EXPERIMENTAL STUDY OF CIRRHOSIS OF THE LIVER

PART I

PRODUCTION OF EXPERIMENTAL CIRRHOSIS OF THE LIVER

by

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CONTENT PART I

I INTRODUCTION

In order to study the efficiency of surgical treatment for cirrhosis of the liver, paying special attention to the efficiency of hepatic artery ligation, I have undertaken to induce experimentally in dogs a condition similar to that of cirrhosis of the liver

in human beings.

In 1937, it was reported that ROUSSLOT and THOMPSON[1] injected particles of silicon dioxide directly into the portal veins of dogs in order to produce BANTI's syndrome, and they succeeded in producing the cirrhotic liver, portal hypertension and splenomegaly 2 or 3 years after the injection.

In 1955, BARET and FITTS[2] administered carbon tetrachloride according to an experiment carried out by BOLLMAN and MANN,[3] and after a period from four to eight weeks, they were able to induce cirrhosis of the liver and portal hypertension. However, likewise to the preceding experiments, they could not produce ascites to any degree.

It was previously reported that when the inferior vena cava between the liver and the heart was constricted experimentally, ascites and portal hypertension were produced. In 1930, ZIMMERMAN and HILLMAN[4] performed a extensive histological studies on congestive liver and fibrosis of the liver in the dogs whose vena cava had been constricted. MCKEE, SCHILLING, TISHKOFF and HIATT,[5] in 1949, examined the influence of sodium chloride and protein on ascites induced by this method, and in 1950, VOLWILER, GRINDLAY and BOLLMAN[5] studied the orgin of these ascites. Reviewing the literatures, it can be said that only the constriction of the inferior vena cava is effective in producing ascites, and actually this method has been most widely used for that particular purpose, the production of ascites.

On the other hand, due to the fact that constriction of the inferior vena cava is accompanied by congestion of the kidneys, adrenal glands and systemic region caudad to the occlusion, it was believed that factors other than the liver should be taken into consideration. Thus, there were several methods reported to induce congestion to the liver only.

In 1944, ARMSTRONG and RICHARDS[7] constricted the hepatic veins according to the method used by SIMMONDS and BRANDES.[8] KERSHNER, BOOTON and SHEARER,[9] in 1946, believed it impossible to make a direct approach to the hepatic veins and they ligated and divided the vena cava immediately caudad to the liver in two successive stages in order to minimize the influence caused by congestion below the diaphragm, then they performed constriction of the inferior vena cava above the diaphragm.

In 1952, WILES, SCHENK and LINDENBERG[10] anastomosed the vein graft end-to-side to the vena cava circumventing that portion of the vena cava within the liver. The proximal anastomosis was just superior to the diaphragm, and the distal anastomosis just inferior to the liver. The inferior vena cava was ligated between the liver and the distal anastomosis. Thus, all the blood from the iliac and renal veins was shunted through the vein graft. Finally the vena cava above the liver but below the diaphragm was constricted to one-half its lumen. Thus, they induced portal venous congestion without systemic congestion inferior to the liver.

In 1953, CROSS, RAFFUCCI, TOON and WANGENSTEEN[11] performed complete individual occlusion of the hepatic veins and, at the same time, carried out portacaval shunt and obtained portal hypertension and ascites. But there was a crux in performing a portacaval shunt.

In order to produce a state similar to hepatic cirrhosis, I placed much stress on the following points,

1) to have symptomatic similarities rather than pathohistological ones,
2) to induce an injury exclusively to the liver,
3) to have an ultimate result within a comparatively short period of time.

II EXPERIMENTAL METHOD

(1) Materials and Examinations

i- Materials

Mongrel dogs of normal health with body weights of approximately 10 kg were used.

ii- Examinations

a : Liver Function Tests

For the liver function tests, Bromsulphalein Test (B. S. P.), Zinc Sulfate Test (Z. S. T.), TAKATA's Reaction in serum, and Sublimate Reaction in serum were adopted.

(a) Bromsulphalein Test

5 mg/kg of Hepatosulphalein (DAIICHI PURE CHEMICALS Co., Ltd.) was injected intravenously and the percentage of the dye retained in the serum was determined. When B. S. P. was administered intravenously to normal dogs, retaining amount of dye in serum were 5 per cent after 10 minutes, 2.5 per cent after 20 minutes, 1.5 per cent after 30 minutes and 0 per cent after 45 minutes, respectively. In the present study, the samples obtained 20 and 45 minutes after the injection were examined.

In the thirteen normal dogs, the percentage of dye retained in the serum ranged from 0 to 5 per cent after 20 minutes with the average of 2.3 per cent and those after 45 minutes ranged from 0 to 2.5 per cent bringing the average of 0.75 per cent. Therefore, those over 5 per cent for the 20 minutes value and over 2.5 per cent for the 45 minutes value were considered as abnormal.

(b) Zinc Sulfate Test

The Zinc Sulfate Test by KUNKEL was performed. In the 21 cases of normal dogs, they ranged from 0 to 11 units with an average of 4.2 units. Excluding the three cases which had high units, 18 cases or namely 85 per cent of all the cases showed less than 6 units. Therefore, those over 7 units were considered as abnormal value.

(c) TAKATA's Reaction

As the percentage of TAKATA's reaction reagent used for humans showed practically no reaction in all normal dogs and even in dogs which were believed to be morbid, the concentration of the sublimate was increased to 1.5 per cent. Four positive test-tubes were obtained on normal dog controls and thus I designated (+) for five positive test-tubes, (++) for six, (+++) for seven and (+++) for eight or more.

(d) Sublimate Reaction

In this test, 0.25 per cent solution of sublimate was used. By using a reagent

of over 0.35 cc on serum of normal dogs, serum began to flocculate. Therefore, when 0.35 cc or more of the reagent was used, I designated the reaction to be negative, and when from 0.34 to 0.30 cc was used, to be (+); from 0.29 to 0.25 cc, to be (++); and from 0.24 to 0.20 cc, to be (+++) (Table 1).

Table 1. Liver Function Tests in Normal Dog

Bromsulphalein Test (13 dogs)
Retention in 20 minutes, 0 to 5%, average 2.3%
Retention in 45 minutes, 0 to 2.5%, average 0.75%
Zinc Sulfate Test (21 dogs)
Turbidity 0 to 6 units 18 dogs
〃 7 to 11 units 3 dogs
Sublimate Reaction (18 dogs)
Volume of Reagent 0.35 to 0.49 in all dogs
TAKATA'S Reaction (5 dogs)
Numbers of positive test-tube 2 to 4 in all dogs

b: Measurement of Portal Vein Pressure.

It can be naturally considered that the portal vein stream flows from the capillaries of the abdominal organs to the sinusoids of the liver with a gradient of pressure. It would be unreasonable to make a comparison unless the position to measure is fixed. Finding the confluent place of the splenic vein flowing into the portal vein to be most reliable, I decided to take measurements at this place and made calculation considering this position as a zero point. Applying the method employed by TAYLOR and EGEERT,[12] I exposed a small branch of the superior mesenteric vein and made a small incision into which the vinyl tube (approximately 1 mm in diameter) was inserted. The tip of this tube was introduced to the place where the splenic vein joined the portal vein trunk, and the measurements were made by using physiological salt solution. In taking the measurement, the dog was laparotomized in dorsal position under anesthesia of amobarbital (0.02-0.03g/kg) administered intravenously.

In the 75 normal dogs, the values of the portal pressure showed an approximate statistical normal distribution, the average value showing 118.37 mm H_2O with 31.8 mm H_2O standard deviation ranging from a minimum portal pressure of 62 mm H_2O to a maximum of 195 mm H_2O. Within the distribution of these values, 95 per cent of the total values were discovered within the $m \pm 2\sigma$, or namely in the values ranging from 55.13 mm H_2O to 182.33 mm H_2O. Therefore, I designated 180 mm H_2O as the top limit for normal portal pressure and that above 180 mm H_2O as portal hypertension (Table 2).

c: Pathohistological Study

In all cases, a specimen of the liver was obtained at the time of laparotomy, which was fixed in formalin solution and given a hematoxylin eosin and VAN GIESON'S stain.

(2) Method of Production of the Condition Similar to the Hepatic Cirrhosis

KERSHNER et al.[9] previously stated, "On the basis of these anatomic findings, no practical experimental approach on the hepatic veins directly suggested itself".

BOLLMAN[13] also stated, "Because of the anatomic relation of the liver to the inferior vena cava, no suitable technique had been devised for direct constriction of the hepatic veins".

As mentioned previously, ARMSTRONG and RICHARDS,[7] applying the procedure utilized by SIMMONDS and BRANDES,[8] carried out a method of collectively constricting the hepatic veins below the diaphragm avoiding the damage to the inferior vena cava on nine dogs. They reported that three cases survived for a long period and that one developed ascites and portal hypertension. KERSHNER, HOOTON and SHEARER,[9] prior to causing constriction of the inferior vena cava above the diaphragm, ligated and divided the inferior vena cava between the liver and kidney in two stages and waited for collaterals to develop sufficiently and then, after having avoided systemic congestion caudad to the occlusion, they performed the constriction of the inferior vena cava above diaphragm on many dogs. Four dogs that survived the three-stage operations developed portal hypertension and ascites.

Table 2. Portal Pressure in Normal Dog

mm H_2O	No. of Dogs
60	3
70	4
80	3
90	13
100	11
110	8
120	7
130	2
140	9
150	6
160	4
170	0
180	3
190	2

Minimum : 62 mm H_2O
Maximum : 195 mm H_2O
Mean Value : 118.73±31.8 mm H_2O

In 1952, WILES, SCHENK and LINDENBERG,[10] by anastomosing the vein graft to the vena cava above and below the liver, were able to establish a shunt of the inferior vena cava. Thereafter they constricted the inferior vena cava between the liver and the proximal anastomosis and they were able to exclude systemic congestion caudad to the occlusion. They reported that a congestion of a uniformed degree in various lobes could be expected. This experiment was performed on ten dogs. Five survived for a comparatively long period of which two were found to have developed ascites.

C. G. CHILD,[14] with the object of causing occlusion of the hepatic outflow, inserted a polyethylene tube within the inferior vena cava and ligated the inferior vena cava without exerting any influence whatsoever to the tube therein, at places above and below the joining of the hepatic veins.

In 1953, CROSS, RAFFUCCI, TOON and WANGENSTEEN[11] reported that they succeeded in ligating the hepatic vein branches separately. They accomplished this with the thoracoabdominal approach and they had simultaneously to perform a portacaval shunt, because complete ligation of the hepatic veins brought about a 100 per cent death rate. Under this condition they were able to induce portal hypertension and ascites to approximately half of their fifteen cases performed.

This report is the first and only one made on such a contemplation that the

hepatic vein itself is to be manipulated. I had an intension, from the beginning of this study, to manipulate the hepatic veins only, and finally I was able to expose the entire hepatic vein branches. I used only the abdominal approach and found it unnecessary to use the portacaval shunt.

i- Anatomical Study of the Hepatic Veins

The liver of a dog can be divided into three main lobes, namely the left, middle and right lobes, and the caudate lobe. The three lobes excluding the caudate lobe can be again separated into two small lobes each, and in the left and right lobes, they were named as the superior and the inferior respectively. For the left lobe, for instance, we called the left superior lobe or the left inferior lobe. As for the middle lobe we divided it to the left and right with the gallbladder in the center and called them the middle left lobe and the middle right lobe (Fig. 1). The portal vein, the

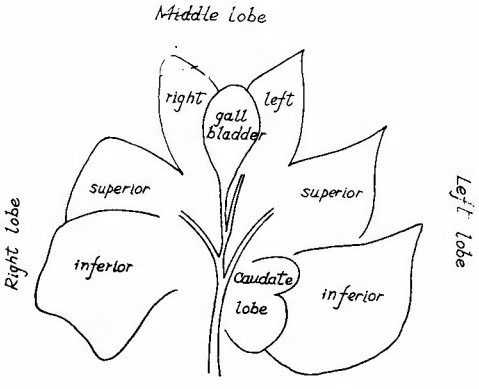

Fig. 1 Diagrammatic view from porta hepatis of dog's liver, anatomical relations and its nomenclature.

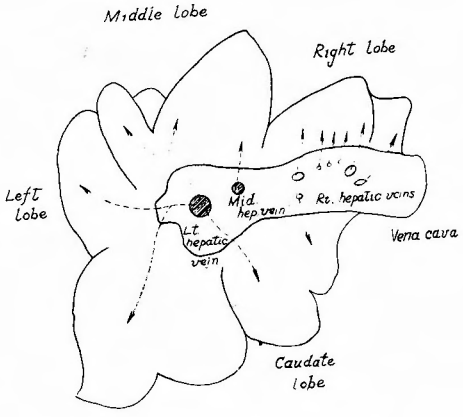

Fig. 2 Diagram illustrating main hepatic veins emptying into the inferior vena cava and areas of liver drained by them.

hepatic artery and the bile duct ramify approximately at the porta hepatis and enter each lobe. Then they were named as the right branch, the middle branch and the left branch respectively.

The hepatic vein can also be roughly divided into the left, right and middle branches but their draining areas are more or less complicated. The left branch is the thickest, being an assembly of the hepatic vein branches of the left lobe, the caudate lobe and the middle left lobe, but as for the middle branch it conveys the blood only from the middle lobe. The left and middle branches join the inferior vena cava immediately below the diaphragm. The middle branch is considerably fine and at times it consists of two branches. The inferior vena cava is imbedded in the right lobe so that the right hepatic vein branches cannot be recognized from the outside. The right branch issues from the right lobe in several thin branches which connect themselves directly to the inferior vena cava in the liver parenchym (Figs. 2, 3 and 4).

As considered from the anatomical standpoint, a procedure to place a thread

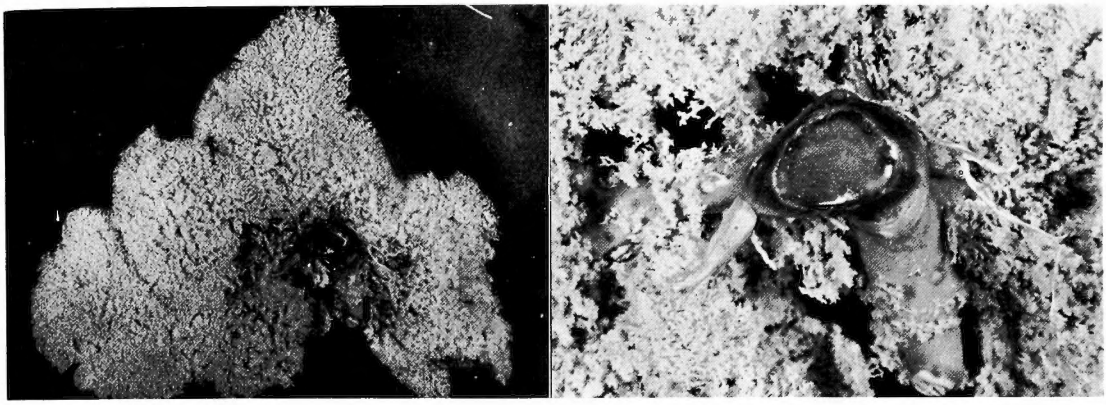

Fig. 3 and 4 Plastic resin specimen of normal dog's liver, showing the relationship between hepatic veins and inferior vena cava. (Plastic resin was injected into inferior vena cava.)

around the left and middle branches of the hepatic vein is possible but as regard the right branches, this is quite difficult as claimed by both BOLLMAN[13] and KERSHNER.[9] Therefore, at the beginning of my study I had conceived a method either to partially resect the right lobe, or to ligate and divide the right hepatic bile duct which empties itself into the right lobe, and later, I succeeded in manipulating the right hepatic vein.

These methods are as follows;

Method A :- Complete ligation of the middle and the left hepatic vein branches. (Right lobe intact)

Method B :- Constriction of the middle and left hepatic vein branches and partial resection of the right lobe.

Method C :- Complete ligation of the middle and left hepatic vein branches and ligation and division of the right bile duct.

Method D :- Individual ligation of the right hepatic vein branches and constriction of the middle and left hepatic vein branches (Fig. 5).

ii- Method of Ligation of the Middle and Left Hepatic Vein Branches.

Anesthesia was generally induced by administering amobarbital soda intravenously (20-30 mg/kg). The dog was placed in a supine position and given a midline epigastric incision. In gently depressing the liver downwards and lifting up the costal margin, the left and middle hepatic vein branches which emptied themselves into the inferior vena cava could immediately be seen below the diaphragm. The falciform ligament was dissected up to its attachment to the left hepatic vein branch, and the left triangular ligament was separated. As the lesser omentum was found to extend to the vicinity of the left hepatic vein branch just in front of the caudate lobe and as it veered toward the diaphragm, this was also dissected. After the left lateral side of the left branch was completely freed, the front side was also loosened carefully. The contour of the left hepatic vein branch became distinct so that a thread was able to be placed around the left branch by using a DESCHAMPS' aneurysm needle without any resistance.

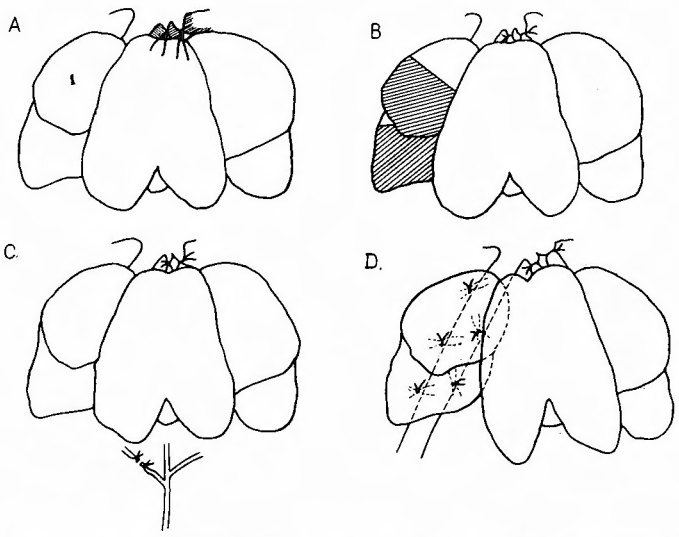

Fig. 5 Schematic representation of the operative methods to produce the condition similar to cirrhosis of the liver.

As for the middle hepatic vein, it was a simple matter to place a string around it, because its vessel wall was comparatively well defined.

In ligating the vein, the string was naturally placed in position and then tightly tied around it. In case of constriction, an appropriate substance was placed beside the hepatic vein and, after ligating them together, it was drawn out.

The method A mentioned above was to ligate the left and middle branches completely, whereas Method B consisted in the constriction of the left and middle branches respectively combined with the partial resection of the right lobe. In Method C, adding to the complete ligation of the left and middle hepatic vein branches, the ligation and division of the right hepatic bile duct was performed.

iii- Method of Ligation of the Right Hepatic Vein Branches.

After inducing anesthesia, the upper half of the body of the experimental dog was placed on its left side on the table and its lower half in a supine position. The abdominal cavity was opened by a mid-line epigastric incision combined with a transverse incision which ran to the right at the level of the umbilicus and extended to the back. The right triangular ligament and the hepatorenal ligament were divided, and the right lobe was pushed aside to the left.

When the peritoneum that covered the part between the diaphragm and the posterior face of the right lobe was opened, the posterior face of the right lobe was distinctly viewed. In some experimental dogs, the wall of the inferior vena cava was able to be seen at the rear of the right lobe. But the right and the caudate lobe, as they were in most cases, were fused together just behind the vena cava so that the wall of the inferior vena cava was covered by them. In the latter cases, the liver tissue which lay on the inferior vena cava was dissected along the vein wall.

As the wall of the inferior vena cava came into a full view, it was carefully

dissected from the liver to obtain a branch of the hepatic vein. After dividing the branch between the two ligatures, the other hepatic vein branches could be attained comparatively with ease. Each of five or six branches was entirely ligated and divided, but sometimes the uppermost branch was only ligated or constricted.

III RESULTS OF EXPERIMENTS

(1) Changes Immediately after the Constriction of the Hepatic Veins, Especially on the Changes of Portal Pressure.

In the cases of Method A and C (complete ligation of the left and middle branches of the hepatic vein), the left and middle lobes became dark red and swollen hard immediately after the ligation, in contrast with the right lobe which remaind soft and in vivid red color.

Immediately after the operation the portal pressure increased while the systemic blood pressure and caval pressure slightly dropped, but after the closure of the abdominal wall, it was found that both of the systemic blood pressure and the caval pressure somewhat recovered (Fig. 6).

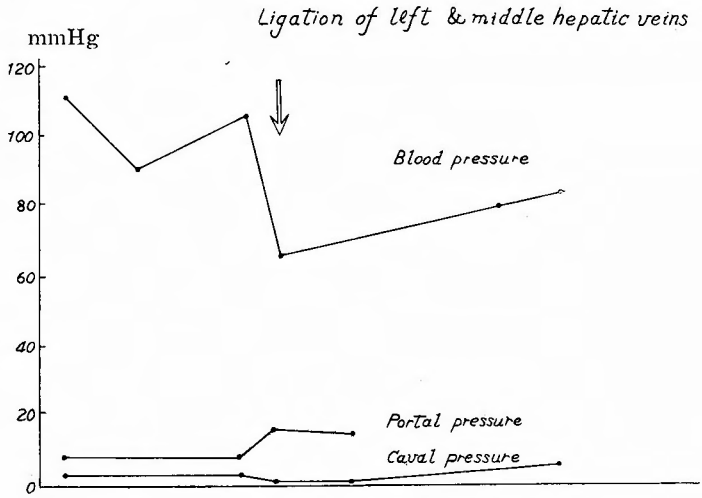

Fig. 6 Changes in the portal, caval and systemic blood pressure after ligation of the left and middle hepatic veins.

In Method B (constriction of the left and middle branches of the hepatic vein), the appearance of the liver lobes was much less remarkable than in Method A and C.

In Method D, due to the fact that the right hepatic vein branches were individually ligated, the right lobe became quite dark and swollen hard whereas the middle and the left lobes were brighter in color because their hepatic vein branches were only constricted.

Generally speaking, whatever the operative procedure, portal pressure increased immediately after the operation. The average increase after applying Method A, was 37.6 mm H_2O, after Method B 65.6 mm H_2O, after Method C 27.4 mm H_2O,

Table 3. Portal Pressure Before and After Constriction of Hepatic Veins. (Average Values in Mm. H_2O)

Operative Method		Before	After	Difference
A	Survived	115.75	166.25	50.5
	Dead	135.45	168.35	32.9
	Total	130.2	167.8	37.6
B	Survived	144.6	199.2	54.6
	Dead	146.85	220.13	73.28
	Total	145.91	211.41	65.5
C	Survived	154.5	186.83	32.33
	Dead	105.5	118.0	12.5
	Total	142.25	169.62	27.37
D	Total	119.5	179.16	59.66

and after Method D 59.6 mm H_2O respectively (Table 3).

(2) Mortality

In the beginning of my study, the overdose of anesthetics and the lack of technical experience necessitated many deaths of experimental dogs after the operation, but later, although an adequate dose of anesthetics was given and operative technique improved, the rate of mortality was yet high.

The death rates of various methods utilized were as followed, namely: 69.2 per cent in Method A, or 9 deaths out of 13 cases; 58.2 per cent in Method B, or 7 deaths out of 12 cases; 30 per cent in Method C, or 3 deaths out of 10 cases, and 67.6 per cent in Method D, or 23 deaths out of 34 cases, respectively (Table 4).

Table 4. Operative Mortality.

Methods	Survivals	Deaths	Mortality
A	4	9	69.2%
B	5	7	58.2
C	7	3	30.0
D	11	23	67.6

The majority of mortalities were mostly due to pooling shock. They died several hours to twenty-four hours after the operation, but some of the experimental dogs died from unknown cause approximately one week after the operation.

(3) Liver Function Tests

In experimental dogs that survived the operation, the liver function tests were performed at various periods.

i- Serum Protein

12 out of 20 cases showed less than 6.0 per cent hypoproteinemia and 2 of the 12 cases showed 4.0 per cent.

ii- B. S. P. Test

Of the 20 minute value, 9 out of 18 cases examined showed over 5 per cent. Of the 45 minute value, 8 out of 19 cases showed over 2.5 per cent.

Of the 20 minute value, there were 5 cases which showed over 7.5 per cent while the maximum showed 25 per cent. Of the 45 minute value, 4 cases showed

over 7.5 per cent and some showed a maximum of 17.5 per cent.

iii- Zinc Sulfate Test

There were 2 out of 16 cases that showed an abnormal value over 7 units.

iv- TAKATA's Reaction and Sublimate Reaction

The abnormal values above (+) were obtained in 5 out of 14 cases examined in the former test, and in 4 out of 17 cases in the latter.

Considering from these data, it could be said that the cases which showed abnormal values in B. S. P. test did not necessarily show the abnormal reaction in the other tests. Even though abnormal values were apparently obtained through the sublimate reaction, the TAKATA's reaction or the zinc sulfate test, a normal value was obtained through the B. S. P. test. From the results of these tests, it was difficult to tell that there was any difference in significance among the various methods of producing similar condition of hepatic cirrhosis. However, almost all of the experimental dogs revealed abnormalities in these results of the liver function tests so that it was considered that they had more or less liver damages (Table 5).

Table 5. Liver Function Tests in Experimental Dogs.

Dog No.	Op. Method	No. of Days after Op.	Serum Protein(%)	B. S. P. 20 Min.	B. S. P. 45 Min.	Z. S. T.	Sublimate R.	TAKATA R.
39	A	8	5.4		10	3		−
58	A	35	6.6	0	0	5	−	+
62	A	37	7.0	10			−	
68	A	83	7.0	7.5	7.5			
41	B	10	4.0		17.5			
85	B	72	8.6	2.5	2.0	16	++	++++
57	C	28	8.6	2.5	2.0	3	−	
64	C	28	5.4	5	5	1	−	−
66	C	36	7.2	5	2.5	7	++	++
67	C	72	5.2	2.5	0	1	−	−
76	C	58	5.8	12.5	7.5	1	−	−
78	C	95	6.4	25		4	−	−
136	D	12	5.0		2.0	4	+	+
135	D	17	4.0	5	2.5	3	−	−
96	D	31	5.0	0	0	5	+	−
149	D	36	5.9	2.5	2.0	4	−	
111	D	38	6.2	5	2.0	5	−	
93	D	41	5.8	0	0	3	−	
113	D	199	5.0	10	2.5		−	−
100	D	66	5.6	2.5	2.0	5	−	+

(4) Finding at Laparotomy

Dogs with distinctly accumulated ascites were found to have the distended abdomen and collaterals on the abdominal wall soon after the operation (Fig. 7) but in all experimental dogs once operated on, a laparotomy was carried out again to examine ascites and to determine the portal vein pressure at various periods

ranging from the first week to 15 months after the operation.

i- Ascites

Ascites was discovered in 12 out of the 24 cases including 6 cases of which the amount of ascites was over ++ (it means the amount was from 500 to 1000 cc). Moreover, in one case, ascites was recognized even 15 months after the operation.

Total protein contained in ascites was 1.5-4.2 per cent with an average of 3.3 per cent.

ii- Portal Pressure

Portal pressure of experimental dogs was determined. Their values ranged from 95 mm H_2O to 360 mm H_2O. The values of 10 out of 24 cases were found

Fig. 7 Photograph of dog No. 96 showing ascites and collaterals in the abdominal wall 16 days after operation (Method D).

Table 6. Portal Pressures and Presence of Ascites in Experimental Dogs

Dog No.	Op. Method	No. of Days after Op.	Ascites	Total Protein in Ascites (Gm. per 100cc.)	Portal Pressure ($Mm.H_2O$)
39	A	23	+	4.0	185
58	A	40	+	3.0	125
62	A	38	−		175
68	A	86	−		105
11	B	54	−		225
41	B	10	++	3.0	195
85	B	72	−		130
57	C	29	+++		250
64	C	29	+		250
66	C	36	−		124
67	C	72	−		205
76	C	58	+		110
78	C	97	−		95
93	D	56	++	3.4	180
96	D	121	++	4.2	250
100	D	70	+	3.4	140
111	D	39	−		150
113	D	204	++		360
113	D	409	++	1.5	226
135	D	41	−		100
136	D	43	−		120
139	D	7	−		125
146	D	29	+	3.9	222
148	D	38	+	3.1	135
149	D	36	−		122

to have over 180 mm H_2O.

Moreover, the dog which survived 15 months after the operation, maintained its portal pressure at 226 mm H_2O (Table 6).

iii- Other Findings during Laparotomy

Generally speaking, fibrinous or fibrous adhesions took place widely around the liver and between the hepatic lobes.

The lobe whose hepatic vein branch had been ligated or constricted, showed a dark red swelling and a round margin and was covered with white fibrous membrane. The lobe whose hepatic vein branch was left intact (the right lobe in Method A) showed a normal appearance without any atrophy or hypertrophy. The remainder of the partially resected liver (the right lobe in Method B) did not show any hypertrophy, while the lobe whose biliary duct had been ligated (the right lobe in Method C) somewhat atrophied and its size, in one case, was reduced to about half of its original size.

In four cases which ascites was found, many small cysts were discovered on the surface of the liver. They had a thin transparent membrane and contained fluid like lymph in it. It could be considered to be hepatic lymph cysts, accumulated under the liver capsule.

iv- Relation between the Operative Procedure and the Ascites and Relation between the Operative Procedure and the Portal Pressure.

Ascites was noticed in 2 out of the 4 cases in Method A, 1 out of the 3 cases in Method B, 3 out of the 6 cases in Method C, and 6 out of the 11 cases in Method D. In regard to the rate of formation of ascites Method D dominated the other methods.

Portal hypertension was obtained in 1 out of 4 cases in Method A, 2 out of 3 cases in Method B, 3 out of 6 cases in Method C, and 4 out of 11 cases in Method D. Concerning the production of portal hypertension, it appeared that Methods B and C were superior to the others.

However, the amount of ascites above (++) was obtained in only one case respectively after Methods B and C, while in 3 cases after Method D: portal hypertension above 200 mm H_2O was acquired in 3 cases respectively after methods C and D, while in one case after Method B and in none after Method A. In other words, it was clear that Methods C and D contributed much to the production of the state similar to the cirrhosis of the liver.

Therefore, I believe that we should pursue the study of Method D due to the fact that experimental conditions produced are simple and clear to interpret, though the operative technique is somewhat complicated and the rate of mortality after the operation is high (Table 7).

(5) Pathohistological Finding of Liver Biopsy Specimen obtained by Laparotomy

At the time of operation, small fragments of the liver were obtained and placed immediately in the 10 per cent solution of formalin. Paraffin sections were made and hematoxylin eosin stain and VAN GIESON's stain were used.

Table 7. Operative Methods and Rate of Formation of Ascites and Portal Hypertension

Operative Method	Total of Dogs	Ascites (+) (No. of Dogs)	Ascites (++) (〃)	Portal Pressure Over 180mm. H_2O	Over 200〃.
A	4	2	0	1	0
B	3	1	1	2	1
C	6	3	1	3	3
D	11	6	3	4	3

In Method A. the specimens were taken from the left lobe. In one case, congestion of the central vein, hemorrhage in the central zone, intraacinous bleeding from the central to the intermediate zone, clear hydropic degeneration in hepatic cells of the central zone and dilation of the portal vein in general were observed. Ascites was found in this case, and as mentioned later, ascites accumulated usually in those cases which demonstrated outstanding histological changes, so that it was believed that there was a parallel relationship between histological changes and ascites.

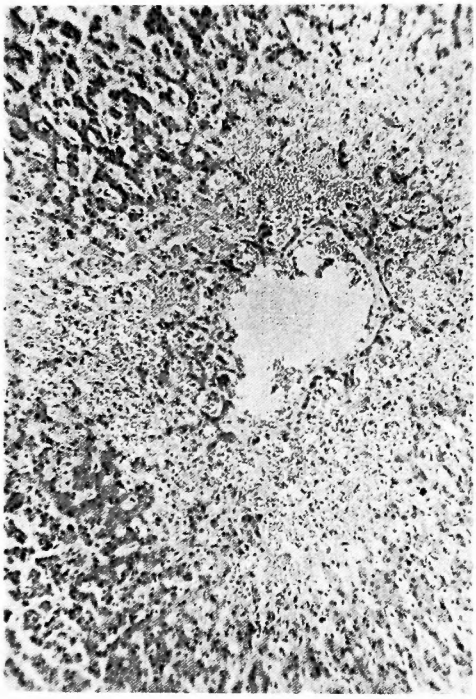

Fig. 8 Photomicrograph showing the dilated central vein, congestion, hemorrhage and hydropic degeneration of the hepatic cells in areas about the central vein. This condition occurred in the left lobe of the liver of dog No. 12, which was operated on by Method B 34 days previously. Hematoxylin and eosin stain: ×200

In Method B, similar to method A the typical changes were dilation of the central vein, congestion and hemorrhage in the central zone, edema of the hepatic cells of the central zone, fibrosis of the central zone. In other words, findings which indicated cyanotic induration were observed (Fig. 8).

In Method C, in which right bile duct had been ligated and divided, specimens were taken from the left and

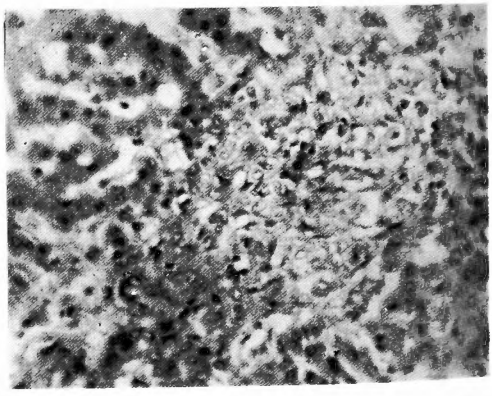

Fig. 9 Photomicrograph of the right lobe of the liver showing fibrosis and proliferation of the cells of the bile ductules in the portion called the ampulla. (Dog No. 67 ten weeks after operation of Method B.) Hematoxylin and eosin stain: ×400

right lobes simultaneously at the time of laparotomy.

The histological findings of the right lobe were noticed to have fibrosis in the GLISSON's capsule and mild proliferation of the cells of the bile ductules in the portion called the ampulla or canal of Hering (Fig. 9).

As for the left lobe, hemorrhage or congestion, edema and fibrosis of the central zone and intra-acinous congestion were noticed to some degree similar to those found in Methods A and B, but in severe cases, marked degeneration and atrophy of the hepatic cells in the central zone were observed. In those cases accompanied by ascites, the histological changes were distinct.

In Method D, the specimens were mainly taken from the left lobe. In severe cases hemorrhagic necrosis or even fatty degeneration of the hepatic cells of the central zone was noticed and dissociation of the hepatic cell cords was generally distinct; congestion of the central zone, fibrosis and hydrops of the hepatic cell of the central zone were also distinct. (Fig. 10). In this group also, the dogs with ascites revealed the most outstanding changes histologically.

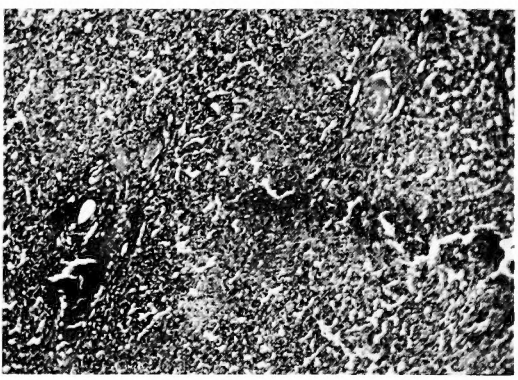

Fig. 10 Photomicrograph of the left lobe of dog No. 93 showing hemorrhagic necrosis and dissociation of the hepatic cell cords. Biopsy was taken 8 weeks after the operation. (Method D). Hematoxylin and eosin stain : ×100.

(6) Relation between the Results of the Liver Function Tests and the Existence of Ascites.

In the ascitic group, 8 out of 11 cases were found to have total serum protein below 6 per cent, while in the non-ascitic group only 4 out of 9 cases were below 6 per cent. The average value of the total serum protein was 5.76 per cent in the former and 6.15 per cent in the latter. Therefore, hypoproteinemia was apt to develop in the dogs with ascites. However, as regards the other results of the liver function tests, no differences could be found significantly between the ascitic and non-ascitic groups. Except for the finding of total serum protein, it could not be asserted that the function of the liver of the ascitic group was lower than that of the non-ascitic group (Table 8).

(7) The Presence of Ascites and Portal Hypertension.

The average portal pressure of the ascitic group was 300.2 mm H_2O while that of the non-ascitic group was 139.7 mm H_2O. This indicated that the accumulation of ascites was accompanied by portal hypertension. In the cases of portal hypertension exceeding 180 mm H_2O, 8 out of 10 cases were found to have ascites while only 2 out of 12 cases in the non-ascitic group had high portal pressure exceeding 180 mm H_2O. There was significant correlation between the accumulation of ascites and the portal hypertension at 2 per cent significant level according to FISHER's exact probability test (Table 9).

Table 8. Relation between the Presence of Ascites and the Results of Liver Function Tests

		Ascites (+)	Ascites (−)
Serum Protein	No. of Dogs		
	Below 6%	8	4
	Over 6%	3	5
	Average (%)	5.76	6.15
B. S. P. Retention	No. of Dogs		
in 20 Min.	Over 5%	4	5
	Below 5%	6	3
	Average (%)	4.0	8.1
in 45 Min.	Over 2.5%	5	3
	Below 2.5%	6	5
	Average (%)	4.4	3.3
Z. S. T.	No. of Dogs		
	Over 7 Units	1	2
	Below 7 Units	9	6
	Average (Units)	4.1	6.1
Sublimate Reaction	No. of Dogs		
	Over (+)	2	3
	(−)	7	5
TAKATA's Reaction	No. of Dogs		
	Over (+)	3	3
	(−)	5	3

Table 9. Relation between Ascites and Portal Pressure

	Ascites (+)	Ascites (−)
Average of Portal Pressure (Mm. H_2O)	200.2	139.7
No. of Dogs Over 180 mm. H_2O	8	2
No. of Dogs Below 180mm. H_2O	1	10

(8) The Presence of Ascites and the Histopathological Findings of Biopsy Specimen of the Liver.

The histopathological findings of biopsy specimens were more remarkable in the ascitic group than in the non-ascitic group. There was invariably definite congestion of the liver. It may be asserted that hepatic congestion—ascites—portal hypertension can be created monistically by the constriction of the hepatic vein (Tables 10 and 11).

IV SUMMARY

In my study, I have mainly constricted the hepatic vein by laparotomy to produce the congestion of the liver, and succeeded in creating portal hypertension and ascites in dogs.

Table 10. Microscopic Examination of the Liver (Group with Ascites)

Dog No.		39	58	41	57	64	76	93	96	100	113	146	148
Central Vein	Dilatation			+				+	++	+	+++	+	+
	Congestion	+	+		+++	++	++	+++	+	±	+++	++	±
	Hemorrhage	+	+	+	+++	+	++	+++	+	−	++	++	
	Thickning of the Wall	+	+				+	++	++		+++	++	+
Degeneration of Hepatic Cells	Central	++	++	+	+++	+	+	++	++	++	++	+	++
	Periphery				+++				++			−	+
Dissociation of Cell Cord		+			+++			+++		+	+++	±	
Central Fibrosis		++	+	−	++	+	+	++	+++	+	+++	+	++
Intraacinous Bleeding		++	+	++	++	+++	++	+++	++	+	++	+	−
Dilatation of Sinusoid			+	++		+	+	+	+	++	++	+	±
Dilatation of Portal Vein		+	+	+	+	+		+	+	+	++	+	+

Table 11. Microscopic Examination of the Liver (Group without Ascites)

Dog No.		62	68	85	66	78	111	135	136	149
Central Vein	Dilatation						+	−	+	+
	Congestion	+	−	+	−	−	±	−	−	±
	Hemorrhage	−	−	−	−	−	−	−	−	−
	Thickning of the Wall	−	−	−	+	−	−	−	−	−
Degeneration of Hepatic Cells	Central	+	−	+	−	−	±	−	+	±
	Periphery	−	−	−	−	−	−	−	−	−
Dissociation of Cell Cord				+			+		+	+
Central Fibrosis		+	−	+	−	+	−	±	−	−
Intraacinous Bleeding		−	−	−	−	−	±	−	+	−
Dilatation of Sinusoid			−	−	+	−	±	+	+	±
Dilatation of Portal Vein		+	+	−	+	−	−	+	+	−

After the constriction of the hepatic vein, portal vein pressure increased immediately and, moreover, in some cases portal pressure maintained its high values for a considerable length of time. In these cases, accumulation of ascites was frequently found, so it appeared that there was close relationship between ascites and portal hypertension. Moreover, in almost all cases in which ascites and portal hypertension existed, histological findings of those livers were remarkable, whereas when ascites and portal hypertension did not exist, the histological changes were only slight. In liver function tests, both diminution of total serum protein and decrease of excretion of bromsulfalein were noticed in approximately half of the cases, and sometimes the other tests (zinc sulfate test, sublimate reaction and TAKATA's reaction) showed abnormal results indicating the liver insufficiency in some degree. ARMSTRONG[7] in 1947, KERSHNER et al.[9] in 1946, and WILES et al.[10] in 1952 attempted to produce hepatic congestion without the associated congestion of the systemic blood system but without results to be noted; again WANGENSTEEN et al.[11] in 1953 succeeded in ligating individually the hepatic vein branches but they were urged to make a portacaval shunt (side to side anastomosis).

J. L. MADDEN et al.[15] in 1954 injected varied colored solutions of neoprene latex Type 571 into fresh cadavers with normal livers, and also to the ones having diseased livers with or without ascites, and then he corroded the liver parenchym to study its vascular bed. In cirrhosis of the liver without ascites, there was a symmetrical deficit in all of the vascular systems but most pronounced in the hepatic venous and portal venous beds. The same thing was also true for cirrhosis of the liver with ascites whose appearance was acute and reversible. Contrary to these cases, in cirrhosis of the liver with irreversible ascites, there was a reciprocal pattern of the circulation within the liver characterized by an absolute and compensatory increase in both the portal venous and the hepatic arterial beds, and a concomitant and absolute decrease in the hepatic venous bed. In the last cases, the majority of both the central and hepatic veins were found to be occluded by obliterative fibrosis.

Thus, irreversible ascites developed in cirrhosis of the liver of humans was believed to be due to the occlusion of the hepatic venous vascular beds. The pathological states of the experimental dogs in my study, therefore, cannot be said to be altogether unrelated to those of cirrhosis of the liver in humans.

BARET et al.[2] in 1955 utilized carbon tetrachloride and experimentally brought about cirrhosis of the liver in dogs. They obtained the microscopical findings which greatly coincided with cirrhosis of the liver and also obtained portal hypertension but no ascites.

In my study, Method D (ligation and constriction of the individual branches of the hepatic vein) brought about the pathological conditions which were simple and clear to interpret, and produced both ascites and portal hypertension in high percentage only with a disadvantage of high operative mortality.

In 1950, BOLLMAN and others[6] demonstrated that ascites, which was experimentally produced by the constriction of the thoracic inferior vena cava, originated from the hepatic lymph, and that ascites and portal hypertension were not parallel. I believe that ascites which occur in hepatic vein constricted dogs, originate also from the hepatic lymph. Moreover, in the hepatic vein constricted dogs, correlation between ascites and portal hypertension was recognized, because ascites and portal hypertension were always accompanied with each other in those dogs.

V CONCLUSION

With the object of studying the patho-physiology of the circulation of cirrhotic liver and of finding theoretical principles in regard to the hepatic artery ligation, I endeavored to produce experimental conditions in dogs analogous to cirrhosis of the liver, and have established the following methods.

Method A :- Complete ligation of the middle and left hepatic vein branches.

Method B :- Constriction of the middle and left hepatic vein branches and partial resection of the right lobe.

Method C :- Complete ligation of the middle and left hepatic vein branches, and ligation and division of the right bile duct.

Method D:- Individual ligation of the right hepatic vein branches and constriction of the middle and left hepatic vein branches.

In these procedures, manipulation of the hepatic veins was performed under the diaphragm, not opening the thoracic cavity.

Immediately after constriction of the hepatic veins, there was an increase in portal pressure, and in many of those cases, thus produced portal hypertension kept its high values for a considerable period. In general, liver insufficiency was noticed to a certain degree. It is presumed that a certain degree of interrelationship existed between ascites and portal hypertension. There is a marked distinction in histopathological findings between dogs with ascites and portal hypertension and those without them.

Of the four methods stated above, Method D is deemed to be the most appropriate despite its difficulty in technique and high operative mortality.

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PART II
EVALUATION OF HEPATIC ARTERY LIGATION

CONTENT PART II

I INTRODUCTION

In 1947, W F. RIENHOFF,[1] for the first time, performed hepatic artery ligation for cirrhosis of the liver and then, in 1952,[2] he made a report of all the cases he had experienced thereafter. J. K. BERMAN, in 1950,[3] also performed hepatic artery ligation for cirrhosis of the liver. Both of them obtained brilliant results bringing about an improvement not only in the disappearance of ascites but also in the other clinical findings.

On the other hand, ROSENEAUM et al. in 1954[4] ligated a hepatic artery for a typical case of portal cirrhosis of the liver with operative death from massive necrosis and anemic infarct of the liver. Moreover, TAYLOR and ROSENEAUM[5] from perfusion studies claimed that the total blood flow through the cirrhotic liver was proportional to the hepatic arterial flow, and any procedure which decreased this flow would be very detrimental.

DESFORGES et al.[6] performed hepatic artery ligation on four patients with cirrhotic liver accompanied by esophageal varices. All of them tolerated this operation, but showed no distinct improvement in histopathological findings or in clinical data, although only a slight degree of improvement was observed in patients with ascites. Thus, despite the good results reported by RIENHOFF and BERMAN this problem about ligation of the hepatic artery has not yet been settled.

RIENHOFF,[2] in 1952, from his results in hepatic artery ligation in cases of cirrhosis of the liver, stated that this method was effective in cases where ascites was the chief complaint but in cases where hematemesis from esophageal varices was predominant, the results was unfavourable. Therefore, we cannot deny the fact that there is a possibility of causing danger to the patient's life by performing ligation of hepatic artery even in cirrhosis of the liver. On the other hand, it has been proven that there must have been some cases to which this procedure would have been indicated. RIENHOFF's report is believed to suggest this indication and the object of my experiment is nothing other than to determine it.

II EXPERIMENTAL METHODS

(1) Materials and Examinations

i- Materials

I performed the following experiments on the dogs whose hepatic veins had been constricted in the previous operation (Part I).

ii- Examinations

I made liver function tests, measurements of portal pressure and histopathological studies at various periods before and after ligation of the hepatic artery.

(2) Method of Hepatic Artery Ligation

The anatomy of the extrahepatic arterial system of normal dogs was examined by HIDEHIKO URABE[7] of our laboratory. Results revealed that the patterns of the principal arterial blood supply to the liver had very little variation and that the coincident ligation of A. hepatica communis, A. gastroduodenalis and A. gastrica dextra, usually interrupt the hepatic arterial blood flow almost completely.

At the outset of my experiment I had ligated and divided only the A. hepatica communis in 3 cases but later, I ligated and divided the three arteries as mentioned above (Fig. 1).

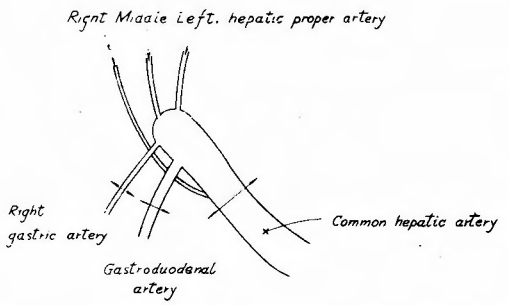

Fig. 1 Anatomy of the hepatic artery and its branches. ←——→: showing the sites of ligation.

For A. hepatica communis, after opening the lesser omentum, it was exposed at a point before entering the duodeno-hepatic ligament after its ramification from the coeliac axis, and divided between two ligatures. At times, by pressing aside the stomach and the duodenum to the left, the A. hepatica communis could be obtained through the WINSLOW's foramen without any necessity of opening the lesser omentum. A. gastroduodenalis and A. gastrica dextra were loosened with great care at hepatoduodenal ligament and then ligated and divided. As anticipated easily, findings by laparotomy differed from that of normal dogs and there were such extensive adhesions around the liver that I met with much difficulty in trying to expose the hepatic artery during the operation.

III RESULTS

(1) Mortality

It was already known that ligation and division of the A. hepatica communis, causing incomplete occlusion of the hepatic blood flow, did not bring about any liver damage. It was also proven by the experiments of BERMAN,[13] LAUFMAN[8] et al. that occlusion of the common hepatic artery cannot become the cause of death.

At first, in my study, I ligated and divided the common hepatic artery on one dog whose hepatic vein had been occluded after Method A and on 2 dogs after

Method C, and found that only one dog of Method A died on the second postoperative day. Yet, this death was due to the existence of a piece of infected gauze which was left in the peritoneal cavity during the operation. The other 2 cases survived for a long period.

On 16 dogs which had originally been prepared for experiment by Methods A, B and D, ligation and division of the three arteries (A. hepatica communis, A. gastroduodenalis and A. gastrica dext.) were performed under the administration of penicillin. Of these dogs only one survived for a long period, and all of the rest died shortly after the operation.

I designated those dogs that had died within the first eight days after the operation as the early stage death group. 9 cases belonged to this group; 6 cases that had died 2 days after the operation, one case each that had died 3 days, 6 days, and 8 days respectively after the operation. As regards the causes of these deaths, 2 cases died from unknown cause, one case from intraperitoneal hemorrhage, and 2 cases from bile peritonitis originated in necrosis of the gallbladder.

In reference to liver necrosis, this took place in 5 cases more or less. In 2 of these cases, liver necrosis was the sole finding at autopsy. Marked changes were observed in one case and slight ones in the others. In the 3 other cases, liver necrosis was found to be combined by intraperitoneal hemorrhage, peritonitis or abscess (Table 1).

Table 1. Causes of Deaths in Early Stage After Ligation of Hepatic Artery

Dog No.	No. of Days After Ligation	Cause of Death
139	2	Liver necrosis (markedly)
62	2	Liver necrosis + Intraperitoneal hemorrhage
135	2	Liver necrosis + Subphrenic abscess
66	2	Intraperitoneal hemorrhage
39	2	Unknown
146	2	Unknown
78	3	Liver necrosis (slightly)
148	6	Necrosis of gallbladder (Bile peritonitis)
68	8	Liver necrosis + Necrosis of gallbladder (Bile peritonitis)

Those that had died 11 days or more after the operation, I designated as the late stage death group to which 6 cases belonged; 3 cases which had died on the 11th day, one case each that had died on the 13th, the 25th and on the 28th postoperative day respectively. Of these none was acknowledged to have liver necrosis. The causes of deaths were peritonitis due to a foreign body left in the peritoneal cavity during the operation, or bile peritonitis caused by necrosis of the gallbladder, or simple diffuse infected peritonitis (Table 2).

Without considering the degree of liver necrosis in the dogs whose hepatic veins had been constricted, the rate of death which was caused by liver necrosis was 31 per cent or, in other words, 5 cases out of 16, almost equally corresponding to that in normal dogs.

Table 2. Causes of Deaths in Late Stage After Ligation of Hepatic Artery.

Dog No.	No. of Days After Ligation	Cause of Death
85	11	Peritonitis on account of gauze left in the peritoneal cavity
113	11	Necrosis of gallbladder + Subphrenic abscess
136	11	Necrosis of gallbladder + Bile fistula
111	13	Necrosis of gallbladder + Abscess
100	25	Necrosis of gallbladder + Abscess
149	28	Panperitonitis acuta

There were 6 cases in which deaths were caused by gallbladder necrosis which occurred 6 days or more after the operation. I believe that long survival could have been attained if, like LAUFMAN et al., the gallbladder had been extirpated in advance. URABE[7] claimed that in normal dogs, gallbladder necrosis occurred, despite penicillin administration, at the rate of 50 per cent of dogs surviving over 7 days after ligation of the hepatic artery.

(2) Changes in Portal Pressure immediately after Hepatic Artery Ligation

In normal dogs, the immediate fall in portal pressure was generally slight. On 11 normal dogs, a slight increase of portal pressure was observed in 3 cases, no change in 1, and a decrease, though slight, in all of the rest. The average value of variation was −4.1 mm H_2O (Table 3). On the other hand, in the hepatic

Table 3. Changes of Portal Pressure immediately after Ligation of the Hepatic Artery in Normal Dogs

Dog No.	Change of Portal Pressure		Difference
	Before	After	
75	95	103	+ 8
77	92	87	− 5
80	180	133	−47
120	85	85	0
129	100	97	− 3
155	101	95	− 6
156	81	96	+15
157	150	140	−10
158	90	113	+23
159	92	90	− 2
161	115	97	−18
	Average		−4.1 mm. H_2O

vein constricted group, a slight portal pressure elevation was seen only in 1 case out of 18, and no change in 3 while all of the remaining 14 cases showed a precipitous fall in portal pressure. The average value was −33.9 mm H_2O (Table 4).

(3) Liver Function Test

The result of the liver function test performed on 3 cases of long survivals, namely 2 cases of survivals (whose common hepatic artery was ligated and divided)

Table 4. Changes of Portal Pressure immediately after Ligation of the Hepatic Artery in Experimental Dogs with Constricted Hepatic Veins.

Dog No.	Change of Portal Pressure		Difference
	Before	After	
39	185	135	−50
62	175	145	−30
68	115	120	+50
85	130	130	0
57	250	150	−100
64	250	206	−44
66	124	111	−13
78	95	70	−25
93	180	83	−97
100	140	135	− 5
111	150	94	−56
113	226	158	−68
135	100	97	− 3
136	120	105	−15
139	125	95	−30
146	222	142	−80
148	135	135	0
149	122	122	0
	Average		−33.9 mm. H_2O

Table 5. Liver Function Studies in the Long Term Survivals of the Experimental Dogs after Ligation of the Hepatic Artery.

Dog No.	57		64		93	
	Before Ligation	After	Before	After	Before	After
No. of Days after Op.		116		150		36
Serum Protein	8.6	6.7	5.4	6.6	5.8	6.2
B. S. P. (45 Min.)	25	0	5	0	5	0
Z. S. T.		3	1	3	3	5
Sublimate	−	−	−	−	−	−

and 1 case of survival (whose three main arteries—common hepatic, gastroduodenal, and right gastric artery—were ligated and divided) suggested improvements of the hepatic function, that is, increase of serum protein, decreased retention of Bromsulphalein, etc. (Table 5). Contrary to these findings, the results in cases of short survivals indicated, only too naturally, a gradual change for the worse (Table 6).

(4) Findings at Relaparotomy

Regarding the cases of 3 long survivals whose hepatic arterial flow had been occluded two to five months before experimental relaparotomy, all of these dogs

Table 6. Liver Function Studies in the Short Term Deaths of the Experimental Dogs after Ligation of the Hepatic Artery.

Dog No.	100		111		113		149	
	Before Op.	After	Before	After	Before	After	Before	After
No. of Days after Op.		6		7		3		24
Serum Protein	5.6	4.6	6.2	6.4	5.9	5.8	5.9	5.7
B. S. P. (45 Min.)	2.5	2.5	2.5	0	0	12 5	2.5	2.5
Z. S. T.	5	7	5	3	10	15	4	17
Sublimate Reaction	−	±	−	+	+	±	−	±
TAKATA'S Reaction	+	±			±	±		±

possessed portal hypertension and ascites before the occlusion of the hepatic artery, but after that the portal pressure returned to its normal value and ascites entirely disappeared.

On the other hand, 2 cases were put under a long period of observation after constriction of the hepatic veins without undergoing the occlusion of their hepatic artery and then experimental laparotomy was performed after 4 months and 7 months respectively. In either case portal hypertension had been maintained and ascites was found. One of them was again laparotomized after 1 year and 3 months and found to have portal hypertension and ascites. In contrast with the facts of a decrease in portal pressure and disappearance of ascites in the hepatic artery occluded group, this finding is quite interesting (Table 7).

Table 7. Long Term Survivals After Ligation of Hepatic Artery

Dog No.	57	64	93	96	Contrast 113	Contrast 113
CONSTRICTION OF HEPATIC VEINS						
No. of Days after Constriction	29	29	56			
Portal Pressure before Ligation	250	250	180			
Ascites before Ligation	±	+	±			
LIGATION OF HEPATIC ARTERY						
Portal Pressure after Ligation	150	206	83			
No. of Days after Ligation	168	150	63			
No. of Days after Constriction	196	178	118	121	204	409
LAPAROTOMY						
Portal Pressure at Laparotomy	153	130	120	250	360	226
Ascites	−	−	−	±	±	±

(5) Histological Examination of the Liver

Histological examination of the liver revealed that slight indications of recovery was observed in the 3 long survivals after the occlusion of the hepatic artery, that is, though congestion and hemorrhage of the central region of the lobule, and moreover, dissociation of the hepatic cell cords remained, yet these changes were reduced in degree, and appearance of the hepatic cell itself was favorable. Pseudo-bile-ducts were found in the hemorrhagic area and in the connective tissues.

On the other hand, of the cases placed under a long period of observation without undergoing the ligation of the hepatic artery, the histological findings of the livers showed the remnants of extensively high grade of changes even 7 months after the constriction of the hepatic veins, but indicated some improvements 1 year and 3 months after,—the liver cells in the peripheral region of the lobule were hypertrophied, the hepatic cell cords were arranged in order, and at the same time

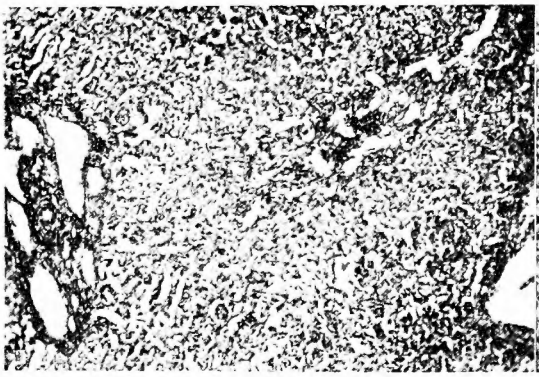

Fig. 2 Photomicrograph of biopsy specimen of liver of dog No. 57 taken at the time of hepatic artery ligation. VAN GIESON stain ×100.

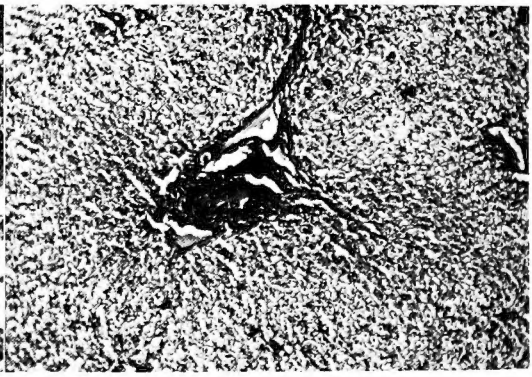

Fig. 3 Photomicrograph of biopsy specimen of liver of dog No. 57 taken 168 days after hepatic artery ligation. VAN GIESON stain ×100.

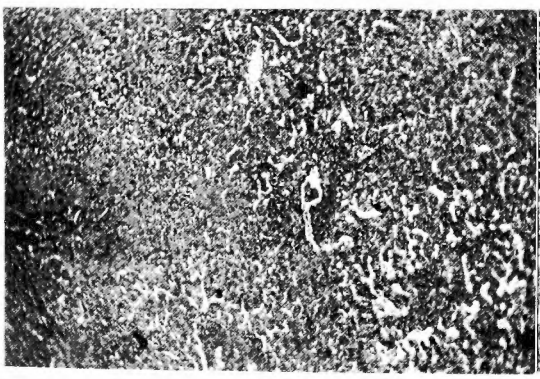

Fig. 4 Photomicrograph ×100 (VAN GIESON stain) of dog No. 93. Biopsy specimen of liver taken at the time of hepatic artery ligation.

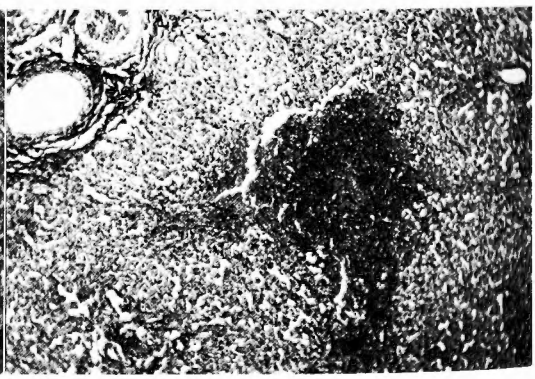

Fig. 5 Photomicrograph ×100 (VAN GIESON stain) of dog No. 93. Biopsy specimen of liver taken 63 days after hepatic artery ligation.

pseudo-bile-ducts were noticed in the central region.

From the histological point of view, it may be said that after constriction of the hepatic veins, a comparatively long period (over 1 year) is necessary to obtain a sign of recovery whereas by applying the hepatic artery ligation only a short period (2 months) is sufficient (Figs. 2, 3, 4, and 5).

(6) Relation of Liver Necrosis with the Existence of Ascites

MARKOWITZ, in 1946,[9] reported that when hepatic artery ligation was performed on dogs, mortality due to liver necrosis was 100 per cent but that by the administration of penicillin it decreased to 30 per cent. Pertaining to this fact, URABE[7] of our laboratory attained similar results. It is understood that there were still approximately 30 per cent of mortality due to liver necrosis. Therefore, even after the administration of antibiotics, we hesitate to apply hepatic artery ligation to cirrhosis of the liver as a method of treatment.

In my experiments, in 9 cases in which ascites had been formed after the constriction of the hepatic veins including 3 cases of long survival, no liver necrosis was found even in the cases of short survival after the ligation of hepatic artery. In those that developed liver necrosis, none had been found to have ascites. (Fig. 6)

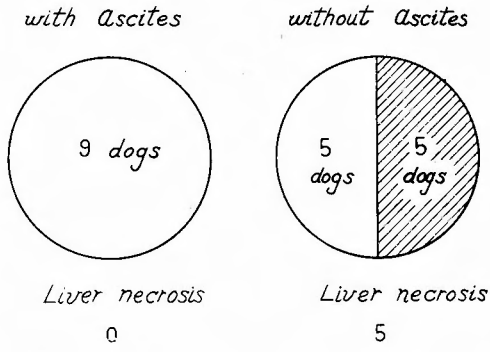

Fig. 6 Relation between Ascites and Liver Necrosis.

The findings here reported coincide with RIENHOFF's report[1,2] in which he stated that in the cirrhosis of the liver in human beings, hepatic artery ligation was effective in cases where ascites was found but that this was not so in cases where the main complaint of the patient was hematemesis, not ascites. Therefore, in cases where ascites is found, ligation of the hepatic artery is not only dangerous to the liver but is effective to cause the disappearance of ascites, to bring about good influence on the liver function, and to decrease portal hypertension.

IV SUMMARY

I undertook to scrutinize experimentally the effect of hepatic artery ligation in the cirrhosis of the liver and had, as stated in Part I in this report, by constricting the hepatic veins of experimental dogs, brought about the congestion of the liver and thus had succeeded in producing a condition similar to cirrhosis of the liver accompanied by portal hypertension and ascites. Then I performed hepatic artery ligation on these dogs thus originally prepared, administering antibiotics.

In making a comparison with normal dogs, hepatic vein constricted dogs showed a definite decrease in portal pressure immediately after ligation of the hepatic artery. Most of them died within a short period after ligation, and only 3 survived a long period. In all of these long survivals, ascites disappeared, several liver functions

were improved, a decrease was seen in portal pressure, and favorable findings were noticed histologically.

Of the 16 cases that had died within a short period, 5 cases or 31 per cent had liver necrosis. This mortality was nearly the same as that of normal dogs. Therefore, generally speaking, alike in normal dogs, we must anticipate the development of liver necrosis in hepatic vein constricted dogs after ligation of the hepatic artery.

However, in all cases where liver necrosis was found, none had ascites. Cases where ascites had been found, no liver necrosis was noticed so that it can be said that in cases where ascites exists, this condition accommodates a resistance against liver necrosis after ligation of the hepatic artery. Moreover, after hepatic artery ligation, ascites disappeared and liver function improved and a decrease in portal pressure was seen so that it is believed that hepatic artery ligation is indicated to the ascitic dogs as a method of treatment.

H. LAUFMAN et al.,[8] in 1953, reported that in ascitic dogs which were produced by constriction of the thoracic inferior vena cava, the more extreme the arterial deprivation of the liver, the better the protection against the formation or re-formation of ascites. On the other hand, the greater the arterial deprivation of the liver, the higher the mortality. However, when a minute examination was made on the causes of these deaths, none was found to have died from liver failure or liver necrosis but that all cases had died from jaundice, infection, or duodenal ulcer which might well be named accidental. Therefore, even in complete arterial deprivation of the liver, it is believed that ascitic dogs have resistance against liver necrosis.

A. C. BARET et al.[10] reported that in experimental cirrhosis of the liver of dogs produced with carbon tetrachloride, out of 7 dogs 2 died of liver necrosis but in his experiment none of them was ascitic dogs.

Again, pertaining to cirrhosis of the liver of human beings, RIENHOFF[2] concluded in his report that in cases where the main complaint was hematemesis from esophageal varices, the results of hepatic artery ligation were not favorable but in cirrhosis of the liver where ascites happened to be its main complaint, hepatic artery ligation showed excellent results, and moreover, the operative mortality was low so that for cirrhosis of the liver with ascites, this procedure was a valuable method. In considering BERMAN's private cases[3] or of CHENOWETH's[11] experiments and again of the clinical results of SMITH[14] et al., they reported favourable results in the cases of ascites.

In 1952, ROSENBAUM et al.[4] had ligated the hepatic artery in one case of typical portal cirrhosis of the liver and had brought about massive necrosis and anemic infarct on the liver but no ascites was noticed whatsoever in this case.

Moreover, in futile examples by JAHNKE,[12] DESFORGES[6] or BERMAN,[15] ascites was not found to be the main symptoms but it is interesting to note that they were cases in which the main complaint was hematemesis caused by esophageal varices.

As mentioned above, in the reports both from clinical and laboratory data, not only did this procedure cause any harm to the ascitic cases but it also brought about the disappearance of ascites, decreased portal pressure and, moreover, it was believed

to have favorable effects on liver function. In other words, cirrhosis of the liver that possessed ascites offered an appropriate "field" for hepatic artery ligation to come into play effectively.

V CONCLUSION

I performed hepatic artery ligation—ligation and division of A. hepatica communis, A. gastroduodenalis and A. gastrica dextra—on dogs prepared originally by the method as have been stated in Part I in this report, namely by constricting the hepatic veins. The results are as follows:-

(1) Rate of deaths due to liver necrosis in hepatic vein constricted dogs after ligation of the hepatic artery was the same as that in normal dogs. Alike normal dogs, it was believed that danger of death due to liver necrosis also prevailed in experimental dogs.

(2) In making a comparison with normal dogs, the hepatic vein constricted dogs did show a marked decrease of portal pressure after ligation of the hepatic artery.

(3) Liver function tests on 3 cases that survived for a long period after hepatic artery ligation indicated favorable results and when these dogs were relaparotomized, it was found that ascites had disappeared entirely and portal pressure had returned to its normal level.

On the other hand, dogs put under a long period of observation without undergoing hepatic artery ligation retained ascites and maintained portal hypertension over 1 year and 3 months after constriction of the hepatic veins.

(4) Histological findings of the liver of long survivals after hepatic artery ligation showed, in comparison with that of dogs put under observation without hepatic artery ligation, that they had already recovered after a short period.

(5) Of the cases that died during a short period after hepatic artery ligation, none of them which had ascites showed liver necrosis. In all cases that developed liver necrosis after hepatic artery ligation, no ascites was previously found.

In completing my report, I would like to express my deepest gratitude to Professor Dr. Chisato Araki for his kind encouragement and supervision and to Assistant Prof. Dr. Ichio Honjo for his zestful encouragement and supervision throughout my work. Also regarding histological pathological findings I am greatly indebted to Instructor Dr. Fumihiro Ichida of the First Division of Internal Medicine of Kyoto University Medical School.

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肝硬変症の実験的研究

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第1篇 実験的肝硬変症の作製について

肝硬変症に於ける肝血流の病態生理を研究し，肝動脈結紮術或はその他の術式の理論的根拠を探索する目的で，先づ，犬に腹水，門脈圧亢進を伴う肝硬変症類似の状態を作製せんとし，次の作製方法を考案した．即ち

A法，肝静脈中枝・左枝結紮

B法，肝静脈中枝・左枝狭窄，右葉部分切除

C法，肝静脈中枝・左枝結紮，右胆管枝結紮

D法，肝静脈右枝各箇結紮，中枝・左枝狭窄

で，主として，肝静脈を横隔膜下で操作したものである．

肝静脈狭窄直後より門脈圧は上昇し，術後時日を経過しても門脈圧上昇を維持している場合多く，実験犬の半数に腹水を認め得た．肝機能は一般に多少とも障害をうけており，腹水保有群では，血清蛋白濃度の低下を認めた．腹水と門脈圧亢進とは或程度の相関性を以て発生すると考えられる．腹水及び門脈圧亢進を有するものは，然らざるものに比べ，開腹時採取せる肝生検の組織学的変化が著明であつた．

以上の如く，主として肝静脈を狭窄することにより肝硬変症類似犬を作製することに成功したが，上述の4つの方法の中，D法が，実験条件が簡明であり，腹水，門脈圧亢進を高率に発生する点で，最も適当と思われる．然し手術死亡率が高いのが欠点である．

第2篇 肝動脈結紮術の検討

第1篇で述べた如き方法で作製された実験犬に対して，肝動脈結紮術を施行した．即ち，A. hepatica communis, A. gastroduodenalis, A. gastrica dextra の結紮切断を行つたのである．その結果，

① 正常犬に肝動脈結紮術を施行した場合と同率に肝壊死を来した．正常犬と同様，肝壊死を来す危険性が，此等実験犬にも存在するものと考えられる．

② 肝動脈結紮直後の門脈圧の変化は，正常犬に比

べ，門脈圧低下の度合が著明であつた．

③ 肝動脈結紮後長期生存せる実験犬の肝機能検査の2,3の成績は好転を示し，之等長期生存例を再開腹するに，何れの実験犬も腹水は消失しており，門脈圧は正常値に復帰していた．

一方，肝動脈結紮せず，長期観察した犬では，最長1年3ヵ月後に至るも尚腹水を保有し，門脈圧亢進を維持していた．

④ 肝動脈結紮長期生存例の肝組織学的所見は，肝動脈結紮せず長期観察した実験犬の肝組織学的所見に比べて，短期間に既に恢復の徴を認めた．

⑤ 肝動脈結紮後短期間に死亡した例の中，腹水を保有していた実験犬には，1例も肝壊死を来さなかつた．肝壊死を来した例は何れも腹水を保有しない例であつた．

そこで，腹水を保有する場合は，肝動脈結紮後最も憂慮せられるべき肝壊死の発生に対して，抵抗を有するものであり，更に肝動脈結紮術が，腹水消失，肝機能好転，更に門脈圧低下の方向に働き，有効に作用するので，肝動脈結紮術の適応症は，腹水を有する肝硬変症にあるのではないかと予想するものである．