mass. Disse's results correspond very closely with those obtained by Budge. He found large lymphatic trunks in the walls of the hepatic veins, and agreed with Budge that they are lined by endothelial cells. The injection material introduced into the wall of the vein quickly spreads under a low pressure, and emerges by trunks in the portal fissure. Only a small portion of the liver in the neighbourhood of the puncture is injected, and in this situation the injection mass penetrates into the lobules and follows the blood-vessels, filling clefts between them and the liver cells, and uniting outside the

lobules with the lymphatics of the portal spaces. Disse could find no endothelial cells lining these clefts, but nevertheless believed them to be lymphatics. Disse, however, found that there is another means of communication between the hepatic and portal lymphatics by large trunks running between the lobules in connective-tissue septa which unite the adventitia of the hepatic veins to the connective tissue of the portal spaces.

Disse also examined sections and teased preparations of healthy livers, and came to the conclusion that the clefts round the capillaries of the lobules are the lymph radicles of the liver parenchyma, and that their walls consist of structureless ground substance and fine fibrils of an equal thickness forming a membrane which is paved at intervals with the star-shaped cells that surround the capillaries. The liver cells, he stated, are contiguous, on the one side with bile capillaries, on the other with lymphatic spaces; and the lymph flow from the lobules can go in two directions, by the portal vein system which emerges from the portal fissure, and by the hepatic system which runs through the diaphragm to lymphatic glands in the posterior mediastinum.

In 1896 Teichmann (60) made an additional contribution to the literature of the subject. He described the lymphatics of the liver as forming networks which surround the branches of the portal vein, but nowhere enter the lobules. He failed to find lymphatics in the walls of the central veins of the lobules and in the walls of the hepatic veins.

In 1898 Reinke (51) described in the liver lobules connective-tissue cells other than Kupffer's cells. He also stated that he had seen the lymph spaces portrayed by Disse lying between the capillaries and the liver cells, and that the connective-tissue cells form sheaths for them; perhaps a lymphatic endothelium.

In the same year Kupffer (38) took up a new position regarding the star-shaped cells he had described in 1876. He now considered them to be endothelial cells of a peculiar nature which belong to the walls of the blood-vessels, and not connective-tissue cells lying outside the blood-vessels.

Browicz (11), at the same time and independently of Kupffer, arrived at a similar conclusion, but went further in regarding these cells as having an intravascular situation. He also stated that the existence of perivascular lymphatics inside the liver lobules is doubtful. In subsequent papers Browicz emphasised the fact that the connection of the liver cells with the blood capillaries is much more intimate than has been generally supposed, and insisted that in all probability the perivascular lymphatics described by MacGillavry and others within the liver lobules do not exist.

Holmgren (28), in 1902, found lymph channels in the liver cells of the hedgehog, and considered that they are in direct communication with lymph spaces lying between the liver cells and the blood-vessels.

In 1902 Schäfer (55) showed that the liver cells can be injected from the portal vein, and that there is a direct communication by means of fine channels between the lobular capillaries and the interior of the liver cells. He could find no trace of perivascular lymphatics within the lobules, but described the injection as having passed in a diluted form into the lymphatics accompanying the branches of both the portal and hepatic veins.

Several authors have argued the presence of perivascular lymphatics in the lobules from the results of work by Fleischl (19), Kunkel, Kufferath (36), and Vaughan Harley (21). These observers showed that ligature of the bile duct in living animals is followed by escape of bile, not directly into the blood, but into the lymphatics of the liver. Ebner* and Oppel (1900) considered that the bile after ligature of the common bile duct finds its way into lymphatics inside the lobules, and is prevented by them from entering the blood-vessels directly. Such a view assumes that the leakage of bile takes place within the lobules either from the liver cells direct or from the bile capillaries, and that the bile is under these circumstances taken up by intralobular lymphatics and passes from them into the lymphatics accompanying the portal vein branches.

Heidenhain (22), in 1881, showed that in jaundice experimentally produced in animals the place where the bile is absorbed by the lymphatics does not coincide with the place where the bile is formed. He found that a pressure of 11 to 15 mm. Hg in the common bile duct causes bile to appear in the lymphatics of the liver, that secretion of bile continues although this absorption is taking place, and that after the obstruction is removed the bile which escapes from the bile duct is not more concentrated than it was before, showing that equal proportions of the solid and watery constituents of bile were passing into the lymphatics. He also showed that in experimental jaundice the lobules of the liver are not stained with bile unless the biliary obstruction is one of long-standing duration and, in such cases, he argued that the bile finds its way from the lymphatics into the lobules by perivascular lymphatics, and so into the liver cells. Heidenhain believed in the existence of intralobular lymphatics, but considered that they become filled with bile secondarily, and then only in cases of long-standing biliary obstruction where pathological changes have taken place leading to obstruction of the larger lymphatics. In experimental jaundice he stated that the bile breaks through the walls of the interlobular bile ducts into lymphatics around the vessels, and never enters the interior of the lobules.

Nauwerck (46), in 1897, criticised Vaughan Harley's results, believing that they did not prove that bile always finds its way directly into the lymphatics

* Kölliker's 'Gewebelehre,' 1899.

when the bile duct is obstructed. Nauwerck stated that in cases of obstructive jaundice in man the *post-mortem* examination of the liver gives no foundation for the view that the bile passes directly into the lymphatics, but that all appearances support the view that the bile passes through the liver cells by a network of fine channels and escapes directly into the lobular blood-vessels.

Nearly all the authors above quoted agree in describing the existence of lymphatic spaces encircling the intra-lobular blood capillaries. Hering is the strong opponent of this view, and he was supported to some extent by Kölliker. Most of its advocates have relied on the results of injection of the lymphatics with watery solutions of Prussian blue, asphalt dissolved in chloroform, or alcannin in turpentine oil. These fluids have been described as making their way along clefts or spaces between blood-vessels and liver cells throughout the lobule. The readiness with which injection material passes from ruptured lymphatics into the blood-vessels has been generally admitted, but its full extent cannot be appreciated when the blood-vessels are injected subsequently with material of another colour, and this method of procedure has been adopted by nearly all workers on the subject. Biesiadecki acknowledged that in the human livers he investigated the injection material found its way into blood-vessels and clefts equally. Had he injected the blood-vessels with another material this important fact would have been disguised, and the typical appearance described by MacGillavry and others would have been obtained. Budge recognised this difficulty, and stated that the first injection employed has a tendency to adhere to the vessel walls, but he believed that a double injection of a blood-vessel could be distinguished from the separate injection of blood-vessel and lymphatic by the sharp definition which exists in the latter case between the two. He described the outlines of the lymphatic injections as smooth and sharp. Budge employed asphalt in chloroform to inject the lymphatics. We made use of a similar solution to inject the blood-vessels, but found it most unsatisfactory. The chloroform evaporates or diffuses away and leaves the asphalt adhering to the vessel walls. It also alters the character of the liver cells and causes shrinkage of the tissues with which it comes into contact, and another great disadvantage in its use is its lack of colour when seen in thin sections. In dealing with the question whether fine clefts exist between blood-vessels and liver cells the injection material used must be such as can be detected in the thinnest of sections, and asphalt does not admit of this. The forcing of chloroform into a tissue is, besides, not unlikely to produce artificial clefts by the shrinkage which it induces, and these clefts will be accentuated by subsequent fixation and hardening.

Watery solutions of Prussian blue have been extensively used; they have the fatal property of diffusibility. Carter (14) urged this objection to the use of Prussian blue. It is, moreover, readily decolourised by the tissues, and although the colour can be restored, it is, as Carter pointed out, both soluble and diffusible in its colourless condition.

Kisselew and Reinke alone of all who have worked on the subject have described endothelial cells lining the clefts between intralobular blood-vessels and liver cells. No one else has been able to find them. Hering denied the existence of natural clefts in this situation in the rabbit's liver, but allowed that they might be produced artificially.

Methods Used in the Present Inquiry and Results Obtained.

The observations of the brothers Fraser, Nauwerck, Browicz, and Schäfer, and the results of our own experiments on injection of the liver cells from the blood-vessels, are against the probability of there being perivascular lymphatics in the lobules of the liver, but are not conclusive in themselves. We have injected the lymphatics of the liver to ascertain if we could get the injection mass to run into the clefts described by MacGillavry.

The animals experimented on were dogs and cats. The injection mass used was carmine gelatine prepared as already described for injection of the blood-vessels. Carmine gelatine is pre-eminently suitable for this purpose on account of its lack of diffusibility, the readiness with which it is solidified and fixed without damage to the surrounding tissues, and the ease with which the smallest amount can be detected. The main lymphatic trunks coming from the portal fissure were clamped immediately after death; they soon become distended with lymph. One of the larger trunks close to the liver was selected, and a thread passed round it by means of a needle, care being taken not to injure any neighbouring trunks or the portal vein. It was then opened, and a fine cannula, connected to the injection bottle and filled with carmine gelatine, was inserted and tied in position. The liver was disturbed as little as possible. The whole animal being immersed in a bath of water at the temperature of the body, the pressure was gradually raised until the carmine gelatine began to distend the lymphatic vessel. The pressure was maintained for from one to three hours. In most cases the inferior vena cava was opened above the diaphragm to prevent any rise of pressure in the blood-vessels. When the experiment was completed the liver was removed from the body, the clamp on the efferent lymphatic vessels being still attached; the tube which conducted the injection mass to the lymphatics was ligatured close to the cannula. These precautions were adopted to prevent any escape of injection fluid from the lymphatics. The liver was at once placed in

cooled 10-per-cent. formalin, and as soon as the gelatine had set was cut into small pieces and replaced in the formalin. Sections were subsequently cut in paraffin and stained with dilute hæmatoxylin.

The injection can be made to pass beyond the valves without any rupture of the vessel wall taking place; it usually spreads first to the neighbouring trunks and then enters the liver substance. We find no evidence of superficial lymphatics on the surface of the liver in the dog or cat, and in this respect agree with MacGillavry, who could not find them in the dog or rabbit. The large trunk which MacGillavry found on the surface of the gall bladder is readily filled if the injection is made in the vicinity of the gall bladder. This trunk receives its radicles from the connective tissue around the gall bladder and pursues a tortuous course along the cystic duct, to end in a lymphatic gland near the portal vein. Another large trunk is often filled which runs from the connective tissue of the portal fissure along the round ligament into the suspensory ligament of the liver; it appears to terminate by joining the lymphatics of the diaphragm, but we have not been able to trace it further. This lymphatic trunk is evidently similar to the one described by Teichmann and others; it arises, however, not from surface lymphatics, but from the connective tissue of Glisson's capsule, and is closely related to the efferent lymphatics which accompany the portal vein. It affords for the lymph of the liver an additional outlet by means of the lymphatics of the diaphragm.

One of the most remarkable features of an injection of the liver lymphatics is the absence of any sign of the injection mass on the surface of the liver; vessels appear close to the fundus and at the sides of the gall bladder and join its central trunk, but they are distinctly confined to the connective tissue separating the gall bladder from the liver, and no vessels join them from the surface of the organ. After a prolonged injection we have found the wall of the inferior vena cava above the diaphragm coloured to a certain extent by the presence of carmine gelatine in it, but the flow along the wall of the inferior vena cava is very slow and does not spread far.

Whenever the liver has shown a distinct appearance of injection on its surface, it has, in our experience, been due to rupture into and injection of the blood-vessels. In such cases the injection mass may or may not have entered in sufficient quantity to reach the opened inferior vena cava.

On cutting up a liver, the lymphatics of which have been injected in the above described way, the injection mass is seen at many points in its substance close to or surrounding the blood-vessels in the portal spaces. In the neighbourhood of the portal fissure there is often a considerable

amount of extravasation of the carmine gelatine, principally in the connective tissue accompanying the blood-vessels and bile ducts, but also in some of the lobules. Injection mass is also seen in the walls of the hepatic veins.

Microscopical examination of sections lightly stained with hæmatoxylin shows that the carmine gelatine is in the connective tissue of the liver, and that large injected trunks accompany the branches of the hepatic artery, portal vein, and bile ducts. In the large portal spaces there is frequently extravasation throughout the connective tissue; the large lymphatic trunks can be distinguished by their being filled with carmine gelatine, but the surrounding connective tissue is lightly stained owing to the presence in it of diluted injection mass.* The carmine gelatine is frequently seen extending into some of the lobules even to the central vein, and presents an appearance very like that described by MacGillavry. In other places no trace can be seen of injection passing into the lobules, although extravasation has occurred throughout the connective tissue bordering them; a sharp line of demarcation exists between injected connective tissue and non-injected periphery of lobule.

In the smaller portal spaces injected lymphatics are sharply defined and extravasation is uncommon. Lymphatics are often seen running parallel with and close to branches of the hepatic artery; they also surround the bile ducts and portal vein. Even in the smallest portal spaces lymphatics are found (fig. 14). The walls of the hepatic veins contain a large plexus of lymph vessels lying principally in the adventitia close to the liver substance, but there may be several layers with anastomosing branches (fig. 16). The mode of connection between the portal lymphatics and those of the hepatic veins is easily seen. Branches of the hepatic artery supply the walls of the hepatic veins, and lymphatics accompany these branches. Further, large portal spaces containing hepatic artery, portal vein, and bile ducts are found in close connection with, indeed joined on to, the walls of the hepatic veins (fig. 13). There is, in fact, an intimate relationship between Glisson's capsule and the adventitia of the hepatic veins, and the large lymphatics of the two systems are directly continuous in these situations. The place of entry of the branches of the portal vein into the liver is, moreover, very close to the large hepatic veins, and the lymphatics of the latter join the large lymphatics which accompany the branches of the portal vein and which leave the liver by the portal fissure. Disse, alone of all who have previously worked at the subject, described this connection between the two systems. Budge did not notice it, and was led to believe

* Is it possible that on dilution with alkaline lymph some of the carmine becomes diffusible?

in intralobular lymph clefts, largely because he could not find any other connection between portal and hepatic lymphatics. Disse described large lymphatic trunks lined by endothelial cells which run in connective-tissue septa between the adventitia of the hepatic veins and the connective tissue surrounding the branches of the portal vein, but he did not emphasise the fact that the lymphatics also accompany the branches of the hepatic artery.

The plexus of lymphatic vessels in the adventitia of the hepatic veins follows them to their small branches, and lymphatics are present wherever there is an appreciable amount of connective tissue in the wall of the vein. In transverse section of the smaller hepatic veins several lymph vessels (fig. 15) are seen in their walls. The number of these channels varies, as Budge stated, according to the size of the vein they surround; the larger the vein the greater the number of lymphatics in its wall.

We have employed Fleischl's method of injection in several experiments, using the same apparatus as before, but removing the liver from the body and introducing the point of a fine cannula into the adventitia of one of the hepatic veins. The injection mass causes a local distension of the wall of the vein, and some extravasation takes place, but the injection gradually finds its way into the lymphatics and appears at the portal fissure. The extent of liver injected by this method is very small. Disse, in his injections, made several punctures in different places, and noted the small areas of liver injected. This is readily explained, and is what one would expect. The lymphatics of the large branches of the hepatic veins have frequent and large communications with the lymphatics of the branches of the portal vein, and the injection mass has a ready means of escape and soon appears in the large efferent trunks at the portal fissure; it spreads very little and simply follows the large lymph channels. The natural flow of lymph from the hepatic veins in all probability takes the same course. Very little spread of injection mass is seen towards the inferior vena cava, and our observations point to the probability that most of the lymph of the liver in the dog and cat emerges at the portal fissure.

It has been mentioned already (p. 476) that after injection of the portal lymphatics some of the lobules, especially those in the neighbourhood of the portal fissure, contain injection mass, and present appearances similar to those figured by MacGillavry. On careful examination of well-fixed specimens in thin sections it is apparent that the injection is undoubtedly inside the blood-vessels, not only in the intralobular vessels, but in neighbouring interlobular branches of the portal vein as well. Sometimes the periphery only of a lobule shows the injection, extending inwards a little way from the portal space; in such cases the interlobular portal vein nearly

always contains some carmine gelatine mixed with the blood corpuscles. Wherever comparatively little injection mass has entered the intralobular blood-vessels, it shows a tendency to adhere to the walls; this gives rise to the appearance of a thin layer of injection close to the liver cells. The injection is frequently found in Kupffer's cells on either side of their nuclei, but Kupffer's cells show exactly the same appearance in imperfect injections of the blood-vessels, and it is not uncommon to find in the normal liver red blood corpuscles lying in the same situation. In many lobules the intralobular vessels are comparatively well filled; in these cases many of the liver cells, both in the dog and the cat, contain the carmine gelatine, but in all cases where the liver cells contain the injection there is no doubt of its presence in the interior of the neighbouring blood-vessels. On the other hand, there are places where the portal spaces are filled with carmine gelatine and not a trace of any has spread into the adjacent lobules. The same is seen after injection from the wall of the hepatic vein; there is often well-marked extravasation into the connective tissue of the adventitia, but not a trace of injection passing into the surrounding liver tissue. The usual site of rupture into the blood-vessels appears to be at the borders of the lobules where the interlobular veins break up to enter between the columns of liver cells. The usual site of extravasation of bile after ligature of the common bile duct is, according to Heidenhain, just outside the lobules, and this appears to be the place where both blood-vessels and bile ducts are weakest; their walls lack the support they receive from the liver cells in the lobules, and have not attained the strength they afterwards possess when united to form the interlobular veins and bile ducts.

In several animals in which we injected the bile ducts, rupture obviously took place in this situation. The injection mass passed into both blood-vessels and lymphatics of the interlobular connective tissue, and did not fill the bile capillaries at all. The passage of bile into the lymphatics of the liver after ligature of the common bile duct in living animals is not an argument in favour of the presence of intralobular lymphatics. It is more reasonable to suppose that rupture takes place into the connective tissue just outside the lobules, and that the bile under a comparatively low pressure finds its way more readily into the lymphatics than into the blood-vessels. When the bile ducts are injected at a pressure higher than can be reached in simple obstruction of the common duct (11 to 15 mm. Hg according to Heidenhain), rupture takes place into the blood-vessels.

We do not believe that the injection appearance in the lobules denotes the presence of lymphatic clefts between the blood-vessels and liver cells;

the lymphatics of the liver are, in our opinion, confined to the obvious connective tissue which accompanies the branches of the portal and hepatic veins; the appearances described by MacGillavry are produced by artificial rupture into the blood spaces.

The parenchyma of the normal liver contains little or no connective tissue; fine fibrils, the "Gitterfasern" of Kupffer, have been shown to exist by many observers, but they are extremely fine and closely applied to the liver cells. We can find no evidence of the existence of any perivascular connective-tissue cells in the lobules. The "Gitterfasern" are more evident in man and the dog than in the cat and rabbit. In the two latter animals it is often impossible to observe any indication even of an endothelial outline between the blood and the liver cells, except where Kupffer's cells occur. Minot has shown that the connective tissue appears late in the development of the liver, and that it is accompanied by the appearance of true capillaries which grow from the hepatic artery. To us it seems probable that the lymphatics of the liver have a similar distribution, and are specially related to the hepatic artery and its branches. As has already been stated, Minot has shown that the blood spaces of the liver are originally sinusoids, and one of the characters of a sinusoid is the non-occurrence of connective tissue between its wall and the adjacent parenchyma. Under these circumstances the wall of the blood space is closely applied to the (liver) cells and there is no natural cleft between them, nor is there, as in other secreting glands, an intervening lymph space. And if, as appears certainly to be the case in some animals, the endothelial wall of the blood space is itself deficient or is only represented by the isolated cells described by Kupffer, the liver cells must be in direct communication with the blood and the blood plasma, and even under some circumstances blood corpuscles may pass directly into the cells.

It is known that a large amount of concentrated lymph comes from the liver, and Rutimeyer (54) found solid particles of cinnabar in the efferent lymphatics of the liver 35 minutes after a suspension of cinnabar in salt solution had been injected into the external jugular vein of a dog; a very intimate relationship between blood-vessels and lymphatics must therefore also exist in the liver.

To explain this relationship, and the ready passage of injection material into the liver cells, it is necessary to consider the structure of the walls of the hepatic capillaries in some detail.

The Structure of the Intralobular Blood-vessels. (*Historical.*)

His (27), in 1860, described the capillaries of the liver and remarked that they differ from the capillaries of other parts of the body in that their walls

present an indistinct contour which is due to their having closely applied to them a thin layer of connective-tissue fibres. The fibres are extremely fine and occasionally stretch from vessel to vessel; the capillary wall consists of a layer of endothelial cells separated from the liver cells by the fine fibres only.

Wagner (61), in the same year, described in the liver a delicate membrane everywhere surrounding the hepatic cylinders. It is different from the capillary membrane, but is in close relationship with it, so that only one delicate membrane separates the blood from the liver cells. The membrane surrounding the liver cells he stated to be perfectly clear and homogeneous, and of the greatest delicacy and transparency when normal. It has also a further and peculiar property in possessing nuclei, and small cell-like structures in its wall. The nuclei are constantly present, but differ in number and arrangement; they are for the most part round and seldom elongated. Other cells of an irregular or pointed shape occur, and belong to the same membrane. A visible cement substance between capillary wall and membrane of liver cells does not exist, but the two membranes are closely applied to one another.

Wagner pointed out that the liver differs from nearly all other organs in the absence of connective tissue within its lobules. He laid emphasis on the fact that the connective tissue of the liver never penetrates into the lobules under normal conditions, and that any thickening of the membrane surrounding the intralobular capillaries is of a pathological nature.

In 1864, MacGillavry (42) described a space between capillary wall and liver cell which he regarded as one of the sources of the lymphatics of the liver. He found that the space is lined by connective-tissue fibres on the side of the blood capillary and by liver cells and the walls of bile capillaries on the other side. MacGillavry, therefore, differed from previous observers in regarding the walls of the blood capillaries as having a less intimate relationship to the liver cells, and being separated from them by perivascular lymphatics.

About the same time Carter (13) described in the capillary walls of the liver nuclei which are filled with injection when carmine gelatine is injected into the blood-vessels. He came to the conclusion that the nuclei instead of being simply embedded in the walls of the capillaries are nucleated tubular swellings which connect the blood-vessels with a "diaplasmatic system" of vessels. Carter described plasmatic channels between the liver cells and called them a "diaplasmatic system of vessels" or "intercapillary plexus." He considered that blood plasma alone passes into them and that the nucleated sheath of the blood-vessels allows the ready permeation of plasma,

but not of red blood corpuscles. He also believed that nucleated fusiform cells in the capillary walls give rise to the lymphatics of the liver.

Carter's paper does not appear to have been seen by many of the subsequent workers, and is seldom mentioned in the literature.

Hering (25), in 1866, criticised MacGillavry's work and was firmly convinced that in the rabbit's liver no space exists between the capillary walls and liver cells. Hering described the neighbouring liver cells as being connected together by a "Scheidewand," which, when seen in profile, has the appearance of a single dark line or a fine double contour with clear space between. The last, he said, is not to be mistaken, as is done by MacGillavry, for the contour of a natural canal; it is due to the "Scheidewand" not being cut in profile. Whether the wall is composed of cement substance between the closely lying cell membranes or of a homogeneous substance is unsettled. In an alcohol-hardened preparation Hering said two cells always separate so that the protoplasm of one cell at least is torn away from the common "Scheidewand." He could find no trace of any membrana propria in the rabbit's liver and believed that the capillary wall is closely adherent to the liver cells. The perivascular lymphatics described by MacGillavry, Hering believed to be non-existent in the normal liver. He also stated that MacGillavry was wrong in describing bile capillaries between blood-vessels and liver cells.

Kölliker (35) recognised connective-tissue cells in the lobules, and a fine ground substance which accompanies the capillaries, but which is very scanty in amount, and only revealed by special stains.

Henle (24) denied the presence of intralobular connective-tissue cells, but found fine threads between the capillaries and liver cells.

In 1869, Ponfick (49) showed that after fine particles of cinnabar have been injected intravenously in rabbits, guinea-pigs and dogs some hours before death, a *post-mortem* examination of the liver shows that many of the granules of cinnabar lie in round or oval amœboid cells which may be mistaken for liver cells. Ponfick described cinnabar-holding cells in the interlobular connective tissue, and in the fine connective-tissue sheath of the intralobular capillaries in what he considered an extravascular situation.

Platen (48) also described cells in the liver lobules which take up fat from the blood, and may be filled with fat droplets.

In 1876, Kupffer (37) described "Sternzellen," or star-shaped cells, in the liver; these cells are now known as Kupffer's cells. Kupffer stained sections of liver with gold chloride, and found that certain cells take up the stain, and show as deep black bodies on a red field. He described them as pointed protoplasmic bodies, varying in size, but always much smaller

than the liver cells, and containing one or more nuclei. They are regularly distributed throughout the lobules, and occur at intervals along the vessels, never in groups. Their position is constant in being always in contact with the capillary wall; but the form of contact varies. Some cells enclose the capillary with their processes, others have one end only touching the vessel while the main body lies on the nearest liver cell; their branches often pass in between the liver cells, and may even reach the bile capillaries.

Kupffer found "Sternzellen" in the liver of the rat, mouse, rabbit, pig, dog, and man, and believed them to be connective-tissue cells lying outside the blood capillaries. He identified them with the cells described by Wagner and Kölliker, and with some but not all of the cinnabar-holding cells of Ponfick, and thought they might prove to be related to the origin of lymphatics. Kupffer also described an intralobular scaffolding of fine non-nucleated, sharply-cut fibres, which, running from the sheath of the central vein, break up into fibres of extraordinary fineness and support the portal vein capillaries throughout the lobule. To this intralobular network of fibres he applied the name "Gitterfasern." Many subsequent observers have corroborated Kupffer's views, but there has been some difference of opinion regarding the character of the cells he described. Ehrlich (18) looked on them as belonging to his group of plasma-cells. Heidenhain (22), on the other hand, regarded them as special connective-tissue cells related, in all probability, to the perivascular lymphatic spaces described by MacGillavry Ribbert (52) laid stress on the property they have of taking up from the blood not only solid particles introduced into it, but also of depositing in their protoplasm granules from materials which enter them in a soluble form.

Rothe (53) found Kupffer's cells in the cat, guinea-pig, sheep, and sparrow; Asch (2) found that they rapidly take up particles of cinnabar and carmine when these are injected into the blood. In the liver of the frog he states that the pigment cells of the liver have the same function. Löwit (41) concluded that Kupffer's cells take up red blood corpuscles, and transfer the hæmoglobin to the liver cells.

Biondi (6) and Lindemann (40) state that Kupffer's cells take up iron-containing pigment in pernicious anæmia and after the intravenous injection of substances like toluylendiamine. Arnstein (1), in 1874, described pigment-holding cells in connective tissue between capillaries and liver cells in cases of melanosis. The phagocytic property of Kupffer's cells has been amply proved by further researches of Kupffer himself, by Rutimeyer (54), Siebel (57), and quite recently by Heinz (23). Kupffer at first, and nearly all subsequent observers, regarded the "Sternzellen" as extravascular cells

lying between capillary walls and the liver cells. Disse looked upon them as helping with connective-tissue fibrils to form the walls of perivascular lymphatics.

In 1898, Kupffer (38) returned to the subject, and stated that he had been mistaken in assigning to the "Sternzellen" an extravascular position, he now believes that they are an integral part of the blood capillary wall. Kupffer found that 12 hours after the injection of defibrinated (rabbit's) blood into the vein of a rabbit, red blood corpuscles are to be seen lying inside the endothelial cells of the capillaries of the portal vein. In older animals, he states, it is not rare to find the capillary walls thickened, and the injection of perivascular spaces from the lymphatics may be due to the injection mass lifting off an adventitial layer from the capillary tube. As a result of his later observations Kupffer concluded that, in the capillary walls of the liver, there are two forms of nuclei, one spherical or ellipsoidal and the other quite flat; the large nuclei belong to cells which are rich in protoplasm, the small flat nuclei to cells which have relatively little protoplasm. The endothelium of the capillary wall has the appearance of a syncytium, and it is impossible to demonstrate limits between individual cells. The capillary wall appears to be made up of a continuous thin lamella in which the protoplasm is arranged as a network of threads with nuclei-holding nodes; larger accumulations of protoplasm surround the large nuclei, and there is less protoplasm round the flat nuclei. The "Sternzellen" belong to the endothelium of the portal vein, and in gold preparations are shown up by the arrangement of protoplasm round the endothelial nuclei. This protoplasm possesses the power of phagocytosis, and foreign bodies and red blood corpuscles are taken up by it from the blood and broken into fine particles.

About the same time that Kupffer's later results appeared, Browicz (11) described in the human liver and in the liver of the dog long and voluminous cells which lie close to the capillary wall and project into its lumen. He found that these cells are phagocytic, and frequently contain red and white blood corpuscles and granules of pigment, and he identified them with cells containing blood corpuscles found by Silbermann in the liver-blood of children suffering from jaundice, and with phagocytic cells also occurring free in the liver-capillaries of ducks and geese, and described by Minkowsky and Naunyn. Browicz states that the cells do not form a continuous layer on the wall of the capillary, and that the latter is sometimes seen lying between them and the adjacent liver cells; in some pathological conditions they even give rise to emboli in the blood-vessels.

In a later paper Browicz (8) identified the cells he had described with

those shown by Kupffer, but insists that they do not form a syncytium, but are well defined cells which occur inside the blood-vessels. He also considers that the blood has a very close relationship to the interior of the liver cells, and argues against the presence of perivascular lymph spaces in the liver lobules.

Reinke (51), in the same year, described a fine membrane which surrounds every liver cell like a capsule, and is composed of connective-tissue cells, some of which resemble Kupffer's cells, while others are more like the tendon cells seen in the tail of the mouse. He also believes in the existence of the perivascular lymph sheath described by Disse, and thinks that the connective-tissue cells take part in the formation of its wall, and are probably endothelial cells lining it.

Little has been said about the lining of the capillary walls by most of the authors who have described Kupffer's cells as extravascular connective-tissue cells.

Ranvier (50), in 1885, described the capillary walls as composed of a granular and very thin sheet, in which nuclei occur at intervals; the latter are flattened, with long axis parallel to the long axis of the vessel, but with a pronounced relief on their internal surfaces. Ranvier could find no cell margins, and explains the absence of impregnation lines after using silver nitrate in the liver by assuming that endothelial cells are lacking, and that the cells which form the walls of the capillaries are embryonic in character and form a syncytium of protoplasm enclosed by two homogeneous sheets containing nuclei at intervals.

Ranvier's views and the later ones of Kupffer are very similar: both believed that the walls of the capillaries are formed by a syncytium in which there are nuclei but no separate cells. Kupffer showed that the amount of protoplasm round the nuclei varies in amount, and that it has active phagocytic properties. Other observers regarded Kupffer's cells as extravascular connective-tissue cells, as Kupffer himself did at first. Browicz, on the other hand, assigned to them an intravascular position.

Berkley (5), by the employment of a modification of Golgi's method, found two kinds of perivascular cells in the liver of the rabbit, and Dogiel (16), by a similar method, described cells investing the capillaries with their branches.

Many observers, from His onwards, have described fine connective-tissue fibres between the capillary walls and liver cells in the lobules. They are demonstrated for the most part by special stains only, and received from Kupffer the name of "Gitterfasern," a term which Oppel (47) revived in 1891. Oppel employed a method of staining by silver nitrate, and described radial fibres passing from interlobular connective tissue to central vein, and

investing fibres which are finer in texture and surround the blood and lymph spaces. The radial fibres are the thicker and coarser of the two sets.

Mall (43) described similar appearances in the lobules after removal of the liver cells by artificial pancreatic digestion. The nature of the fibres is uncertain. Kupffer states that they have the appearance of elastic fibres, but cannot certainly be classified as such, as they are not stained with orcein. Mall believed them to be elastic. Ebner (17) showed that in preparations stained with orcein, fibres in the interlobular connective tissue, which are undoubtedly elastic, take on a deep stain, while the "Gitterfasern" are unaffected. He believes them to be composed of collagen, and classifies them as fine white connective-tissue fibrils.

In 1900 Minot (45) described a hitherto unrecognised form of blood circulation without capillaries in the organs of vertebrata. Among other organs the liver is one in which, according to Minot, the blood-vessels of the lobules are not true capillaries, but blood spaces or "sinusoids." He states that a sinusoid differs from a capillary in that its wall consists of an endothelial or endotheloid layer without any strengthening addition of adventitia or media. It is of relatively large size; its epithelium is closely fitted against the cells of the organ in which the sinusoid is developed, and it has numerous wide and frequent communications with the neighbouring sinusoids of the organ, while a capillary follows its own shape and is chiefly or wholly embedded in connective tissue. A sinusoid, on the other hand, has little or no connective tissue between it and the adjacent parenchyma, and in those cases where this occurs it is a secondary or late acquisition, and the amount remains usually, perhaps always, very small. The development of capillaries and sinusoids differs. Minot states that a capillary arises from pre-existing vessels or from vaso-formative cells, and is an addition by new histogenesis to vessels previously differentiated, while a sinusoid, on the contrary, is not the product of a new histogenesis, but results from ingrowth of the endothelial wall of a pre-existing blood-vessel. In the development of the liver the hepatic cylinders grow into and subdivide the portal vein, the endothelial cells retaining their embryonic character, and being closely applied to the ingrowing columns of liver cells. The blood-vessels or sinusoids between the columns have irregular shapes and numerous connections with one another, and are typically many times wider than capillaries. Minot states that in the liver the sinusoids come to resemble capillaries by secondary change, and may be called "capilliform sinusoids." The fine fibres of connective tissue he believes to be a secondary development, but one that does not change the essential intimate relation of the wall of the sinusoid to the adjacent parenchyma.

Minot further believes that the supposed endothelial cells lining the liver sinusoids do not form a true endothelium, but a layer of widely separated mesenchymal cells, and he states that the physiological processes that take place between the blood of a sinusoid and its adjacent parenchyma occur under conditions very different from those where the blood flows in true capillaries.

Ebner, in the last edition of Kölliker's "Gewebelehre" (Bd. 3, S. 664, 1902), admits that the embryonic liver contains wide capillaries, but does not think that the fully-formed liver agrees with Minot's description, and regards the employment of the term "sinusoid" as superfluous.

In 1904 Lewis (39) emphasised the importance of Minot's discovery. Lewis re-affirmed the particulars regarding the development of the liver given by Minot, and states that the liver contains at first the smallest possible amount of connective tissue, but that later an increase of connective tissue along the inferior vena cava transforms certain sinusoids into veins, while another and more extensive growth along the bile ducts and portal veins takes place, but never spreads into the lobules. Sinusoids persist between the hepatic columns throughout life, and the so-called central veins of the lobules are large vessels which replace several smaller ones, and in structure remain sinusoids rather than veins.

All who have worked at the subject are agreed that the blood-vessels inside the liver lobules present special features. There is a unanimity of opinion that very little connective tissue exists between their walls and the adjacent liver cells. Most recent observers agree that there is a scaffolding of fine connective-tissue fibres which can only be seen after the employment of special stains. The connective-tissue cells described by earlier authors have been identified as Kupffer's cells, and shown to possess special phagocytic properties; they have been proved, moreover, by Kupffer himself, by Mayer, Browicz, and Minot to enter into the formation of the walls of the blood-vessels. Ranvier and Kupffer believed that the walls are composed of a syncytium with nuclei at intervals and accumulations of protoplasm around them, varying in amount in different places. Browicz described cells actually projecting into the blood-vessels.

Hering could find no space between the cells lining the blood-vessels and the liver parenchyma. Kupffer latterly, Browicz, Schäfer, and Minot have insisted on the same thing. On the other hand, MacGillavry and many subsequent observers claim to have injected perivascular lymph spaces, and Kisselew and Reinke describe lymph spaces lined by endothelial cells. Hering believes such injections to be artefacts, and Kupffer states that they might be due to the injection lifting off an adventitial layer from the capillary tube.

Results of our own Observations.

We have been unable to find any perivascular lymph spaces in the lobules, but have seen appearances which might be mistaken for such; in every such case we have been able to satisfy ourselves that the injection is inside the wall and not between it and the liver cells.

We agree with Minot that the blood-vessels have more the character he assigns to sinusoids than that of capillaries. In injection preparations the distended blood-vessels follow the outlines of the liver cells, and at places penetrate for some distance between them. This appearance was described by Carter, and by J. W. and E. H. Fraser. In the liver of the bird it is extremely well marked (fig. 9), but it is present in all livers we have examined. In injected preparations of the livers of fœtal rabbits the blood-vessels appear as large sinuses closely investing the liver cells and passing between them. Wherever the injection penetrates between the cells it shows a broad connection with the injection mass in the lumen of the blood-sinus and gradually tapers to a fine point between two liver cells (figs. 4 to 9). There is no evidence of any wall lying between the intercellular injection and the lumen of the blood-vessel.

The cells which occur at intervals along the blood-vessels may be divided into two classes, which agree with those described by Kupffer. Small nuclei with very little protoplasm are found closely applied to the liver cells; they have the general appearance of endothelial cells, and their long axes are parallel to the direction of the vessel which they line. One of these cells is shown in fig. 3. They do not appear to form a continuous sheet of endothelium, and long intervals frequently occur between them.

The other class is composed of much larger cells with comparatively large amounts of protoplasm. They vary in shape, and in section may appear as long, pointed cells or star-shaped with several processes. Each cell contains a nucleus which is usually elongated, but varies in size and shape in different cells. Occasionally a large nucleus projects into the lumen of the vessel and lies with its base closely applied to the wall of a liver cell (fig. 3). The protoplasm round the nucleus is sometimes difficult to see, in which case it looks as if nucleus alone were present, but in other cases the protoplasm is large in amount and granular. We take it that these are Kupffer's cells, but they frequently have the appearance described by Browicz, of projecting into the lumen of the vessel, and in thin sections may even seem to be lying free. Fig. 3, which is a drawing made from a section of liver of cat stained with eosine and methylene blue, shows two such cells. Red blood corpuscles are often seen lying between the processes of these cells and

adjacent liver cells. Carmine gelatine when injected into the blood-vessels passes undiluted into the same position. It is difficult to determine whether any of the connective-tissue fibrils are continuous along the side of the projecting processes which is turned towards the wall of the vessel. They are not visible in ordinary preparations, and, if present, do not hinder the ready passage of injection mass and red blood corpuscles behind the processes. Cells of a similar nature are sometimes found lying between liver cells, but some portion of them always borders a blood-vessel and is in direct contact with the blood stream.

They are phagocytic, and frequently contain red blood corpuscles, either entire or in process of disintegration. We can find no evidence of their processes passing into the interior of adjacent liver cells in the manner described by Holmgren. Neither are we able to find any cells occupying the position in which Reinke described connective-tissue cells.

The livers of different kinds of animals vary to some extent in the appearance of the vessel walls; chiefly in the amount of connective-tissue fibrils present.

Whether the walls are composed of a syncytium or of an incomplete layer of cells, we are not in a position to state. In the animals we have examined the evidence seems to us in favour of the latter view. The connective-tissue fibrils do not hinder the passage of carmine gelatine from the blood-vessels into the liver cells, and in the dog's liver red blood corpuscles also pass into the liver cells. Whatever the composition of the wall of the blood spaces, assuming a wall to exist, it is very permeable and closely applied to the liver cells. The blood has an intimate relationship to the liver cells, and the exchange of material between the two takes place without the intervention of lymph spaces.

Kisselew (34) found lymphoid tissue inside the lobules of the pig's liver. The probability is that any lymphoid tissue found in the lobules, assuming it is not the result of tubercular or other pathological changes, is a secondary acquisition, and has passed in with the connective tissue accompanying the branches of the hepatic artery.

The Origin of Lymph in the Liver.

Kiernan (33) as long ago as 1833 recognised that when the portal vein is injected, the injection material soon appears in the lymphatics. This is easily verified in any well-injected preparation of the blood-vessels of the liver. Even if the injection is continued for a short time only, the large interlobular lymphatic trunks are distended with diluted injection mass; an injection continued for a longer time fills them with undiluted material.

The permeability of the vessel walls, which is so marked a characteristic of the intralobular blood-vessels, is also a feature of the blood-vessels at the junction of interlobular connective tissue and liver parenchyma. Starling (58) pointed out that the capillaries of the liver are the most permeable of all the capillaries of the body. He also showed that the lymph which comes from the liver contains from 6 to 8 per cent. proteids, almost as much as the blood plasma itself.

We believe that the lymph is collected at the periphery of the lobules in lymphatic spaces immediately surrounding the branches of the interlobular veins as they enter between the columns of liver cells; it passes into these spaces from the cells at the periphery of the lobules. In all probability the intracellular plasmatic channels of the liver cells act as an intermediate system linking the blood-vessels in the lobules to the lymphatics outside. If this is the case the plasma must flow from cell to cell on its way to the periphery of the lobule. In the injection preparations there is evidence that the channels do communicate from cell to cell, although it is difficult to wash the injection out of the cells by the perfusion of large quantities of salt solution through the blood-vessels. But injection of the lymphatics does not favour the idea that the intracellular channels open into them, for even where the interlobular connective tissue is full of injection material none passes into the liver cells at the periphery of the lobules.

How, therefore, the blood plasma passes from the cells into the commencing lymphatics must be left for the present undetermined. Whenever colouring matter which has been used in injecting the lymphatics is found in the liver cells, there is not the slightest doubt that the injection has passed into the cells from the interior of the blood-vessels, into which it has entered by rupture of their walls.

The large amount and the concentrated character of the lymph which comes from the liver must be ascribed to the peculiarities of the connection between the blood and liver cells and the sinusoidal character of the circulation in the lobules, the incomplete endothelium allowing the ready passage through it of fluid and even of small solid particles both into the liver cells and into the lymphatics.

Whether the endothelium possesses the power or not of altering the degree of permeability is uncertain. Mayer (44) believes that the walls of the liver capillaries can alter their permeability for fluid and solid constituents of the blood, and this quite independently of pressure changes or vaso-motor influences. The question is one which has an important bearing on theories of the formation of lymph, and requires further investigation.

If, as we believe, the liver cells lie in the direct path between blood stream

and lymphatics we should expect that any agencies which increase the activity of the liver cell would also increase the amount of lymph flowing from the liver.*

Summary.

1. The liver cells are permeated by fine anastomosing channels which can be filled with injection mass from the blood-vessels. These channels undoubtedly receive plasma from the blood. In the dog, red blood corpuscles are occasionally seen within the liver cells, and crystals which closely resemble hæmoglobin are frequently found inside the cell nuclei. There must, therefore, be an intimate connection between the blood in the intralobular blood-vessels and the liver cells.

2. The lymphatics of the liver (dog, cat) are confined to the visible connective tissue of Glisson's capsule and the adventitia of the hepatic veins. The lymphatic vessels accompany the hepatic artery and its branches, forming networks around these vessels as well as around the branches of the portal vein and bile ducts. There are no lymphatics within the lobules. The perivascular lymphatics described by MacGillavry do not exist. Both portal and hepatic lymphatics leave the organ at or near the portal fissure.

3. The endothelium which lines the intralobular blood spaces (sinusoids in the sense of Minot) is incomplete and allows the passage through it both of fluid and of fine solid particles into the liver cells. The endothelial cells are of two kinds, large and small. The large cells (Kupffer's cells) are phagocytic, and often project into the blood spaces.

4. The concentrated character of the liver lymph is explained by the incomplete nature of the endothelium lining the intralobular blood-vessels, thus permitting the plasma to pass directly into the liver cells. It is possible that the cells of the lobule form a syncytium, and the lymph is thus able to pass from cell to cell. It is probably passed at the periphery of the lobules into the interstices of the connective tissue which lies between the lobules; here it enters the lymphatics. All conditions which would tend to promote the activity of the liver cells should, by virtue of these arrangements, also tend to promote the flow of lymph.

* *Cf.* Asher, "Untersuchungen über die Eigenschaften und die Entstehung der Lymphe;" Asher and Barbera, 'Zeitschr. f. Biol.,' vol. 36, p. 154, 1898; Asher, 'Zeitschr. f. Biol.,' vol. 37, p. 261, 1898; Asher and Gies, 'Zeitschr. f. Biol.,' vol, 40, p. 180, 1900; Asher and Busch., 'Zeitschr. f. Biol.,' vol. 40, p. 333, 1900; Asher, 'Zeitschr. f. Biol.,' vol. 45, p. 121, 1904.

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DESCRIPTION OF PLATES.

PLATE 22.

FIG. 1.—Liver cells of normal dog. Eosine and methylene blue.

The upper part of the figure represents four liver cells, one with two nuclei. One of the nuclei contains a crystal, which has extended the nuclear membrane to more than twice the diameter of an average-sized nucleus.

The lower part of the figure represents two liver cells. The nucleus of one contains a shorter crystal, and its nucleolus lies to one side, close to the nuclear membrane and opposite the middle of the crystal. The cytoplasm of the same cell contains a semicrystalline body, which stains more lightly with eosine and resembles hæmoglobin. In the other cell a red blood corpuscle lies in the cytoplasm close to the nucleus.

FIG. 2.—Liver cells of normal dog. Eosine and methylene blue.

Four liver cells are shown, in three of which the nucleus contains a crystal. The crystal is of a different size in each. The nucleolus lies close to the nuclear membrane opposite the middle of the crystal. The nucleus of the fourth cell contains a rounded mass of a material which stains like the crystals; its nucleolus lies on one side, close to the nuclear membrane.

FIG. 3.—Part of a section of liver of cat. Eosine and methylene blue.

The sinusoidal character of the blood-vessels and the incomplete nature of their endothelial lining are seen.

One small endothelial cell is closely applied to the junction of two liver cells in the middle column. Two of Kupffer's cells are shown. They are large cells with processes and appear to lie free in the sinusoids. A space exists between each of them and the neighbouring liver cells. In the lower cell represented this space is partly occupied by red blood corpuscles. The cytoplasm of both cells contains granules staining intensely with eosine.

FIG. 4.—Section of rat's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 80 mm. Hg; inferior vena cava open. The injection mass occupies channels between and inside the liver cells.

FIG. 5.—Section of rat's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava ligatured.

The liver cells contain more injection mass than do the cells in fig. 4, but it is largely in the form of separate drops.

FIG. 6.—Section of monkey's liver. Hæmatoxylin.

The portal vein was injected with carmine gelatine at a pressure of 60 mm. Hg; inferior vena cava open. One of the liver cells contains a fine network of injection mass; in others there are small accumulations of the injection in the cytoplasm.

FIG. 7.—Section of dog's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava ligatured. There are channels filled with injection between and inside the liver cells.

FIG. 8.—Section of cat's liver. Hæmatoxylin.

The aorta was injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava ligatured. The injection mass appears in fine channels in the cytoplasm of the liver cells.

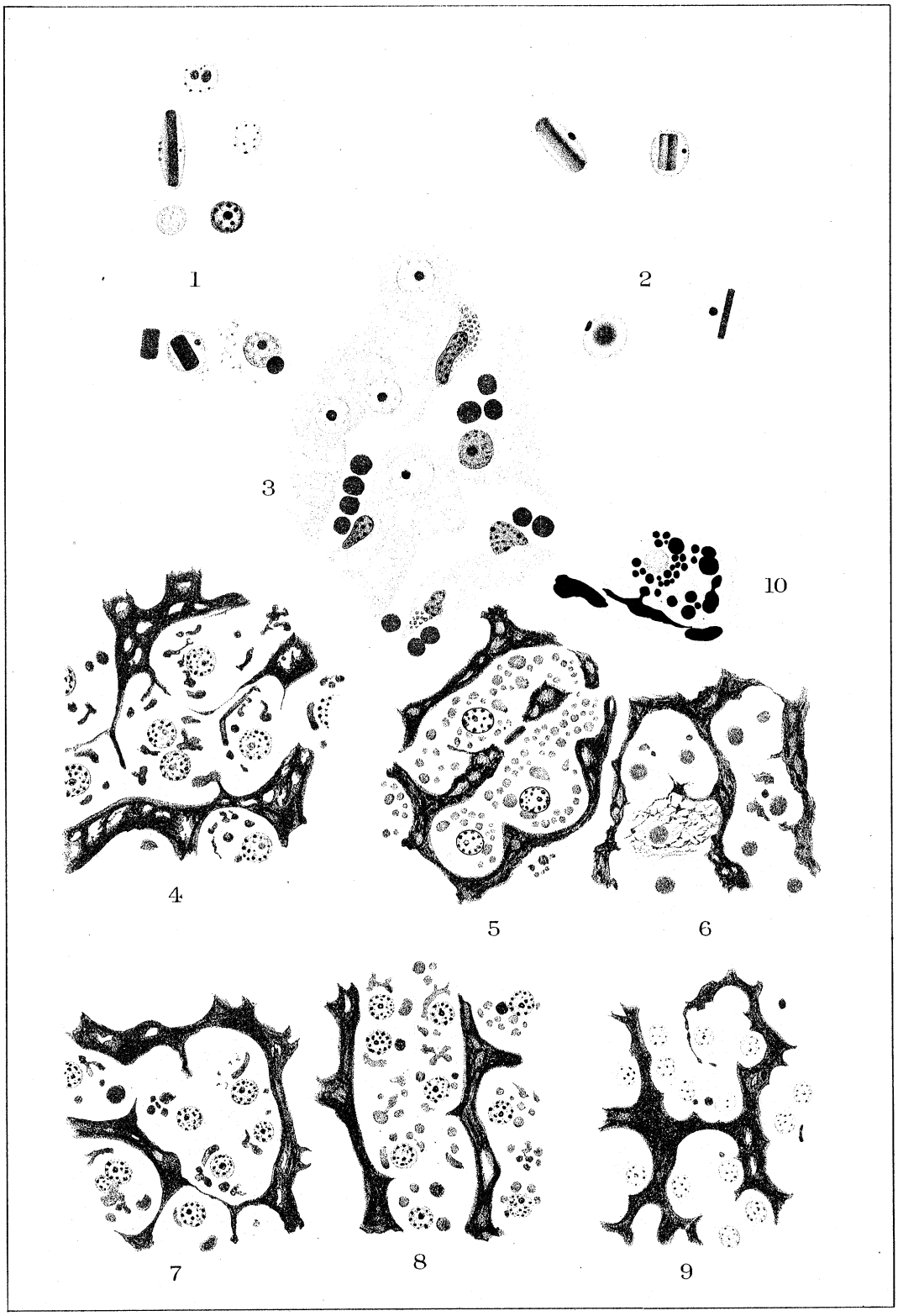
FIG. 9.—Section of fowl's liver. Hæmatoxylin.

Right aortic arch injected with carmine gelatine at a pressure of 100 mm. Hg; inferior vena cava open. The sinusoidal character of the blood-vessels is seen. Fine channels pass between some of the liver cells, and the cytoplasm here and there contains injection.

FIG. 10.—A liver cell and part of an adjacent blood-vessel from child's liver. Osmic acid and methylene blue.

From a case of fatty embolism due to chloroform poisoning.

The liver cell contains globules of fat, and a direct connection is apparent between the fat in the cytoplasm of the cell and fat in the blood-vessel.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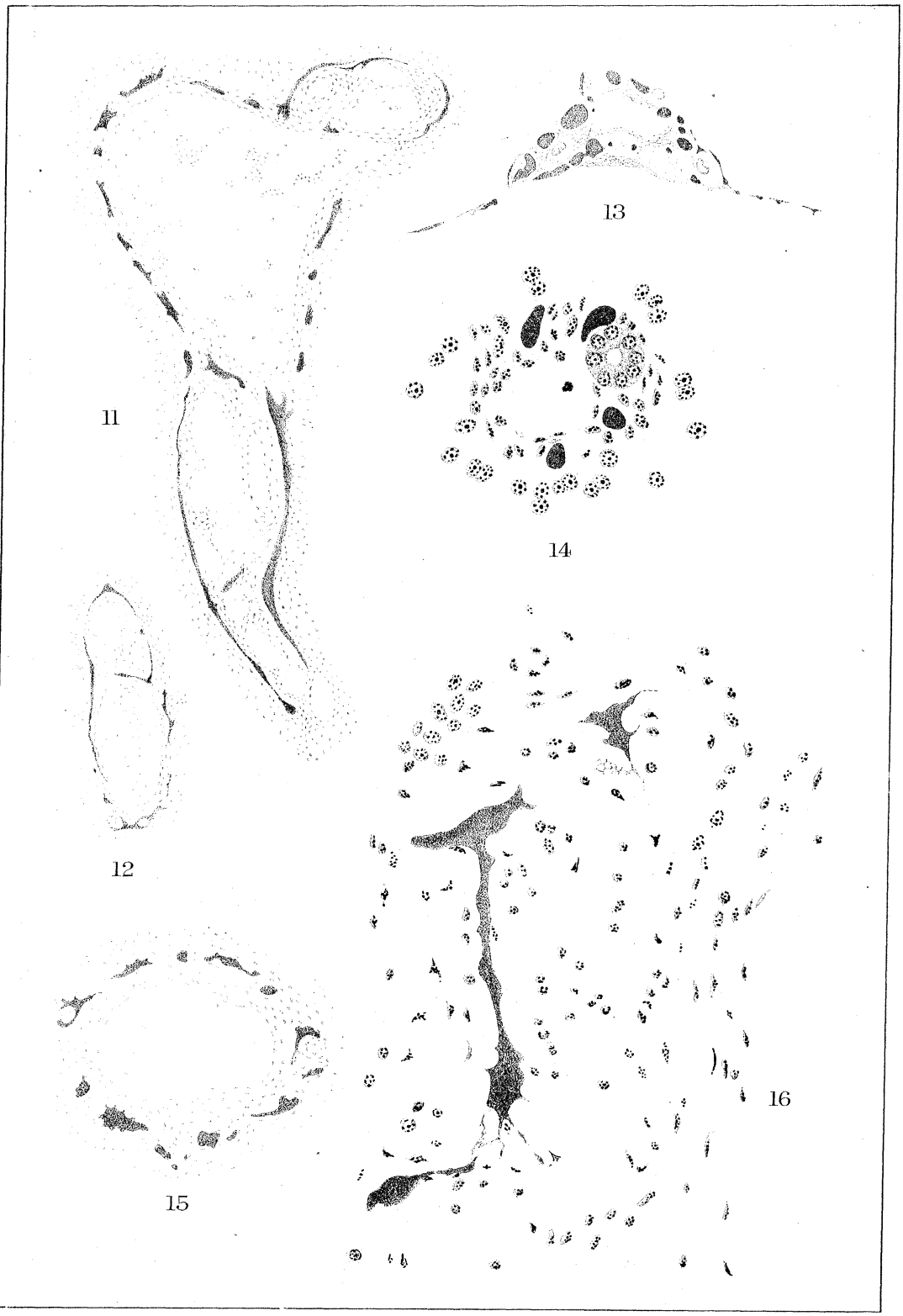
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Plate 23.

Fig. 11.—Portal canal from cat's liver. Hæmatoxylin.
Lymphatics injected with carmine gelatine.
A network of lymphatics surrounds blood-vessels and bile ducts.

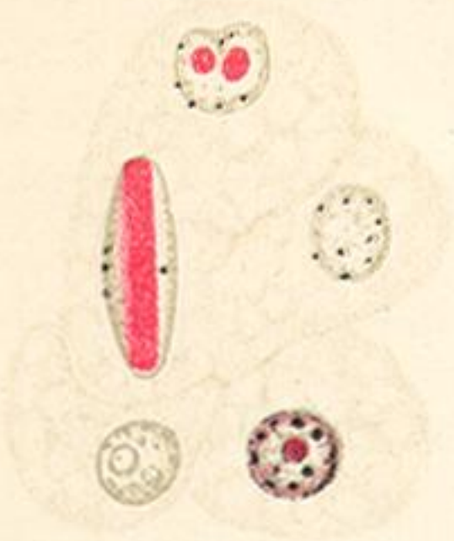
Fig. 12.—Small portal canal from cat's liver. Hæmatoxylin.
Lymphatics injected with carmine gelatine.
Shows a smaller portal space and the lymphatics occupying the connective tissue round the blood-vessels and bile ducts.

Fig. 13.—Portal canal attached to wall of hepatic vein from dog's liver. Hæmatoxylin.
Lymphatics injected with carmine gelatine.
Shows a large portal canal lying in the wall of one of the large hepatic veins. The inner surface of the vein forms the lower limit of the figure. The lymphatics of the portal space and those of the adventitia of the hepatic vein are seen to be continuous with one another.

Fig. 14.—A small portal canal from the dog's liver. Hæmatoxylin.
Lymphatics injected with carmine gelatine.

Fig. 15.—Hepatic vein of dog's liver in transverse section. Hæmatoxylin.
Lymphatics injected with carmine gelatine are seen in the wall of the vein. Small branches of the hepatic artery are also seen in its wall.

Fig. 16.—Longitudinal section of wall of a large hepatic vein in liver of dog. Hæmatoxylin.
Lymphatics injected with carmine gelatine.
Shows lymphatic trunks in the adventitia of the hepatic vein.



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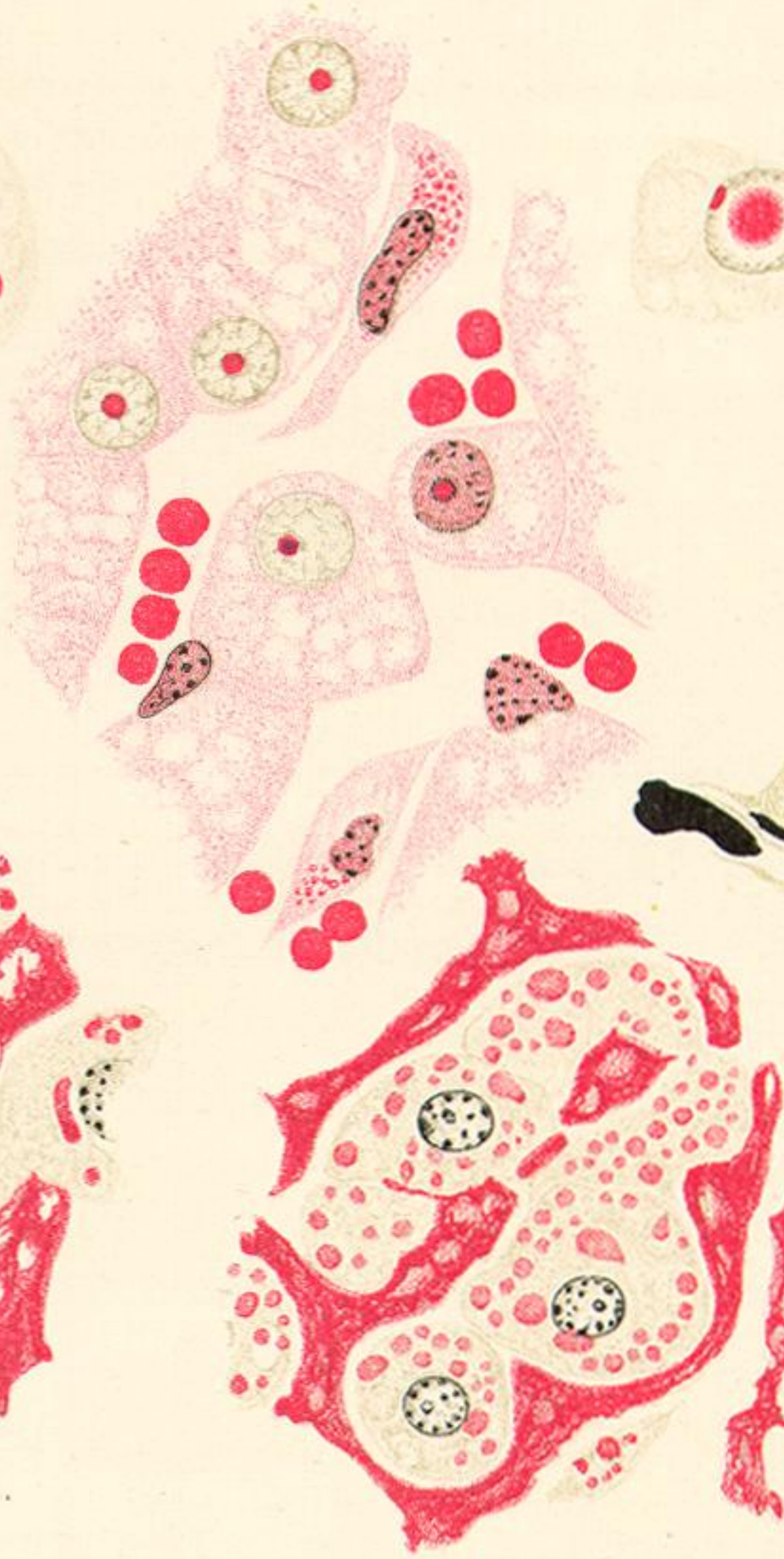

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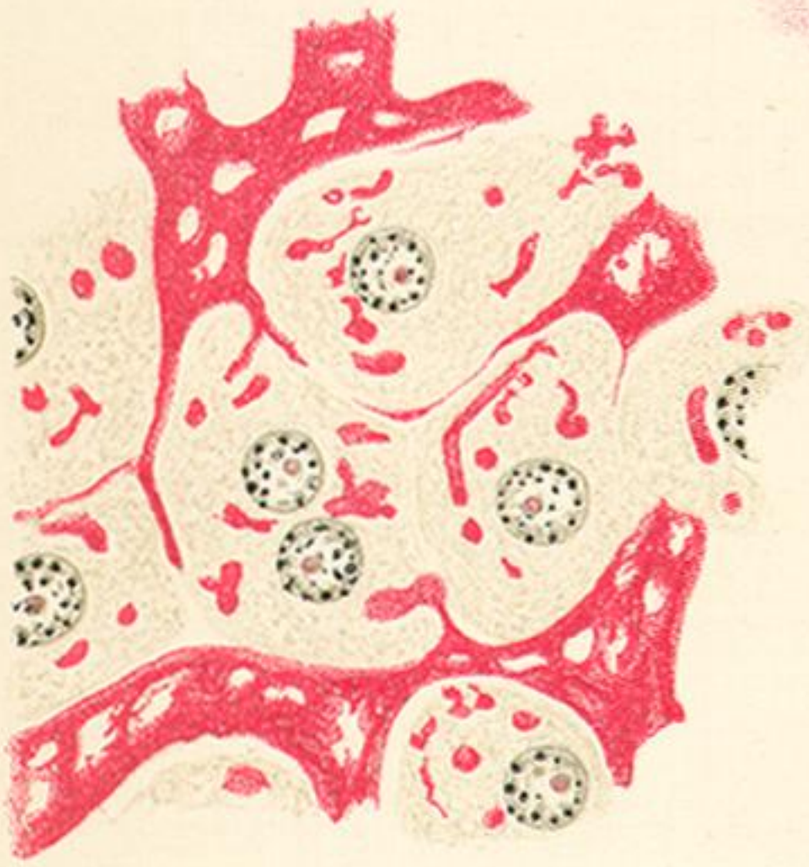

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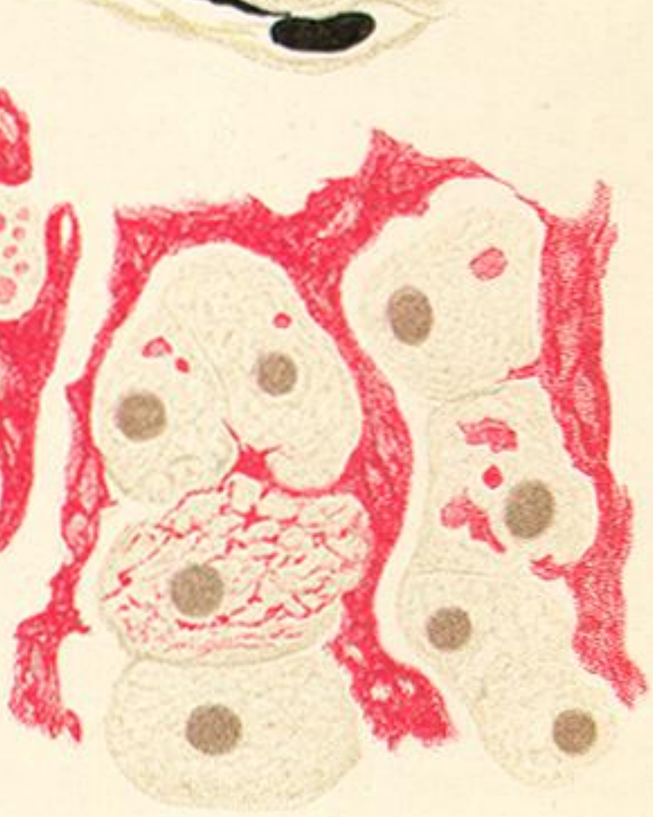

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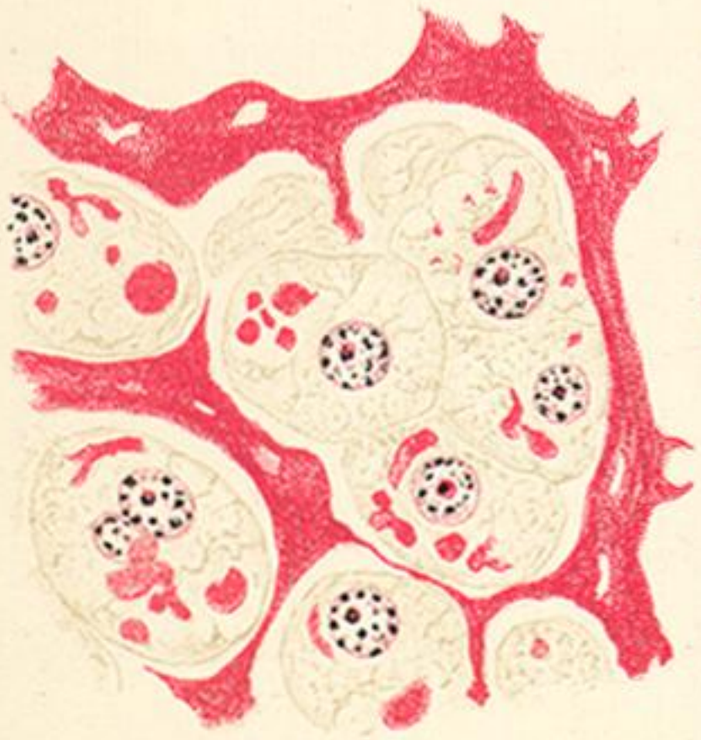

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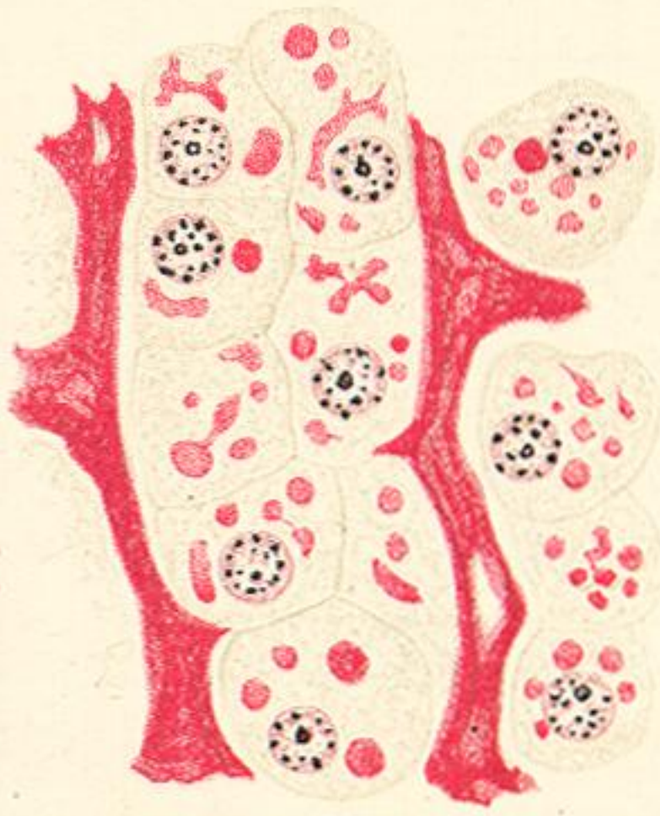

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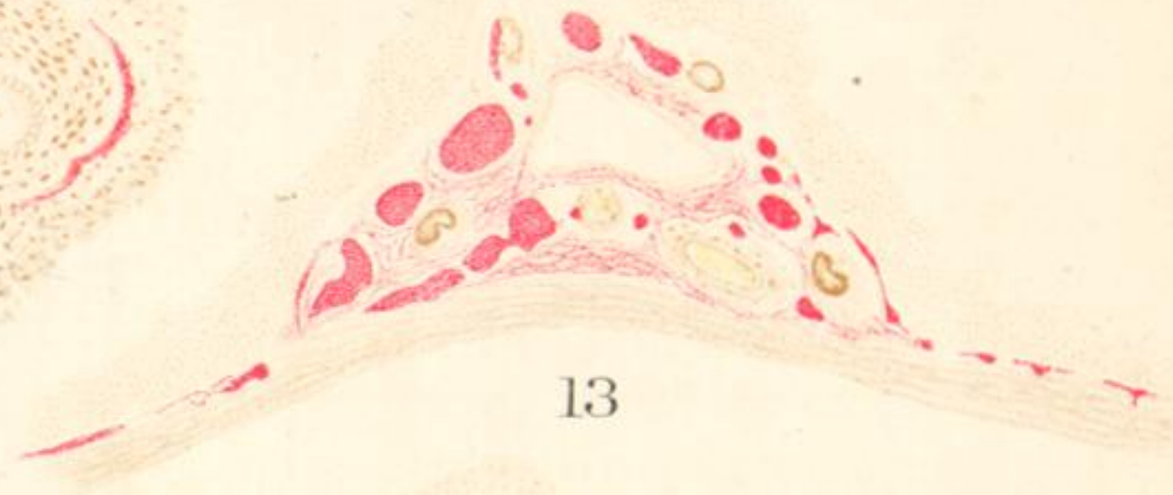

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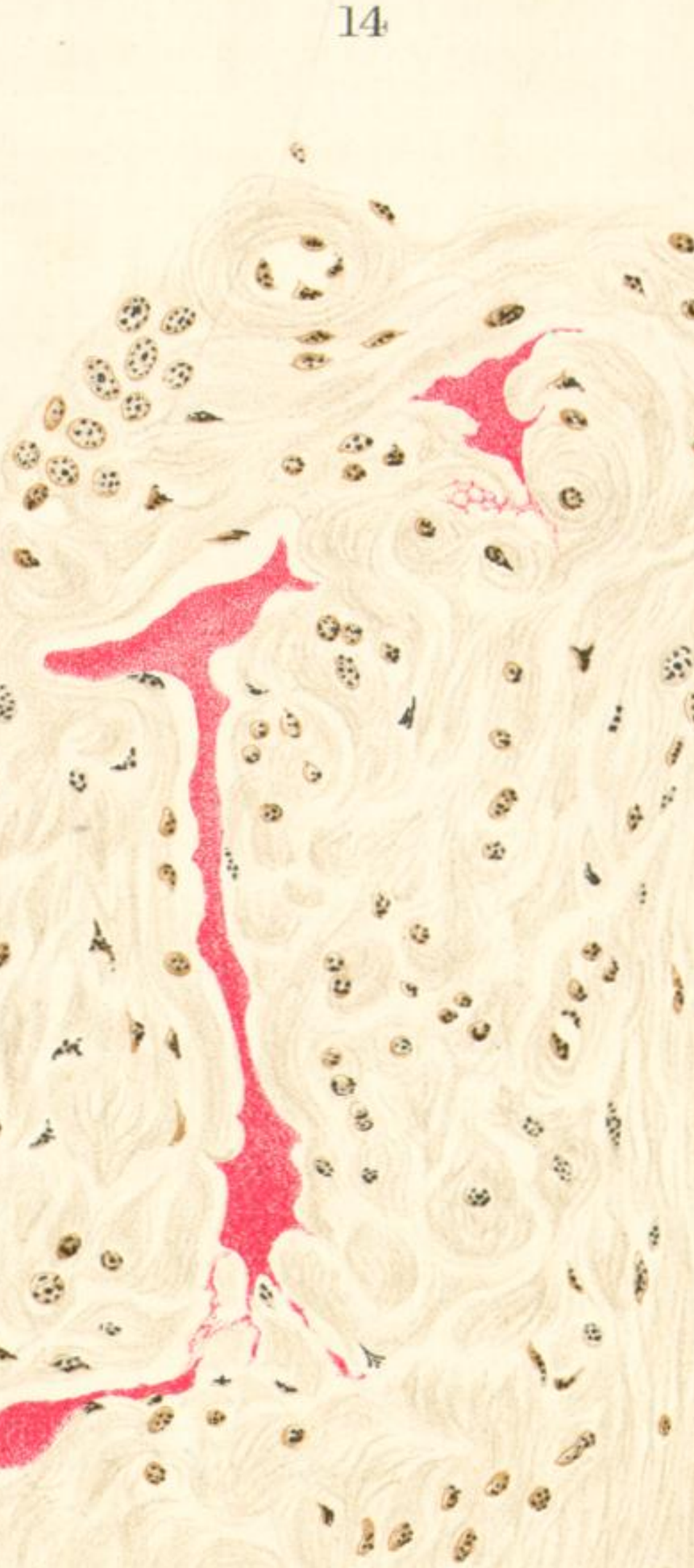

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