THE EFFECT OF ARTERIAL OR VENOUS OBSTRUCTION UPON THE NUTRITION OF THE LIVER CELLS

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Introduction

These experiments were undertaken in order to ascertain more definitely the relative parts played by the arterial and venous blood, which supply the liver, in the nutrition of the liver cells ; and attention was directed more especially to the appearance of fatty changes in these cells. Widely differing views have been held by various writers as to the extent to which the hepatic artery directly supplies arterial blood to the liver cells. The observations of Cohnheim and Litten led them to believe that no arterial blood entered directly the capillary network of the lobules ; they supposed that the hepatic artery supplied blood to the connective tissue framework of the liver, and nourished the walls of the portal vessels running in that framework. Rindfleisch, however, concluded—on the ground of the changes occurring in amyloid disease—that the hepatic arterioles joined the capillary network in the middle zone of the lobule, and that the outer zone received only portal blood.

Recently, Kowalewsky and Opie have independently come to the conclusion that both the artery and the portal vein supply the entire lobule.

Cohnheim and Litten, working with rabbits, found that ligature of the hepatic artery caused necrosis of the entire liver, and death in twenty-four hours. After tying the artery going to a single lobe, the animals lived two or three days. The lobe was necrotic, and showed scattered haemorrhages ; no fatty change was observed.

They concluded that after cutting off the arterial blood supply the nutrition of the walls of the portal veins was impaired. The blood, therefore, stagnated and clotted in these veins, so that anaemic necrosis of the liver occurred.

Ligature of a branch of the portal vein caused death in 24 hours. But the production of multiple embolism in the liver by the injection of wax into the portal vein left the liver cells unaffected. On the other hand, Solowieff, after ligature of a branch of the portal vein, observed at first atrophy of liver cells, and later general fibrosis of the interlobular connective tissue.

More recently, Doyon and Dutourt observed that ligature of the hepatic artery in dogs caused death in twenty-four to forty-eight hours. In such cases areas of necrosis were observed. The results were not constant owing to variability in the collateral anastomoses. In successful cases the proportion of urea in the urine to the total output of nitrogen was diminished. Ligature of a branch of the portal vein caused no necrosis.

Tischner found that in rabbits ligature of the hepatic artery caused thrombosis of branches of the portal vein owing to stagnation of the blood, and necrosis occurred. Infarction occurred in some of the lobules, and the liver cells round the infarcted area showed an increase of fat.

In dogs, Dujarier and Castaigne found that ligature of the hepatic artery, under aseptic conditions, had no effect, but that if sepsis supervened necrosis occurred.

In cats, Ehrhardt obtained general necrosis of the liver after tying the hepatic artery.

Ligature of a branch of the portal vein caused decrease of the fat in the liver, and damaged the nutrition of the cells. After two to three months cirrhosis occurred, and even ascites.

There is no doubt that the collateral blood supply to the liver, after tying the main hepatic artery, varies greatly in different species of animals, and even in different animals of the same species; it is least developed in the rabbit, and most so in the dog. These variations largely account for the divergent results obtained by various

observers. Most investigators have found, however, that the occlusion of arterial blood is followed by acute necrosis of the liver, whereas ligature of a branch of the portal leads after a considerable time to atrophy of the liver cells and to fibrosis.

Methods

A. Experimental.—Most of the experiments were carried out on cats; three dogs were employed. The cats were anaesthetised with ether only, and the dogs with A.C.E. mixture after a preliminary dose of ½-1 gr. morphia, the anaesthesia being subsequently maintained with the same anaesthetic. Strict antiseptic precautions were taken throughout the experiments, and healing always occurred by first intention, except in the case of one dog, which became infected and was killed on the day after the operation.

The hepatic artery was tied one-quarter to half an inch beyond its origin from the coeliac axis as it passed forward towards the gastro-hepatic omentum. In some experiments, which will be referred to later, all the visible small arteries running to the liver in the gastro-hepatic omentum were also ligatured.

In two experiments only that branch of the artery was tied which supplies the right lobe of the liver.

In many experiments a small piece of the liver was snipped off for microscopical examination at the time of the operation, and served as a control in studying the histological changes resulting from the ligature. In two experiments most of the left lobe of the liver was removed at the time of the operation.

Portal Vein.—The right lobe of the liver receives a large branch of the portal vein, which can readily be isolated and tied without disturbing the arterial supply to that lobe or interfering with either the other vascular connexions of the liver or the bile ducts.

The effects of ligature of this branch were confined to the right lobe, so that the rest of the liver served as a control.

Portions of the liver removed at the time of operation and after death were hardened in Müller's fluid; frozen sections were cut and

stained with Soudan III; other pieces were treated by Marchi's method, or stained with the ordinary dyes.

B. Chemical.—The pieces of liver taken for the fat determinations were freed from blood, dried, and powdered, and the powder dried in vacuo at 100° C. The dry powder was weighed and extracted with chloroform in a Soxhlet apparatus after being boiled for a short time in alcohol, according to the method recommended by Rosenfeld. The extract so obtained was then boiled with alcoholic potash, and the fatty acids insoluble in water, set free by acidification of a dilute aqueous solution of the soaps, were collected, taken up in light petroleum, freed from solvent, and weighed. The weight of the insoluble higher fatty acids thus obtained was calculated in percentage of the total solids of the tissue.

Results

A. Ligature of the Hepatic Artery

Owing to the presence of small collateral arteries supplying the liver in cats it is difficult, perhaps impossible, absolutely to shut off all arterial blood from the liver. Consequently, the morbid changes observed vary in degree corresponding to the extent to which arterial blood is excluded. Ligature both of the main artery and of all visible collaterals causes death in twenty-four hours, and very intense changes in the liver cells; ligature simply of the main artery modifies the nutrition of the liver cells to a variable extent in different animals. The pathological alterations vary greatly in degree, and in any case last only for a few weeks, since recovery takes place by the development of the collateral circulation.

The morbid appearances to be described are the result, therefore, of a partial (and variable) deprivation of the arterial blood supply.

I. Histological Changes.—The two points observed which seem of most importance are in the first place the increase in the amount of fat visible in stained sections, and secondly the absence of gross necrosis of the liver. When death occurred within twenty-four hours the liver looked abnormally pale to the naked eye; no haemorrhages or necrotic areas were visible.

Microscopically the portal vessels supplying the lobules were very full of blood; some, but not all, of them were thrombosed. The columnar arrangement of the cells had to some extent disappeared. The cell-nuclei stained well throughout the lobule, except in the inner zone, where some necrotic cells were visible, which stained very badly. There was, however, no necrosis of the lobule as a whole, although all the cells were obviously severely damaged. In the outer zone of the lobules the protoplasm was shrunken, granular, and apparently free from glycogen; in the inner zone many of the cells contained large vacuoles; sections stained with Soudan III showed abundance of fat in the inner zone, corresponding to the vacuoles observed in paraffin sections.

In one experiment, in which the occlusion of arterial blood was almost complete, there was no increase of fat histologically; even in this case there was no general necrosis.

If the animals survived more than twenty-four hours they were killed by chloroform from two to twelve days after the operation. In such experiments the liver looked pale and fatty on inspection.

Microscopically, the outer and middle zones of the lobules were normal in appearance; the inner zone contained abundance of fat. A few days after the operation the fat existed chiefly in the form of large globules; later on it was found as small drops only, and in gradually diminishing amount. In animals killed two or three days after the operation scattered necrotic cells were visible in the inner zone close to the intralobular vein; some of the cells also contained granules of brown pigment, probably bile.

With one exception, the capillary network was not unusually engorged with blood, and the general appearance of the liver was not that of infarction. In one experiment the animal was killed twenty-six days after the operation. The liver appeared normal both microscopically and on section. This may have been due to recovery by the establishment of a collateral circulation, or the animal may, in the first instance, have possessed a good collateral blood supply, so that ligature of the main hepatic artery only slightly damaged the liver.

It should be pointed out that in normal cats the amount of fat found (histologically) in the liver is extremely variable; in some livers the entire lobule may be full of drops of fat, in others the fat may be almost confined to the outer or inner zone, and in others hardly any fat may be visible. It is most desirable, therefore, to snip off a small piece of liver as a control at the time of the operation; even this, however, is not satisfactory if the control piece is full of fat.

Metabolic changes have been described in animals after ligature of the hepatic artery. In our experiments the urine contained traces of sugar for a day or two after the operation; later the urine was normal, and the relation of urea to the total nitrogen was unaltered. For a few days the animals lost weight; subsequently, however, they regained or even exceeded their original weight.

B. Ligature of the Portal Vein

The lobe which was deprived of its portal blood never showed gross necrosis, nor did any appreciable increase in the amount of fat take place.

The animals usually survived, but one or two died within 24 hours. In such cases the columnar arrangement of the cells was lost; the cells throughout the lobule stained badly, and many of them were necrosed, and the lobules as a whole looked atrophied, as though many cells had broken down altogether and disappeared. These necrotic cells were scattered throughout the lobule, and were not confined to any one zone. A few globules of fat were visible, but they were not numerous.

Four days after the ligature the outer zone appeared normal; in the middle and inner zones the nuclei stained well, but the cell substance stained very lightly indeed. Sections stained with Soudan III showed practically no increase of fat. Eighteen days after the ligature the liver was tough and firm, the portal canals were very obvious, and the lobe appeared atrophied. Microscopically, the liver showed a general fibrosis round the arteries and ducts of the portal canals, and the interlobular connective tissue was everywhere increased in amount.

The inner zone was represented chiefly by extravasated blood; the liver cells were scanty, stained badly, and often contained pigment granules. There was no increase in the amount of fat.

The analytical determination of the amount of fat was carried out by the method described above in altogether four animals in which the hepatic artery was tied, and in one in which the vessel ligatured was the branch of the portal vein supplying the right lobe.

In one only of these experiments was the absolute amount of fat in the liver found to be increased after the ligature.

Experiment No. 4 [January 24].—The branch of the hepatic artery to the right lobe was tied, and two days later the fat estimated in this lobe and in two portions of the rest of the liver. In the former the insoluble higher fatty acids amounted to 21·3 per cent. of the dry substance, in the latter to 14·8 and 14·5 per cent. respectively.

But since the characteristic microscopic appearance of fatty change was not evident in this case, the increase in the fat here does not help us to determine the nature of this change.

In all the other cases the amount of fat was either unaltered or diminished, and it was diminished in that case in which the histological appearance of increase was most marked.

Experiment No. 1 [October 28].—The left lobe, removed at the time of the operation in which the hepatic artery and its branches were tied, contained 24·6 per cent. of fat; two days later two samples of the liver contained 19·15 and 19·5 per cent. respectively.

Experiment No. 3 [December 8].—The branch of the hepatic artery supplying the left lobe was tied; two days later this lobe contained 10·5 per cent., and the rest of the liver 10·4 and 10·25 per cent. in two portions taken for analysis.

Experiment No. 5 [July 3].—The left lobe, removed when the hepatic artery and all its branches were ligatured, contained higher fatty acids, amounting to 11·2 per cent. of the dried liver substance. The liver at death on the next day contained 9·7 per cent.

Experiment No. 2 [December 4].—The branch of the portal vein to one lobe was tied. Two days later this lobe contained 13·7 per cent., and the portions of the rest of the liver analysed 13·0 and 13·6 per cent. respectively.

Conclusions

(1) Ligature of a branch of the portal vein is followed by atrophy of the liver cells, and by fibrosis of the interlobular connective tissue, probably as a replacement fibrosis. It is possible, therefore, that in some cases of hepatic cirrhosis associated with portal thrombosis the thrombosis precedes rather than follows the cirrhosis.

(2) Ligature of the hepatic artery produces (*a*) necrosis of scattered cells in the inner zone, but no general necrosis of the liver, even when the animals die within twenty-four hours, and (*b*) considerable injury to the whole of the inner zone, as indicated by the increase of fat (histologically) in the cells.

As regards the necrosis, it is probable that complete occlusion of all arterial blood would cause general necrosis of the liver ; in our experiments this condition was never absolutely fulfilled, and the bulk of the liver cells, though severely damaged, were not killed. There is no doubt that a partial occlusion of arterial blood, if associated with septic infection, can set up necrosis of the liver ; and the combination of these two factors may account for the results obtained by some observers, as was pointed out by Dujarier and Castaigne.

The possible sources of the apparent excess of fat are (1) an infiltration from without ; (2) the formation of fat from proteid by the process of ' fatty degeneration ' hypothesised by Virchow ; and (3) the setting free in a form recognisable by the microscope of fat previously bound up in some complex combination in which it is not detectable by microscopic examination—a process comparable with the liberation of simple fats from myelin in nerve degeneration.

The chemical analyses show clearly that there is no *real* increase of fat in the liver ; the fat, therefore, cannot be either an infiltration or a product of ' fatty degeneration ' of proteid. We consider that when the liver cells are deprived of arterial blood a process of autolysis occurs whereby pre-existent fat recognisable by chemical analysis is set free in a form which stains with Soudan III. It seems clear that this autolysis results from the lack of oxygen, since it is not produced by portal obstruction ; and as the deficiency of oxygen is most marked in the centre of the lobule, the inner zone is chiefly affected.

A very similar condition of the liver (histologically) has been observed by Drummond in animals, and by Langdon Brown in man after intravenous injections of adrenalin ; it may be surmised that in this case, too, the effect is partly the result of a deficient arterial blood supply to the liver.

There was nothing to suggest that the fatty changes were due to toxic or infective causes, since the animals rapidly recovered after the operation, and showed no signs of sepsis.

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