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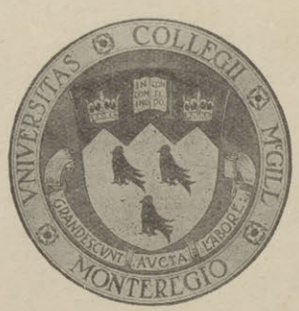

PARTITION OF THE BLOOD SUPPLY OF THE LIVER.

INTRODUCTION: The problem of independence of function of different parts of the liver has long been discussed. Evidence along embryological, histological and anatomical lines has been given in detail, and as workers in each department have laid their claims there has appeared no great similarity in their theories and deductions. However important the bearing of the above evidence may have on the ultimate solution of the problem there remains only one real test,- one that will give everlasting proof - physiology.

Bradley[1] has given accurate information as regards the embryological data. He states that the present anatomical division of the liver is convenient but does not rest upon an embryological or true morphological basis. The ligamentum teres is the remains of the left umbilical vein and the gall bladder must be regarded as the true centre of the liver, in embryo and adult. On either side of the gall bladder are the left and right central lobules, and together they form the central lobe of the liver. These are bounded laterally by the right and left umbilical fissures, the remains of the original umbilical veins. Lateral again to the fissures are the left and the right lobes with their appendages spigelian and caudate lobules. These three principal lobes appear independently and develop in connection with the embryonic veins. The central lobe is constructed around the umbilical veins and the right and left lobes grow along the course of the right and left omphalomesenteric veins. Three large hepatic veins drain the three principal lobes. This

description will probably be cleared up by consideration of the following table by Bradley, giving his modifications of Flower[2]'s Nomenclature in regard to the divisions of the liver.

BRADLEY		FLOWER
Central Lobe:	right lobule	right central lobe
	left lobule	left central lobe
Right lateral lobe:	Main part	right lateral lobe
	Processus caudatus.	caudate lobe
	Processus omentalis or papillaris.	spigelian lobe.
Left Lateral lobe:		left lateral lobe.

Bradley states that there is a great deal to be said in favour of Cantlie's idea that a line running from the attachment of the gall bladder to the hepatic sinus, divides the liver into right and left halves.

This contention has been the mainstay of the arguments given by numerous experimenters for the anatomical and functional independence of the lobes of the liver. Sérégé[3] has studied the question in a variety of ways. In 1902 he experimented by feeding dogs about 250 grams of finely chopped fresh meat, and analyzing their livers for urea after killing 2,4,6 and 8 hours after the meal, he found that in the 2-hour estimations the left lobe held a greater supply than the right, and vice versa with the 6-hour test. The 8-hour test showed the quantity of urea equally distributed. Another experiment of Sérégé[4] which coincides partially with the above is his observation that in animals fed on a mixed diet in nitrogenous equilibrium, there is a rise and fall in urea excretion, the

maximum increase occurring about the sixth hour of digestion. He draws a close analogy between this idea and that mentioned above, i.e., the retention of urea in the liver at different moments of digestion.

Later Sérégé[5] analyzed the liver of animals for glycogen and obtained results similar to the foregoing analysis of urea in the liver. Unfortunately strong objection can be raised to the method he employed in the estimation of glycogen (precipitation of proteins by tri-chlor-acetic acid). Another[6] method followed by this observer, in studying "La Circulation du Sang dans le foie gauche et dans le foie droit chez les chiens", was that of injecting ferricyanide of copper in the portal vein and observing its time of exit in the inferior vena cava. It was found that the circulation times for the two lobes were different, and that the blood flow through the right lobe was always more rapid. Strong objection can be made to this procedure in that the operative injury which must be present would materially influence the liver circulation.

His deductions may be briefly outlined:-

(1) that the circulation of the right lobe is always more rapid than the left, and the left lobe parts with its glycogen less rapidly when the circulation is intact.

(2) that each lobe works independently of the other at different moments during digestion.

(3) that there is a distinct line of separation passing from the gall bladder to the hepatic sinus.

(4) In the early hours (2) of digestion, the gastric digestion proceeds chiefly to the left lobe, while later (6 hours)/in pancreatic digestion the right lobe is working, and not the left. At the end of eight hours both lobes are working at the same rate.

Gilbert and Villaret[7] in 1909, working on "la circulation du lobule hépatique" arrived at a somewhat different conclusion. The method used by them was that of injecting gelatin stained with Prussian glue into the various veins of the portal system,and observing microscopically the distribution of the injection mass. They found that on injecting an intestinal or mesenteric vein, the left lobe was equally affected with the right; a direct contradiction to Sérégé's view. Out of twenty experiments only three were performed on animals with the circulation of the liver intact, and the results of these were only comparable to the results of the other seventeen. Part of these were injections immediately afterbleeding the animal to death; the others were made six hours after death. They, no doubt, have demonstrated accurately enough the anatomical arrangement of the portal capillaries in the liver, but whether this result is of physiological importance is a matter of great doubt. However, they suggest that the blood may be helped along its course by an active contraction of the intra-hepatic veins, the lobule,and also the elasticity of the liver itself may come into play. Under their terminology they designate this quality as "la rétractilité du parenchyme hépatique".

Bauer[8], working independently in the same year with the injection method, discovered an equal distribution of the injection mass in the liver on injecting the intestinal vein. He repudiates the idea of the line of separation in the liver, claims it is only hypothetical in nature, and states that if there is any difference in blood supply to the different lobes of the liver, it must be due to variations in the branches of the portal system. Above all, the difference cannot be absolute.

In 1910, McLeod[9] investigated the normal distribution of glycogen over the liver. He used dogs starved for five days, then fed with cane-sugar solution. Then he analyzed their livers for glycogen at different periods after feeding. He claims to have found no such discrepancy as indicated by Sérégé and the other observers. In the first of his experiments he encountered a percentage variation of about 15%, but later with knowledge of the method and technique, he argues that the greatest variation will fall between five and ten per cent, and that these are chiefly due to the error in the method used for the estimation of glycogen.

In 1914, Bartlett, Corper and Long[10], used the injection of an emulsion of olive oil and defibrinated blood into the various branches of the portal systems of dogs and microscopically examined portions of the liver tissue. They also made use of fistulas, and thus studied the absorption of copper salts by way of the portal vein with subsequent analysis of the liver for copper. The results of their methods corroborate the original view put forth by Sérégé. These observers contend that the blood from the stomach, spleen, part of the jejunum, and also from the rectum, flows principally to the left and central lobes. The blood from the lower jejunum, ileum, and first three-fourths of the colon, flows in greatest amount to the right lobe. The point in their argument to notice is the particular stress they lay on the unequal mixing of the different streams in the portal system. The blood from the lower portions of the system has a longer way to travel and is consequently more sharply defined, whereas that from the upper portion is more mixed and less independent.

We must conclude from the observations made by the above

workers that there does exist an embryological and anatomical difference in the portal branches to the right and left lobes of the liver. Histologically, however, we must say that no definite distinction can be outlined between the various portions of the liver.

We must not lay stress and apply all the tests to the portal importations alone, because natural infiltrations, chiefly fat, by way of the hepatic artery occurring simultaneously with those by the portal vein, are just as vitally important. The most valuable observations on this branch of Physiology have been made by Leathes 11, Hartley 12, Kennaway 13, Meyer Wedell 14, and Mottram 15. A short while ago, observers misled by the fact that the fat absorbed from the food is diverted from the portal stream and carried by its own system of channels to the systemic circulation, thought that the liver is not concerned with the fact in any way. It is now known, however, that the liver may be flooded with fat more so than any other organ. This means either active production of the substance by the organ itself or importation from other parts of the body. Evidence is at hand which points to the liver as a factor in the synthesis of fatty acids, and also still more accurate data which support the idea that fat may be transported to the liver.

If dogs are starved for five days and then given phlorrhizin on the sixth and seventh days, they show livers that have large quantities of fat present, even as much as 70% of the dry weight of the organ. Also, if dogs are fed on linseed oil, the acids of which are laid down in the adipose tissue and then given phosphorus, their livers contain a large amount of fat similar in nature to that of the adipose tissue.[16] The increase of fat in the liver due to importation may reach 300% to 400% as

compared with a similar increase of 50% to 100% in other organs.[17]

The most striking and convincing way of studying the difference between liver fat, adipose tissue fat, and the fat from other organs, has been shown to be a comparison of the iodine value of the unsaturated fatty acids. The iodine values of the fatty acids in organs range from 110 to 140, whereas those of adipose tissue vary from 40 to 65.[18] Hartly and Mavrogordato[19] examined a series of human livers and found that corresponding with the percentage increase of fat in the liver there is a decrease of the iodine value. In fact in extreme fatty change the iodine value may be no higher than that for subcutaneous fat.

The obvious explanation for this seems to be that when there is an unusually large amount of fat in the liver it is because of greater quantity of the stored fat in the body has been brought to it. This fat is carried to the liver with the express purpose of acquiring a higher iodine value, or in other words, it becomes desaturated. The liver prepares fat in this way for other organs, and there the oxidation of the compound is completed. This may be illustrated by the following experiment. Dr.L.Meyer Wedell[20] fed white rats on cod liver oil, the iodine value of which is 150. The normal iodine value of the rats' livers is 130. It was discovered that the iodine values of the fatty essence in their livers rose as high as 160-180: 215 in one instance. The iodine value of the fat depôts was also found to have increased but nearly so so great an extent.

The character of the fat in the liver is determined by the nature of the fat offered to it by the blood. If this is the case,

then in the rats fed on cod-liver oil, the liver should have a corresponding iodine value but such was not the case. It must be granted then, that the liver possesses the power to desaturate the fatty acids brought to it. More than this can be said of the liver. Besides the/desaturation, it has further been demonstrated that the liver may alter the chemical bond of union[21] in the fatty acid molecule to a more oxidisable form.[22] Leathes suggests that this transformation of the character of the fat by the liver prepares the compound for other organs of the body, there to be oxidised and used as a source of energy. It is obvious that too active a mobilization of fat in the liver will result in an accumulation of the unfinished product and a fatty liver.

The mobilization of fat may also occur in several physiological conditions.[23] Mottram has shown in starvation, late pregnancy and early lactation[24] that the amount of the fat in the liver is increased. He experimented by feeding rabbits and guinea-pigs on a constant liberal diet for a long period. Some were then killed to serve as controls, others were killed at the end of one, two, three, four and five days' starvation. He found at the end of one, three, four and five days starvation the iodine value of liver fatty acids lowered and a percentage increase of fat in the liver. At intervals the liver was working on its supply and the iodine value rose. The point that Mottram emphasizes is that this infiltration is not pathological or harmful but due to simple hunger of short duration; also that the importations are a building-up process, a regeneration.

The problems that arise on consideration of above description of the results of previous workers are:-

(1) to test for a difference in distribution in hepatic blood.

(2). If Sérégé, Bartlett, Corper and Long are correct, to discover if natural infiltrations, chiefly glucose, etc., from the portal vein will be divided in any preferential way,.

(3) If infiltration from the hepatic artery (simultaneously with the protal vein) are subject to a regular gross difference in their distribution.

In investigating the possibilities the three following methods were devised:-

(1) Injections of hepatic artery with an emulsion of olive oil and defibrinated blood, accompanied by histological examination.

(2) The estimation of glycogen carried out on the livers of dogs starved for a period, then fed with cane-sugar.

(3) The estimation of the non-volatile fatty acids in the infiltrated livers of starved rabbits.

SECTION 1.

INJECTIONS OF HEPATIC ARTERY.

Gilbert and Villaret[25] carried out observations on the branches of the hepatic arteries in the liver. The technique employed by them was that of injecting gelatin stained with Prussian blue or carmine into the hepatic artery immediately after the animal was bled to death. An important point in their protocols was that they ligated the portal vein before injecting. It was considered a necessity by them in order to obtain a true hepatic artery injection. A legitimate procedure in their case, it cannot be used with safety when one desires to study

the branches of the hepatic arteries as described in this section of the paper. They have, however, demonstrated perfectly well histologically the anatomical ramifications of the hepatic artery in the liver.

It may be wise to cite a few of their discoveries:

(1) The perilobular capillaries of the hepatic arteries are filled with the injection as well as the branches which supply the bile ducts.

(2) The peripheral capillaries of the lobule are injected.

(3) The larger arterial branches are in direct union with the intralobular capillaries.

(4) No injection mass is found in the sub-hepatic veins.

(5) The hepatic artery acts as a conveyor of nutrition to the cells.

It will be noticed that the quantitative distribution and its bearing on infiltration is not discussed.

A serious objection to their method is that not one of six experiments was performed on an animal with the circulation intact, i.e., under anaesthesia. Though the results obtained from the injection of the hepatic artery in an animal under anaesthesia and with portal vein left free are singularly complicated to interpret, yet it is without a doubt the only fair way to tackle the problem.

TECHNIQUE AND METHOD IN DETAIL.

GENERAL OUTLINE OF A PROTOCOL. An animal (cat or dog) was anaesthetised and the abdomen opened to the right of the linea alba. The hepatic artery was located and a cannula inserted. Next, a cannula

was placed in the femoral artery, twenty-five to fifty ccs. of blood withdrawn and defibrinated. An emulsion was made consisting of one part of olive oil with eight parts of defibrinated blood,and 5 to 10 ccs. of the emulsion weere injected into the hepatic artery. The animal was immediately bled to death, the liver excised and the portions of it at once placed in the fixative.

METHOD IN DETAIL. Small portions of the liver were taken from the left center and right lobes, ten in all. (See fig.5). The sections were made from different parts,periphery and hilus, of the same lobe, because one section cannot be considered as representative of a lobe. The pieces were fixed for 24 hours in a solution of 4% Formaldehyde with 5 ccs. of 5% Glacial Acetic acid in every 100 ccs. of the Formaldehyde solution. They were then placed over night in formol gum: a micture of 4% Formaldehyde and Gum Acacia. Next day the hardened tissue was frozen and cut in sections of approximately 5u thickness with a Sartorius Microtome. The sections were immediately removed when cut to watch glasses, containing a dilute solution of Formaldehyde, by means of a camel's hair brush,moistened with the same solution. The sections were stained with Delafield's Haematoxylin, rinsed in distilled water, then in tap water for about half a minute to"blue" them. Finally, they were stained for fat, in Sudan III for one hour, washed free of alcohol with water, and mounted in glycerine.

There are some modifications that must be discussed. In series A exps. II to X inclusive, the emulsion was made up with olive oil, previously ground up with Sudan III, and defibrinated blood. It was thought that in this way the fat globules would be already stained.

On examination, however, it was found that they were not.

Experiment VI to IX inclusive, the sections were stained in the usual manner with Sudan III, regardless of the fact that the olive oil was previously stained in the emulsion. In the later experiments X to XIV inclusive, the emulsion of unstained olive oil and defibrinated blood was used.

A departure from the usual protocol was made at this stage. Three white rats were fed for three days on a mixture of olive oil stained with Sudan III and porridge. At the end of the time they were killed and portions of their livers fixed, hardened, frozen and cut and subject to histological observation before staining with Sudan III. There was not the slightest trace of Sudan stained fat present in the cells. However, after staining the usual way there proved to be an excessive amount of fat in the liver, both peri and intra-lobularly infiltrated. This experiment was carried out with the hope of clearing up the difficulty presented above.+ It had to be abandoned at this stage, due to pressure of the other work, but it showed clearly that the Sudan III must have been deprived of its staining qualities, and most probably the liver was the active agent.

In series A seven sections were chosen from each portion of liver tissue, making a total of seventy slides. The fat emboli were counted in sixteen high power observations. Then the area for the sixteen readings was calculated, and the number of fat globules per 10 sq.mm. for each slide reckoned. The figures for the seven sections

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+ Here a few tests were made. The stained olive oil was mixed thoroughly with portions of the fixing solution, hardening solution, defibrinated blood and Haematoxylin, and also with some fresh liver cells. No loss of stain was observed.

were averaged and the numbers in Table I represent the average amount of fat globules per 10 sq.mm. of section. In series B a similar procedure was adopted, though here fifty slides were made, five from each original portion of tissue. Counts were made with the lower power generally over the whole slide, with the exception of experiment 14. Here the unusual amount of fat present necessitated a return to the high power method. The above two methods were considered a fair way of settling the matter. Numbers in Tables I and II must only be taken as a rough estimate. Probably the most serious difficulty of all was that of distinguishing between fat globules situated outside the cell, belonging to the injection mass and those normally present in the liver cells. (See Plate III). In the experiments in Series A this difficulty was almost insurmountable, as Plate II shows the manner in which a normal cat's liver may contain a great deal of fat.

In the observations in Series B, however, the interference of intracellular globules was reduced appreciably by the fact that the dog's liver did not contain nearly as much fat as the cat's liver, though experiment /4 (Plate V) was an exception to this statement. In Table II the averages for the left center and right lobes are given. There was always the possibility that the injection mass may have been washed through into the sub-hepatic veins. Too long a wait in the protocol between the injection and the excision of the organ would account for this. Also the injection was always made rapidly so as to simulate as nearly as possible the pressure of the normal arterial inflow. However, after the injection the removal of the organ was carried out as quickly as possible so that the above difficulty would be practically overcome.

Some of the stained emboli appearing in the sections outside of the cell might be due to the scraping of the razor but this was easily got rid of by ensuring that the microtome razor was always in the best of condition. Trouble with the clotting of blood was encountered in the earlier part of the work but later this was abolished by taking particular care to whip the blood thoroughly. It may not be out of place here to emphasize strongly the fact that all experiments in which there was the slightest doubt concerning the technique were discarded entirely.

PROTOCOLS OF EXPERIMENTS.

SERIES (a)

Exp. I. CAT ♂ 281015. INJECTION OF HEPATIC ARTERY WITH AN EMULSION OF OLIVE OIL AND DEFIBRINATED BLOOD.

ANAESTHETIZED 10.13
RECTUS INCISION 10.17
HEPATIC ARTERY located 10.30
FEMORAL INCISION 10.33
INSERTED CANNULA IN FEMORAL 10.38
DEFIBRINATED BLOOD about 30 ccs. 10.41
Defibrinated blood by whipping it with a feather until the fibrine clot was removed.
Mix the emulsion of olive oil and defibrinated blood.
CANNULA IN HEPATIC ARTERY. 11.
INJECTED 5 ccs. Emulsion 11.05
BLED TO DEATH 11.06
LIVER EXCISED 11.07
PIECES OF LIVER PLACED IN FIXING SOLUTION.

HISTOLOGICAL REPORT: Great quantity of fat present in liver; infiltrated cells; portal canals injected.

Exp. VI. CAT ♀ 121115. INJECTION OF HEPATIC ARTERY; GASTRODUODENAL ARTERY LIGATED; PORTAL VEIN LIGATED; EMULSION OF OLIVE OIL STAINED WITH SUDAN III AND DEFIBRINATED BLOOD.

HISTOLOGICAL REPORT: Large amount of fat in cells of lobules; injection mass found near the periphery of the lobules and in the portal canals; variation:- nil.

EXPERIMENT VII. CAT ♀ 161115. INJECTION OF HEPATIC ARTERY; GASTRO-DUODENAL ARTERY LIGATED; EMULSION AS IN EXPERIMENT VI.

HISTOLOGICAL REPORT: Results similar to preceding; variation: nil.

EXPERIMENT VIII. CAT ♂ 191115. PROCEDURE as in Experiment VII.

HISTOLOGICAL REPORT: Fat present in smaller quantity than Experiment VII. Variation: left.

EXPERIMENT IX: CAT ♀ 241115. INJECTION OF HEPATIC ARTERY; PORTAL VEIN LIGATED, GASTRODUODENAL ARTERY LIGATED.

HISTOLOGICAL REPORT: Preparations satisfactory; large amount of fat; variation: right and center.

SERIES (b).

EXP. X. DOG ♀ 061215. INJECTION OF HEPATIC ARTERY; GASTRODUODENAL ARTERY LIGATED; PORTAL VEIN FREE; EMULSION CHANGED TO OLIVE OIL AND DEFIBRINATED BLOOD ALONE, NO SUDAN III.

HISTOLOGICAL REPORT: Sections satisfactory, very small quantity of fat in the cells; portal canals injected; variation: left.

EXP. XI. DOG ♂ 161215. INJECTION OF HEPATIC ARTERY; PORTAL VEIN FREE; GASTRODUODENAL LIGATED.

HISTOLOGICAL REPORT: Very small amount of fat; portal canals injected; variation: nil.

EXP.XII. DOG ♂ 171215. SIMILAR DATA.

HISTOLOGICAL REPORT: Preparations satisfactory; variation: left.

EXP. XIII. DOG ♀ 290316. SIMILAR PROCEDURE.

HISTOLOGICAL REPORT: Variation: center.

EXP.XIV: DOG ♀ 300316. SIMILAR PROCEDURE.

HISTOLOGICAL REPORT: Infiltrated liver; large amount of fat present; variation: left.

DISCUSSION: It must be mentioned again that the possibility of experimental error hampering the results obtained was eliminated by discarding any experiment in which there happened to be the slightest mistake. Eight results were entirely abandoned through dissatisfaction of the technique. Series (b) may be considered as the crucial test and

TABLE I. FIGURE I.

		LEFT		CENTRE		RIGHT						
No. Exp.	Subject	Peri	Hil	Peri	Hil	Peri	Hil	Left C.	Right	Spig.	Caud.	
1	281015	54.1	42.3	54.7	45.2	53.7	49.1	53.1	61.3	19.3	49.1	
6	121115	38.7	38.4	36.9	27.6	36.9	35.4	27.6	29.7	33.3	33.	SERIES A
7	161115	16.9	15.8	16.	19.5	15.3	27	16	25.2	24.3	16.5	
8	191115	38.	34.	50.3	49.2	22.	46.	11.2	23.9	14.7	43.2	
9	241115	30.4	27.3	19.3	15.2	22.7	52.4	49.6	29.1	51.3	46.6	
10	061215	53.8	56.8	31.7	38.1	48.7	24.9	30.1	27.9	12.6	73.2	
11	161215	18.6	18.2	18.7	24.2	19.3	16.6	15.2	23.4	22.5	12.3	SERIES B
12	171215	32.	43.2	45.4	41.6	13.2	46.3	38.	17.4	24.8	30.5	
13	290316	44.4	69.5	89.	98	23.9	30.7	33.3	13.8	15.3	40.9	
14	300316	45.3	47.	36.1	38.4	24.3	27.	49.7	86.8	65.3	67.3	

TABLE II. FIGURE II.

Exp.	Subject	Left	Centre	Right	Lobe with Largest Am't
1	281015	49	65	43	Centre
6	121115	35	33	32	----
7	161115	17	17	19	----
8	191115	43	26	27	Left
9	241115	23	41	43	Centre & Right
10	061215	60	34	38	Left
11	161215	19.9	17	17	---
12	171215	40	32	24	Left
13	290316	75	29	23	Left
14	300316	41	33	73	Right

the portal canals as shown in Plate VI clearly demonstrate how the injection travels. Absolute similarity of experimental procedure was adopted, and particular care was exercised in making the count and in estimating the area of the sections so that the figures in the Tables convey a distinct meaning. Probably the most severe criticism that might be urged against the above results is that injury and mechanical stimulation of the hepatic plexus of nerve fibres in the operation would produce local vaso-constriction, and this materially influence the injection flow.

Burton-Opitz[26] has shown that the arterial inflow of the liver is under the direct control of the hepatic plexus surrounding the hepatic artery. It is true that to insert a cannula in the hepatic artery one must destroy sometimes, or at least, injure some of the fibres of the hepatic plexus. For this very reason a ~~weight~~ wait between the insertion of the cannula and the injection was made a necessary part of the protocol. This would allow a great part of any vaso-constriction that might occur to pass off.

The histological reports may be briefly summarized, viz:

(1) The portal vein on no occasion was injected.

(2) The hepatic veins usually held some injection.

(3) The portal canals were always injected.

(4) The majority of cats had a large amount of fat present in their livers. This is not the case with dog's livers; only one was infiltrated, and that mildly.

(5) The interlobular capillaries were easily distinguished, as also were the intralobular anastomoses.

(6) The biliary channels were always infiltrated.

It has also been demonstrated by Burton-Opitz[27] that the hepatic

blood flow may become a highly important compensatory factor whenever the portal inflow is lessened or entirely obstructed. The flow in the hepatic artery can be varied passively quite aside from vasomotor influences in that a greater or less quantity of portal blood may mechanically hinder the arterial flow into the liver. This may in part explain the wide variation in hepatic distribution in the liver, and it is possible that the mechanical influences might alter the portal inflow in different regions of its final ramifications.

Figures IV, V and VI show plainly how an analogy can be drawn between the portal systems of rabbit, dog and man.

SUMMARY:

(1) There is a variation in the amounts of blood carried to different lobes.

(2) There is found a great variation in different parts of the liver in the same animal and also in the same parts of livers in different animals. This is not true altogether of the left lobe, but is so of the right.

The main result is that in any one animal we cannot predict that the hepatic blood will be supplied in the various lobes or even parts of lobes, according to any definite plan. In one animal one lobe will receive more than its proportionate share. In another it will vary to another lobe. It may happen that at one time there is an equal distribution of the flow.

SECTION II.

THE INFILTRATION OF THE PORTAL VEIN.

BRIEF OUTLINE OF A TYPICAL PROTOCOL. Dogs were starved for three,

four and five days, and on the last day of the fast they were fed with a solution of cane-sugar by means of the stomach tube. Three and a half to five hours after the meal the animal was anaesthetized, the liver excised, perfused with ice cold .9% Saline solution, portions of the liver weighed, minced and introduced into Erlenmeyer flasks containing about 100 ccs. of boiling hot 60% K.O.H. (pure Merck). The flasks were previously fitted with rubber stoppers and reflux condensers on a boiling water bath (constant level). The whole procedure from giving the anaesthetic to the water bath occupied only half an hour's time.

METHOD IN DETAIL. The method for the estimation of glycogen was that described by Pfluger[28], with a few slight alterations. Allihn's method for the estimation of sugar was employed. About 20 to 30 grams of tissue from each of the three lobes were placed in three separate beakers. These portions were immediately weighed and cut into small pieces and introduced into three Erlenmeyer flasks containing about 60 ccs. of boiling hot 60% KOH. Beakers containing the mixed tissue were rinsed with potash and the rinsings poured into the flasks. These were fitted with reflux condensers and placed on a boiling water bath for three hours, during the early part of which time they were frequently shaken. At the end of the three hours they were cooled, and the contents of the flask introduced into large beakers. The flasks were thoroughly rinsed and the washings added to the large beakers, containing the original solutions. Now 600 ccs. of 95% alcohol were added to each beaker and the flocculent precipitates of glycogen appeared at once. The beakers were covered and left stand over night.

Next morning the supernatant fluids were decanted and the precipitates each washed with about 500 ccs. 60% alcohol, containing 1 cc. of saturated Sodium Chloride per litre. These were let stand. The decanted fluids were filtered by means of water pumps through hard Swiss filter papers: then the 60% alcohol washings were decanted and filtered similarly. About 500 ccs. of 95% aocohol were added again to each precipitate for further purification. Finally the precipitates were washed on to the filter papers with 95% alcohol and ether, and dried.

The glycogen precipitates were then dissolved in about 100 ccs. of hot water and these concentrated solutions were filtered by means of the pumps. The filtrates were diluted up to 250 ccs. with distilled water, in graduated volumetric flasks and 100 ccs. of each of these solutions were measured out with the burette into three beakers. 5 ccs. of con. HCL added to each and the beakers were then covered and placed on a boiling water bath for three hours to convert all the glycogen into dextrose. About 25 minutes before the required time elapsed, the lids were removed from the beakers so as to permit a quantity of the acid solution to evaporate, though this was not allowed to go too far. At the end of three hours the acid solution was carefully neutralized with 20% KOH, filtered into 100 ccs. measuring flasks and the beakers on filter paper, washed with distilled warm water. The volume was finally made up to 100 ccs, though in one case the sugar-solution was made up to 250 ccs. Up till this stage the three solutions were carried along at once, but from now on in the method only one was used in the operation.

50 ccs. of Fehling's solution (25 ccs. from solutions I and II) were placed in a porcelain bowl on a boiling water bath until the solution had attained the temperature of the water bath, about six minutes. A delivery pipette 25 ccs. of the sugar solution accurately measured with a pipette calibrated to deliver 24.95 ccs, was introduced at the end of the six minutes into the Fehling' solution. Boiling water was added to the mixture until the volume was about 100 ccs. This solution was left on the water bath for exactly twelve minutes. Then it was immediately filtered through a previously weighed Allihn tube with platinum gauze and asbestos fibre. The porcelain bowl was thoroughly washed into the filter. The precipitate of Cu_2O which settled on the filter was washed with hot water, distilled water, 10 ccs. of 95% alcohol, 10 ccs. of ether and finally dried. Then the precipitate was oxidized by gentle heating in a current of oxygen to cupric oxide, then cooled, and reduced by gentle heating in a current of hydrogen to metallic copper. It was finally cooled and weighed. The weights of copper were referred to tables[29] containing corresponding amounts of dextrose to weights of copper. The amount of dextrose in the 250 ccs. of glycogen solution was readily determined. This amount was multiplied by the constant .927, converting it to glycogen and the glycogen finally calculated in 10 grams of fresh tissue. Three parallel estimations were carried out on each sugar solution, the final calculation made from the mean of these weighings. A blank test was carried out before every three estimations. The reason for this is that there is a small amount of self-reduction in Fehling's solution when boiled. The amount was generally estimated as .001 grams.

There are some points in detail that have to be strictly followed in the same manner in every test. Speed and accuracy were necessary factors in removing the liver, perfusing it with ice-cold Saline for about six minutes, weighing and placing it on the water bath. The perfusion was undoubtedly a decided help. It removed any blood and excessive pigment that might be present. Also the action of glycogenase (a glycogenolytic ferment in the liver) was interfered with. This ferment breaks down the glycogen in the liver and the process reaches a maximum twenty minutes after excision of the organ.[30] Care was exercised in drying the glycogen precipitate for if this is neglected glycogen is made useless in a very short while through exposure to the air. In converting the glycogen to dextrose, on one occasion the solutions were let boil dry, and the remaining portions of the original glycogen solutions (250 ccs.) were used.

The Fehling's solutions were made up fresh in one litre quantities and with exactly the same constitution. The use of Fehling's solutions of different composition obviously would give extremely inaccurate results. The use of the water pump was found to be a decided saver of time in the filtration processes. The Allihn tubes were used only when their weights had remained constant after four or five preliminary heatings and weighings. The weights remained fairly constant, changing only 3 mgms. in two or three weeks. After each test a precipitate of copper was dissolved in nitric acid and the filter washed with distilled water, alcohol, ether and dried. The tubes were then heated in a current of hydrogen and cooled and weighed.

The oxidation of a final precipitate to Cupric Oxide was a

TABLE III. FIGURE III.

Exp	Subject		Glycogen. Right	Center	Left	Greatest Diff.	% Diff	Lobe that contains largest amt.	Liver wt.	Body wt Kilos	Hours after feeding	Remarks
15	090216	♂	.540	.540	.523	.017	3.1	Right	287.71	6.52		Control
16	110216	♀	.560	.412	.325	.235	41.9	Right	415.97	9.605	3-1/2	Fed on 3rd day.
17	160216	♀	.293	.515	.672	.379	56.9	Left	342.79	6.736	4	Fed on 4th day.
18	210216	♀	.794	.860	1.168	.374	32.	Left	341.49	5.16	3-1/4	Fed on 5th day.
19	240216	♀	1.40	1.10	1.25	.302	21.4	Right	632.56	11.51	5	" "
20	280216	♂	2.00	1.839	1.738	.262	13.2	Right	415.26	7.17	3-3/4	Fed on 4th day.
21	010316	♂	1.13	1.077	1.01	.12	10.6	Right	326.05	5.69	16	Fed on 5th day.

part of the procedure adopted in case any organic material was present. The most important part of the technique which was followed implicitly was the adoption of a method that ensures the same amount of dilution, boiling,etc., in every estimation. Without these precautions no accurate or trustworthy results could ever be obtained. In Experiment XXI digestion was let proceed for sixteen hours and then the estimation of glycogen was carried out. Two experiments at the outset were discarded as worthless, because a gross error was discovered in the preparation of Fehling's solution.

DISCUSSION: Results of seven experiments are compiled in Table III. Glycogen is calculated per ten grams of fresh tissue. There appears a great variation between the amounts of glycogen deposited in the lobes of one animal's liver. As high as 56.9% variation is recorded in the case of Experiment XVII. The control shows only 3.1% variation, and thus coincides with the view of Macleod on the distribution of glycogen over the liver of a normal animal. He also believes that after digestion has reached its height, there is a similar equality of distribution of glycogen. Experiment XXI with 10.6% variation nearly agrees with this view. The right lobe seems to be favoured slightly more than the left, the center not appearing first in any case. This directly contradicts the view of gastric digestion proceeding almost entirely through the left lobes, as outlined by Sérégé, Bartlett,Corper and Long.

SUMMARY:

(1) The natural importation of portal blood is not distributed according to a fixed plan.

(2) In no case of early digestion is there an equal distribution of portal blood.

SECTION III.

INFILTRATION BY WAY OF HEPATIC ARTERY.

Throughout these experiments by "Fatty Acid" is meant the non-volatile higher fatty acids which are soluble in petrol ether, (B.P. 40-60° C.) and form potassium salts soluble in 50% alcohol. The method of estimation used is that devised by Mottram[31].

GENERAL OUTLINE OF THE METHOD. The liver tissue is clear from visible fat and connective tissue, and minced. A weighed portion is dissolved in 60% potash. The fatty acids are precipitated with con. HCL acid and extracted with ordinary ether. The resulting extract (consisting of acid haematin, fatty acids, cholesterol and other lipoids) is dried, dissolved in petrol ether and filtered. The resulting solution is converted into soaps and unsaponifiable substances by means of a saturated solution of potash in 50% alcohol. The soaps are separated, the fatty acids precipitated and extracted with petrol ether. The solution of purified fatty acids is then filtered and dried in vacuo at 100° C. and weighed.

METHOD IN DETAIL AND TECHNIQUE. Rabbits were used in these experiments and were killed by a blow on the nape of the neck. The liver was removed immediately and perfused for about 5 minutes with cold Saline .9%. Three portions of liver (10-15 grams each) were taken, freed of connective tissue and any visible fat, weighed and introduced through short wide-mouthed tubes into pressure bottles of about 100 ccs. content. As many ccs. of 60% KOH as grams of fresh tissue were added and three bottles fitted with rubber stoppers and exhausted of air

by means of water pumps. Then they were immersed in a boiling water bath for three hours, and were frequently shaken during the earlier part of the time.

The caustic potash solutions were transferred hot to separating funnels of about 250 ccs. capacity, by means of ordinary funnels. The pressure bottles were thoroughly rinsed with warm water as well as the ordinary funnels. The separating funnels were fitted with taps and stoppers previously moistened with glycerine, and cooled under the tap. Then 20 ccs. of Con.HCL were added to each funnel slowly and heavy precipitates resulted. The acid solutions were cooled under the tap, and then each shaken for 30 seconds with 50 ccs. of distilled ethyl ether. A separation occurred very rapidly and the acid solutions were run off into beakers until the precipitate in each which rose to the junction of the ether and acid just began to leave the funnel. The ethereal solutions of fatty acid,etc., were then siphoned off into Co_2 flasks and the funnels rinsed twice with 10 ccs. of ether. The rinsings were siphoned off in a similar manner.

Each of the precipitates was dissolved in a few drops of 60% KOH and shaken for 30 seconds with 50 ccs. of ether. Before the emulsions had time to settle the acid solutions from the beakers were added to the separating funnel and the mixtures each shaken for a further 30 seconds. Separation was helped at this stage by slightly shaking the funnels. When separation was complete, the acid solutions were run off into the beakers and the ethereal solutions of fatty acids siphoned off into the original Co_2 flasks. The funnels were rinsed, the rinsings siphoned off as before. Solutions of each of

the precipitates was carried out again but this time with 25 ccs. of ether. The siphonings each time were added to the original Co_2 flasks. The united ether extracts of each portion were evaporated in a current of Co_2 nearly to dryness. Absolute dryness results in pigmentation of the fatty acids. The extracts just moistened with ether were left to stand over night in a covered jar in an atmosphere of Co_2. The ether evaporated during this time and left a brownish crystalline masses. Each of these was dissolved in 50 ccs. of petrol ether and the mixtures filtered through asbestos fibre filters into three separating funnels. The Co_2 flasks were thoroughly rinsed with petrol ether and the washings filtered into the funnels. The filters were also washed thoroughly, especially the lower ends. 50 ccs. of 50% alcohol with a small piece of Potash were added to each funnel, and each mixture was shaken for 30 seconds.

Just as separation was thoroughly well defined a drop of phenolphthalein was added to each funnel. This greatly helped in the judgment of the line of separation. The subnatant fluids were then run off into graduated cylinders of 250 ccs. capacity. The petrol ether solutions were washed further with 50 ccs. and 25 ccs. of the potash in 50% alcohol. The alcoholic solutions of the potassium salts of the fatty acids of the three portions were thus collected into the graduated cylinders. 5 ccs. con.HCL were added to each cylinder and the mixtures shaken with a few drops of methyl orange. This was added just to make sure that the solutions were of acid reaction and the addition of the acid precipitates the fatty acids afresh.

The contents of each cylinder were extracted three times with

petrol ether(twice with 50 ccs. and once with 25 ccs) and the ether extracts siphoned off into Co_2 flasks. Between each extraction the cylinders were rinsed twice with 10 ccs. of petrol ether and the washings siphoned off into the correct Co_2 flasks. The combined extracts of each portion were evaporated in a current of Co_2 till the fluid measured about 20 ccs. These concentrated solutions were cooled with the Co_2 passing through and filtered through the asbestos fibre filters into three clean weighed glass bottles (about 35 grams) fitted with ground-in stoppers with short entrance and exit tubes.

The Co_2 flasks and filters were carefully washed into the small flasks. The petrol ether were distilled off from each flask and just as this was about to be completed the stopper was carefully removed slightly in each and washed with petrol ether, and this again evaporated. The small flasks were then cooled with Co_2 still passing through, and the fatty acids dried in a vacuum oven at 100° C. for six hours(usually over night) and were weighed next morning.

As in section II the liver was perfused with .9% cold Saline, for this was found a great help in freeing the tissue from any surplus pigment. Hartley[32] has shown the rapidity with which the fatty acids oxidize when exposed to air, especially at higher temperatures, and every precaution was taken to prevent this. This explains the evacuation of the pressure bottles, the evaporation of the ether in a current of Co_2, and the final drying in the vacuum oven. In the filtration the occurrence of bubbles was eliminated as much as possible. The asbestos fibre filter used was the same as employed by Mottram.[33] It was found a saving of time to renew the asbestos after each estimation.

TABLE IV FIGURE IV.

Exp.	Subject		Liver wt.	Initial Body wt.	Final Body wt.	Fatty Acid Left.	Fatty Acid Centre	Fatty Acid Right	% Variation	Remarks
22	130316	♂	72.82	2227	2227	.3355	.2338	.3771	38.2	Control
23	170316	♀	64.25	+	1765.85	.3605	.2898	.4137	12.8	2 day
24	180316	♀	56.61	2967.21	2967.21	.3163	.3061	.2731	13.6	Control
25	210316	♀	46.97	+	1927.8	.2721	.3024	.3755	27.5	2 day
26	220316	♂	44.46	1802.2	1814.4	.4050	.4589	#	----	1 day
27	230316	♂	54.92	1899.4	1859.7	.5583	.6459	.5009	22.4	3 day
28	240316	♀	45.99	1972	2005.5	.8706	.9650	1.2779	31.9	3 day
29	250316	♂	41.08	2069.5	1984.5	.5016	.5408	.6133	18.2	3 day
30	260316	♂	44.55	2005.5	1998.6	.3479	.3672	.3505	5.2	3 day.
31	270316	♂	52.42	2005.5	2069.5	.5086	.4271	.4088	19.6	3 day

\+ Unfortunately neglected.

\# Accident.

DISCUSSION: The Table IV gives the results of 10 experiments with the fatty acid calculated per 10 grams of fresh tissue. It was found out that the starvation period had to be hit upon by chance, which would give a moderate degree of infiltration. Experiments 23-27 are fairly good examples, with the possible exception of Experiment 26. It is interesting to note that in one day's starvation there seemed to be more infiltration than in the two days' fast: similar fact discovered by Mottram[34]. The possible explanation for the peculiarity is that the liver was at work on the fat already imported to it, with the consequence of a rise of iodine value, a decrease in the amount of fat present. The apparent discrepancy in experiment 26 is easily explained by this. Even in a control there is no equality of distribution of the fat, a noteworthy fact. The percentage variation shows a great difference, the greatest appearing with the largest infiltration. The liver at work shows a variation of 5.2%. The reason for the great variation may be explained by supposing that more fat is brought to the liver than it can handle at one time. The results are comparable to Section I.

SUMMARY:

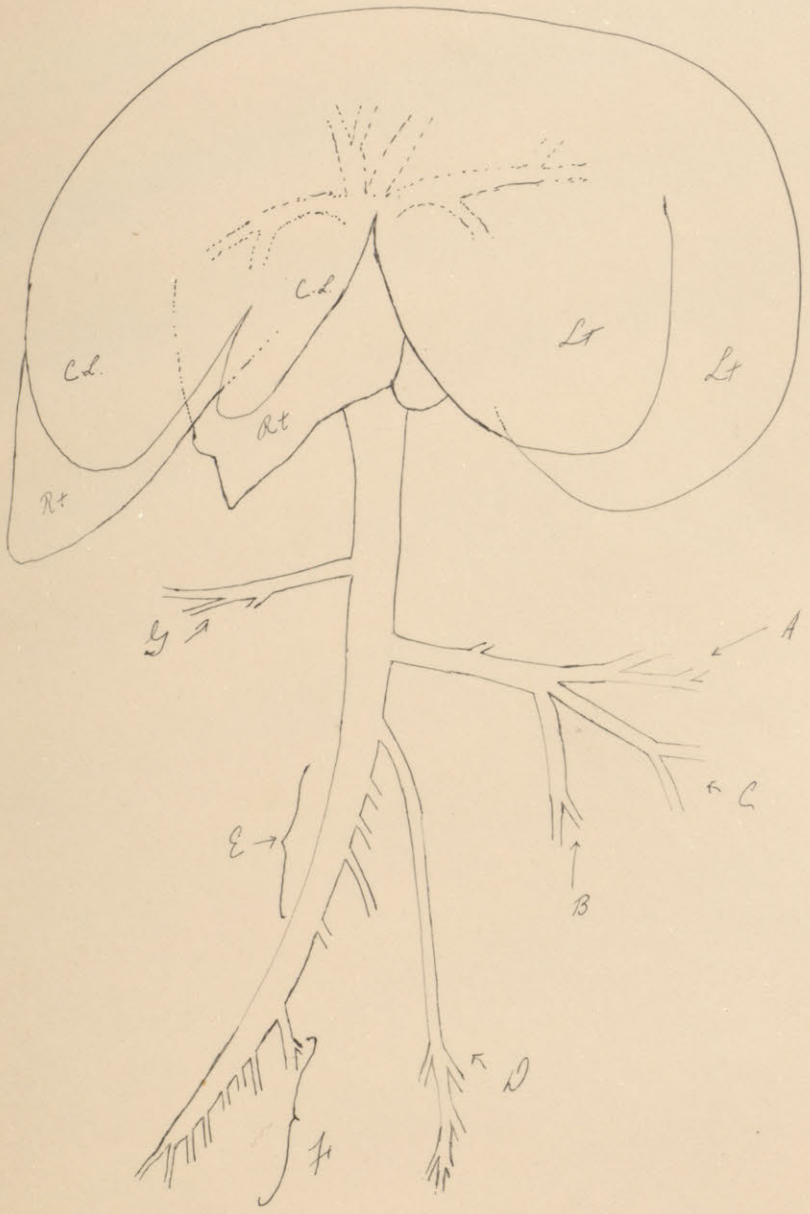
(1) The hepatic blood not distributed to the liver in any definite way. At one time one lobe will receive the greatest amount of blood; at another time, a different lobe.

Before concluding I wish to most heartily thank Messrs Bond, of the Histological Department, E.G.Cassidy, the Laboratory Assistant, and E.G.Cracknell, the Technician, for their valuable assistance. To Dr.

Harding I am very grateful for many helpful suggestions throughout the year, most especially do I wish to express my sense of indebtedness to Professor Mottram for the way in which he so willingly sacrificed his time and energy to give suggestions,criticisms and encouragement.

Fig. 4.

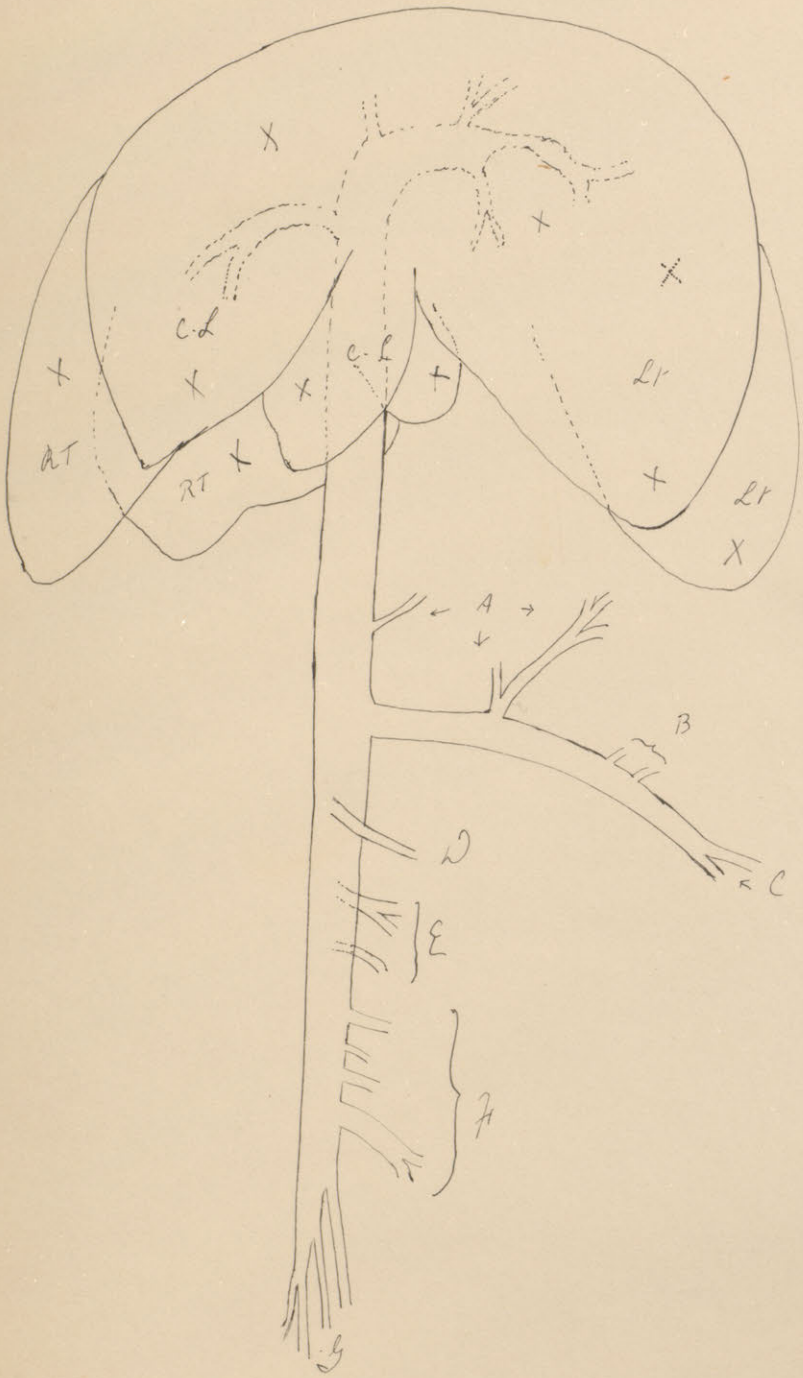
Portal System of Rabbit



A: veins from cardiac portion of stomach. B: from pylorus C: splenic veins. D: Posterior mesenteric vein. G. Duodenal branches F: Ileal veins. E: Jejunal veins. Rt: Right Lobes C.L. Central lobules. Lt. Left lobe

Fig. 5.

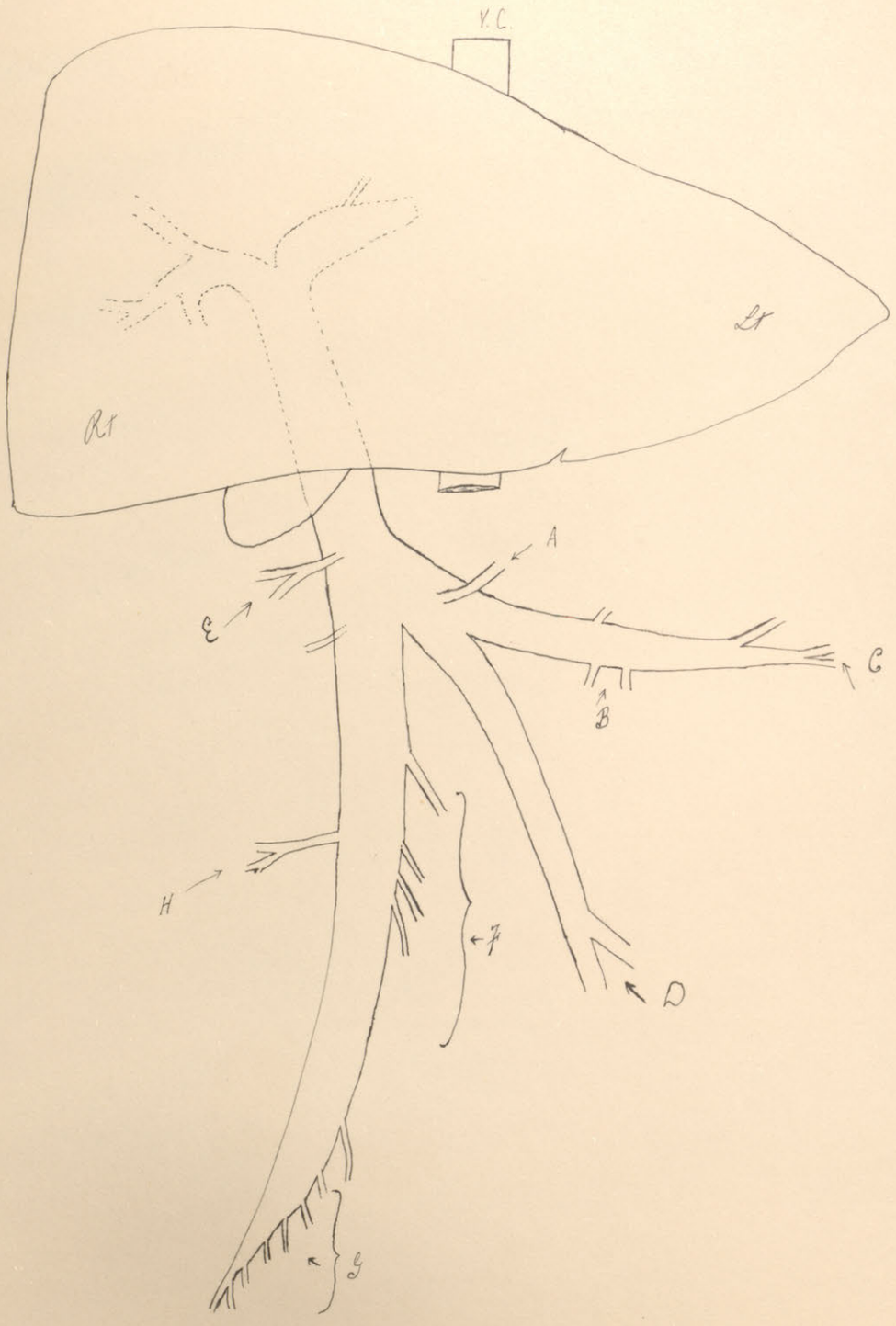
Portal System of Dog



A and B: Veins from stomach C. splenic vein D: small mesenteric E: Duodenal veins F. Jejunal veins. G. Ileal and ascending colon branches Rt. Right Lobe C.l. Central lobules. Lt. Left Lobe. X. Sections taken.

Fig. 6.

Portal System of Man



A: vein from lesser curvature of stomach. B: pancreatic veins. C: splenic veins. D small mesenteric vein. E: vein from greater curvature of stomach F: jejunal veins G: ileal veins. H: vein from ascending colon. V.C: Inferior Vena Cava. Rt: Right Lobe. Lt: Left lobe.

EXPLANATION OF PLATES.

I. Normal Cat's Liver Cells.

II. Same as I infiltrated.

III. Cells of Injected Cat's Liver.

IV. Normal Dog's Liver Cells.

V. Same as IV infiltrated.

VI. Portal Canal.

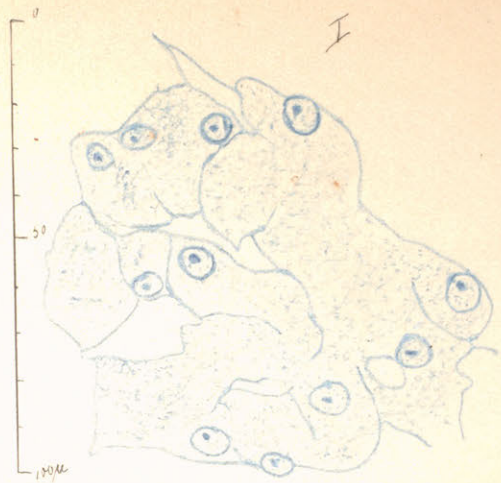
(a) Portal Vein;

(b) Bile Duct.

(c) Hepatic Vein.

(d) Hepatic Artery

(e) Injected Capillaries of Hepatic Artery.


I
0
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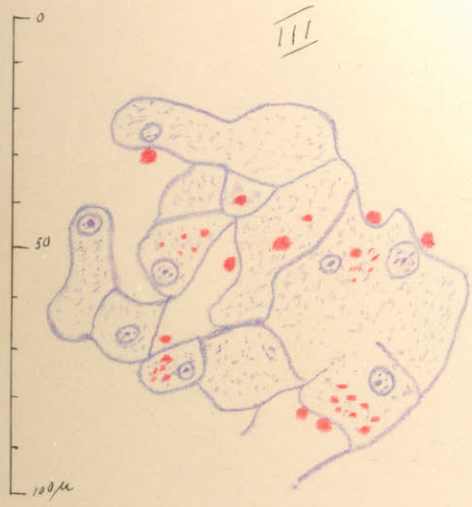
100μ


III
0
50
100μ


II
0
50
100μ

IV

V

VI

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