REEVALUATION OF THE REGENERATIVE ACTIVITY OF THE LIVER IN DOGS WITH ECK FISTULA AFTER PARTIAL HEPATECTOMY*

YASUHISA YOKOYAMA

Second Department of Surgery, Nagoya University School of Medicine
(Director: Prof. Hajime Imanaga)

INTRODUCTION

Since Whipple,[1] and Blakemore and Lord[2] initiated the modern area in the treatment of portal hypertension by establishing venous shunts between the portal and caval veins, as a definitive operative measure to control the bleeding of esophageal varices, a rather large number of reports have been published to support the view that these shunts are effective in saving patients from the life-threatening, recurrent hematomesis.[3~16] In 1960, based on the types of intrahepatic disturbances in hepatic circulation, Imanaga proposed a new classification of portal hypertension, and showed a rationale of performing the end-to-side portacaval anastomosis on patients with intrahepatic circulatory obstruction.[17][18] He stated that, after this operation, the patients with presinusoidal obstruction of the liver showed favorable postoperative courses; whereas, the patients with postsinusoidal obstruction of the liver temporarily recovered after the same operation, but gradually their postoperative condition deteriorated.

In the past few years, long-term follow-up studies of a large, carefully controlled series, covering approximately ten years, have been accumulated by several leading investigators in this branch of surgery, in order to evaluate whether or not it is possible to prolong the lives of the patients by performing these shunt operations.[19~26] Those investigators unanimously agreed that portacaval shunting after variceal bleeding favorably affected the patients' postoperative condition and increased their survival rate, even though the selection of the type of portacaval shunting still remains open to controversy.

However, some skepticism still remains in the minds of some workers, as to whether or not this shunt operation can be justified as the treatment of choice to give patients having esophageal varices caused by portal hypertension.[27~36] Among these workers, some suggest that an exact clinical evaluation of this operation should be made, on the more rigid basis of a large number of cases and a properly selected control group. Further they suggest that since some esophageal varices regress spontaneously, and do not always

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bleed, enthusiastic sentiments for surgery, especially a prophylactic one, should be avoided. Another segment of surgeons[13)29)33)] tends to believe that the later postoperative complications after shunting, namely hepatic impairment and neuropsychologic disturbances, might in some patients outweigh the benefits obtained by protecting them from further variceal bleeding.

This latter critical view is supported strongly by the many experimental results which have been reported mostly by workers researching in fundamental medicine, the first of these reports having been made by Mann and Magath[37)] as early as 1922 during their research on "Production of chronic liver insufficiency". They stated, "If an Eck fistula has been made sometime before the removal of liver tissue, regeneration does not occur, or not to the extent which it occurs with the portal circulation intact". Subsequent to their initial observations, Mann and associates,[38~41)] and other workers[42~45)] ascertained this phenomenon through various experimental approaches, and, in addition, numerous reports were published to describe the impaired hepatic function in dogs having an Eck fistula with regard to bile pigment metabolism,[46)] glucose metabolism,[47)] plasma protein synthesis,[48)] hemoglobin production,[49)] dye clearance,[50~52)] hepatic blood flow[53~55)] and ammonia tolerance.[56~59)]

On the other hand, however, it should be remembered that an equally large number of workers reported, "Eck fistula animals can live and appear to be essentially normal for more than several years, although slight abnormalities are found in their livers".[48)49)60~67)] This fact was already known in 1917 by G. H. Whipple and associate,[46)] who described, "Dogs with Eck fistula on a suitable diet may live a normal life for two years or longer, and the wonder is that the liver stands operation as well as it does without showing more atrophy and degeneration".

Consequently, considering the inconsistencies in the experimental results plus the above-mentioned controversies on the clinical evaluation of portacaval anastomosis, it may be assumed that the precise effect of this operation on the liver still is undetermined. Since Child and associates[43)] and Fisher and associates[44)] emphasized the role played by the volume of blood entering the liver in hepatic regeneration, the post-shunt ability of the liver to adapt itself to the altered circulatory state is considered to be of prime importance for the continuance of its function. Granting that the liver will adapt itself to the changes in blood circulation sometime after the diversion of the portal blood, these studies have been undertaken to reevaluate regeneration of the liver in dogs by performing the various operations as follows:

1. 40 per cent partial hepatectomy (control).
2. Eck fistula accompanied by 40 per cent partial hepatectomy.
3. Eck fistula followed at various later intervals by 40 per cent partial hepatectomy.

MATERIALS AND METHODS

Healthy adult mongrel dogs of both sexes, ranging in weight from 5.5 to 16.6 kg were used. Throughout the experiments they were fed with daily

hospital table scraps and dietologically special considerations were not taken. Before and after the operations all dogs were kept in the fixed kennels.

The experiments were carried out in the fasting state and the operations were performed under preoperative medication with 2 to 4 ml of 2 per cent morphine hydrochloride administered subcutaneously, and, with sterile technic, under intravenous sodium pentobarbital anesthesia adminstered in a dose of 30 mg per kg body weight.

An end-to-side portacaval anastomosis was created according to the method, which had been employed in our laboratory.[58] A partial hepatectomy was performed on dogs with normal hepatic circulation, and on dogs of the Eck-fistula groups at the same time, and at the following various later intervals after the creation of Eck fistula; 2, 4, 7, 12, and 13 weeks, and 4, 7, 9, and 12 months. The operative procedures of the hepatectomy were as follows. After opening the abdomen through a midline incision, the stomach and the intestines were retracted away from the liver and the omentum minor was opened at the pars flaccida. Then the ligamentous attachments from the diaphragm and the porta hepatis were dissected as far as to make the ligature easily around the pedicle of the left central lobe. The ligature was made with No. 7 braided silk at the pedicle after manually separating the proximal portion of the left central lobe along the line of the cleavage between this and the gall bladder lobe. Then the two left lobes were removed distal to the ligature with taking utmost care to leave as small a remnant as possible at the ligated stump. By this technic the hepatectomy was easily performed with least amount of bleeding. Then the stump was covered with the omentum minor by attaching it loosely to the root of the diaphragm. After administering 250 mg of oxytetracycline intraperitoneally, the abdomen was closed, and for a successive few days, 200 mg of the antibiotic was injected intramusclarly.

Two months after the hepatectomy, all these dogs were sacrificed by intravenous injection of 20 ml of saturated KCl solution and were autopsied. At every instance, the stoma between the portal and caval veins was dissected carefully to examine its patency. The data of the animals, in which the stoma was occluded, were discarded from the experimental series. The remaining liver was removed and the gall bladder was excised. After pushing out blood gently, and as far as possible, the liver was weighed. The same manner was applied for weighing the two left lobes obtained at the previous partial hepatectomy.

At the times of partial hepatectomy and sacrifice, studies on the following items were carried out in dogs with normal hepatic circulation and those with Eck fistula.

1) Hepatic regenerative activity.

Hepatic regeneration, or hepatic regenerative activity, was estimated by evaluating the following two points. Namely,

i) Weight of the liver per kg of body weight

ii) Hepatic regeneration rate.

According to the report of Fisher and associates[44] and the one from our

laboratory,[68] the two left lobes of the liver occupy about 40 per cent of the whole organ. Therefore, the hepatic remnant at the time of partial hepatectomy was estimated by multiplying the weight of the resected two left lobes by 6/4. The gain in hepatic weight during the period from partial hepatectomy to autopsy was obtained by subtracting the weight of the remnant from the weight of the liver removed at autopsy. The hepatic regeneration rate was expressed in per cent in which the gain in hepatic weight was devided by the weight of the lobes removed and was multiplied by 100. That is:

$$\text{Hepatic regeneration rate} = \frac{(C)-(B)}{(A)} \times 100$$

where (A)=the weight of the two left lobes resected at partial hepatectomy; (B)= the estimated weight of the hepatic remnant at the time of partial hepatectomy; (C)=the weight of the liver at autopsy.

2) BSP clearance.

As a way to evaluate hepatic function, BSP clearance was employed. By the method of Gornall and Bradawill,[69] 10 mg per kg of body weight of BSP was adminstered, and a venous blood sample was withdrawn 30 minutes after the injection. Concentration of the dye was determined by Hitachi spectrophotometer at the wave length of 575 mμ.

3) Hepatic blood flow (HBF).

Along with the method described by Vetter and associates,[70] disappearance rate of radioactive colloidal gold (Au^{198}) in peripheral blood was measured. Au^{198} colloid was obtained from Radiochemical Center, Amersham, England, and was used immediately after its arrival, in order to avoid the effects of changes in the colloidal particle size. However, this particle size was not examined at each time of the experiments, but usually was known to be less than 250 mμ according to the maker's instructions. About 20 μC of Au^{198} colloid diluted in 2 ml of distilled water was injected rapidly into one of the lingular veins, and from the polyethylene catheter indwelling in the femoral vein, blood samples were taken into heparinized tubes at 2, 3, 4, 5, and 10 minutes after injection. Then 1.0 ml of whole blood from each blood sample was taken and placed in each of flat-bottomed glass tubes with 1.1 cm in diameter and the radioactivity was determined in a well-type scintillation counter (TEN Model EA-14) and TEN 1 000 scaler (Model SA-1 000 C). When plotted on semilogarithmic paper, the disappearance curve of the colloidal gold appeared in a linear fashion, and the disappearance rate constant K was calculated from the following formula:

$$K\ \text{min}^{-1} = \frac{0.693}{T^{1/2}}$$

where $T^{1/2}$ represents the disappearance half time, *i.e.*, the time when the radioactivity comes down to the half of the initial value, obtained by extrapolating the curve back to zero time. Since the two left lobes, to which

hepatic catheterization was most easily done, were removed at partial hepatectomy, the extraction ratio could not be determined in this experiment, and therefore, as some workers reported,[71][72] HBF was expressed in terms of K value.

4) RNA and DNA in the liver.

It has been known that nucleic acids, ribonucleic acid (RNA) and desoxyribonucleic acid (DNA), are closely related to protein metabolism and cell proliferation. In order to evaluate hepatic regenerative activity in view of nucleic acids metabolism, the following studies were performed.

i) Concentrations of nucleic acids in the liver.

Wedge biopsy specimens of the liver, usually of 0.7 to 1.5 gm, were taken from the left lateral lobe before partial hepatectomy, and from one of the remaining lobes at the time of sacrifice. The specimens were weighed and were homogenized in 4 times amount of the ice-cold distilled water. One ml of the homogenate was decanted into a centrifuging tube, and was washed twice with 2.5 ml of ice-cold 10 per cent TCA solution. Lipid fractions were removed by washing the above precipitate, successively with 1 ml of distilled water plus 4 ml of ethanol, 5 ml of ethanol, and 5 ml of ethanol-ether (3 : 1) solution.

Extraction of nucleic acids was made by the procedure of Schmidt and Thannhauser.[73] Namely, the dried precipitate was incubated at 37°C for 20 hours with 2 ml of 1 N potassium chloride solution. The RNA fraction was extracted by acidifying the alkaline digest with 0.4 ml of 60 per cent perchloric acid and by washing the precipitate twice with 3 ml of 5 per cent perchloric acid.

The amounts of RNA and DNA were estimated by determining the phospherus content as RNA-P and DNA-P. These two fractions were mineralized by heating with 60 per cent perchloric acid for 45 minutes and, adding a few drops of hydrogen peroxide, were heated until the liquid became colorless (about 15 minutes). Amounts of phospherous were assayed by the following method of Allen.[74] After adding Amidol reagent (2, 4-diaminophenol) and ammonium molybdate, the color was developed and 15 minutes later, the color intensity at 720 mμ was measured by Beckman spectrophotometer. The results were expressed as RNA-P and DNA-P μM per gm of fresh tissue and the total amounts of these two fractions in the liver were calculated.

ii) Incorporation of P^{32} into RNA and DNA of the liver.

Incorporation of P^{32} into RNA and DNA of the liver cells was examined in dogs with Eck fistula, dogs with 40 per cent partial hepatectomy, and dogs with 40 per cent hepatectomy following Eck fistula. Orthophosphate P^{32} was obtained from Radiochemical Center, Amersham, England. About 600 μC of this material in 5 ml of distilled water was injected intravenously and wedge liver specimens were excised 2, 4, 6, and 8 hours after the injection. The two fractions were separated as described in the above. Each 1.0 ml of the two fractions was taken into each of the counting dishes and was dried up by an infrared lamp. The radioactivity of incorporated P^{32} was determined by G-M

counter (TEN GM 131) and 1 000 scaler (TEN SC 1 000). The results were expressed by per cent of administered P^{32} in RNA and DNA per gm of fresh tissue. In each sample, RNA and DNA concentrations of liver tissue were determined as described above, and specific activity of P^{32} in the two fractions was calculated, by the following formula:

$$\text{Specific Activity} = \frac{P^{32} \text{ radioactivity \% administered dose/RNA or DNA per gm of fresh tissue.}}{\text{RNA-P or DNA-P } \mu\text{M/gm of fresh tissue.}}$$

5) *Histological changes.*

All liver specimens were examined histologically after fixing with 10 per cent formalin. Only hematoxylin-eosin staining was made on each specimen.

RESULTS

Of the six dogs with normal hepatic circulation on which only partial hepatectomy was performed, one dog (Lx-23) died possibly due to postoperative

TABLE 1. Hepatic Regeneration after Partial Hepatectomy in

No. of dogs	Interval between Eck fist. and part. hepatect.	Weight of dogs at part. hepatect. (kg)	Weight of liver removed (gm)	Est. weight of hepatic remnant (gm)	Est. weight of liver at part. hepatect. (gm)	Est. weight of liver at part. hepatect. per body weight (gm/kg)
(1) Normal hepatic circulation						
Lx 1	—	8.5	112	168	280	32.9
5	—	10.6	111	167	278	26.2
11	—	11.0	139	232	371	33.6
21	—	8.8	116	173	289	32.9
22	—	9.3	115	173	288	30.9
23	—	6.7	83	125	208	31.0
(2) Eck fistula						
20	0	9.7	107	161	268	27.6
25	0	11.3	157	236	393	34.8
26	0	11.2	168	252	420	37.5
27	0	7.8	114	171	285	36.6
17	0	9.8	118	177	285	30.1
2	2 weeks	11.2	104	154	258	23.2
10	2	5.5	47	71	118	21.5
19	2	8.5	95	142	237	27.9
34	2	9.3	115	173	288	18.5
9	4	9.0	77	116	193	21.4
18	4	7.8	86	129	215	27.5
32	7	8.7	68	102	170	19.6
3	12	8.1	74	111	185	22.8
6	13	5.8	50	76	126	21.7
29	4 months	8.5	85	116	201	25.0
8	7	6.9	47	71	118	17.1
33	7	9.5	53	80	133	14.0
15	9	6.2	64	96	160	25.8
24	12	11.0	80	120	200	18.2
28	12	7.9	53	80	133	16.8

peritonitis, and the remaining five dogs constituted the non-shunt group of the experiment (Table 1).

Among the Eck fistula dogs, one dog (Lx-17) on which a partial hepatectomy was performed at the same time, died within one week after the operation due to a distemper-like pulmonary complication. One dog (Lx-8) with partial hepatectomy 7 months after the shunt, died on the second postoperative day due to left hemothorax. One dog (Lx-29) with partial hepatectomy 4 months after the shunt, died of unknown causes 4 weeks after the hepatectomy, and the one, Lx-2, with partial hepatectomy 2 weeks after the shunt died 5.5 weeks after the hepatectomy. The autopsy of the latter dog revealed that a large, white thrombus extended from the orfice of the left hepatic vein into the inferior vena cava. Neither of the two dogs of late death showed any typical picture of Eck fistula syndrome. Namely they never manifested excited movements and convulsions, or recurrent attacks of unconsciousness, but gradually lost their activeness, and in their last periods, they sat most of the time, while consciousness remained.

Dogs with (1) Normal Hepatic Circulation and (2) Eck Fistula

Weight of dogs at autopsy (kg)	Weight of liver at autopsy (gm)	Weight of liver at autopsy per cent original weight (%)	Weight of liver at autopsy per body weight (gm/kg)	Est. gain in liver weight (gm)	Hepatic regeneration rate (%)
9.6	327	117	34.1	159	142
12.2	406	146	33.3	239	210
10.3	396	107	38.5	164	118
8.9	326	113	36.2	153	132
9.8	315	109	32.2	142	124
Postoperative death					
8.0	153	57	19.1	− 8	− 8
11.9	246	63	20.7	11	7
11.2	270	64	24.1	22	13
7.0	135	47	19.3	−36	−31
Postoperative death (Eck fistula combined)					
Died 5.5 weeks after partial hepatectomy					
5.7	106	90	18.6	35	74
7.7	186	79	24.2	44	46
Sacrificed for the studies of P^{32} incorporat.					
7.2	185	96	25.7	69	89
8.1	165	77	20.4	36	42
8.5	162	95	19.1	60	89
8.0	170	92	21.2	59	79
5.5	129	102	23.5	53	105
Died 4 weeks after partial hepatectomy					
Postoperative death					
Sacrificed for the studies of P^{32} incorporat.					
6.0	130	81	21.4	34	53
10.8	195	98	18.1	75	94
8.1	135	101	16.7	55	102

Consequently 14 dogs with Eck fistula and 5 dogs with normal hepatic circulation were presented to evaluate the experimental results, as listed in Table 1.

Throughout the test period all of the dogs maintained an apparently healthy condition which can be speculated from the finding that changes in body weight were small, and decreases, if any, were slight in the shunt group as in Fig. 1.

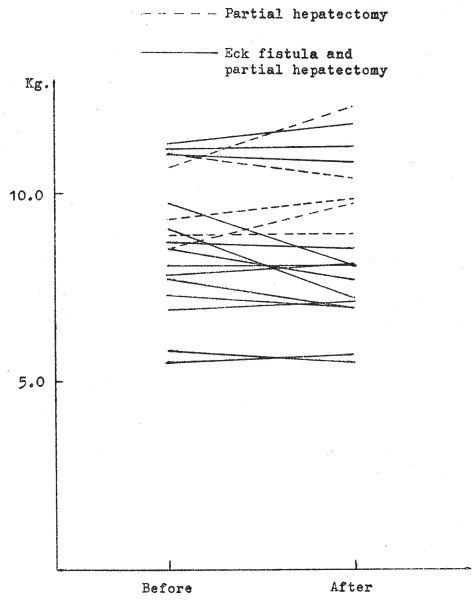

FIG. 1. Changes in body weight before and 2 months after partial hepatectomy.

1) Hepatic regenerative activity.

Hepatic regenerative activity was appraised in the following two points and the results were presented in Table 1.

i) Changes in the weight of the liver per kg of body weight (Fig. 2).

In dogs with normal hepatic circulation it was found that the averaged weight of the liver per kg of body weight increased from 31.4 to 34.9 gm per kg of body weight, *i.e.*, 11 per cent of the original weight, between the times

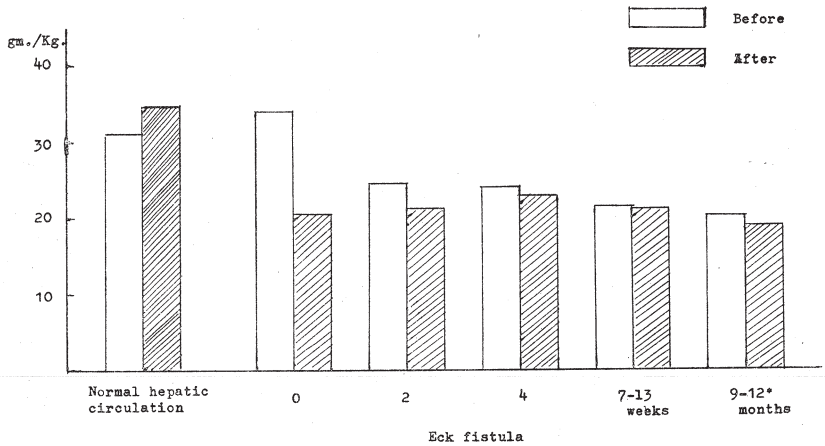

FIG. 2. Average weight of liver per kilogm body weight before and 2 months after partial hepatectomy in dogs with normal hepatic circulation and dogs with Eck fistula.

* Interval between Eck fistula and partial hepatectomy.

of partial hepatectomy and sacrifice.

Before partial hepatectomy it was noted in the established shunt group that the estimated weight of the liver per kg of body weight was considerably reduced, about 30 per cent in average, as compared with that of the normal dogs, and as the postshunt period became longer, a slight decreasing trend in these liver weights was observed (Fig. 3).

In 4 dogs, on which Eck fistula and partial hepatectomy were performed simultaneously, the averaged weight of the liver per kg of body weight at the time of sacrifice was reduced to about 39 per cent of the preoperative value. This finding was quite contrary to that of the dogs with the delayed hepatectomy following the shunt, in which the reduction in the hepatic weight became much less than the animals with simultaneous shunt and hepatectomy, and further the more the intervals between the two operations was prolonged, this reduction tended to be smaller, and the hepatic weight returned nearly to the prehepatectmy level. In these dogs the averaged reduction in the hepatic weight was as follows: in the two dogs with partial hepatectomy 2 weeks after the shunt, 3.3 gm per kg of body weight or 13.4 per cent; in the two dogs with the hepatectomy 4 weeks after the shunt, 1.4 gm per kg of body weight or 5.7 per cent; in the three dogs with the hepatectomy 7 to 13 weeks after the shunt, 0.1 gm per kg of body weight or 0.5 per cent; in the three dogs with the hepatectomy 9 to 12 months after the shunt, 1.6 gm per kg of body weight or 7.9 per cent, respectively (Fig. 2).

ii) Hepatic regeneration rate (Fig. 4)

When hepatic regeneration rate was calculated at the time of sacrifice, the results were similar with those obtained in the foregoing study. Namely, after 40 per cent of the liver was removed, dogs with intact portal circulation restored the hepatic weights to 145 per cent, in average, of the original value. Whereas, in 4 dogs with Eck fistula and simultaneous partial hepatectomy, the average hepatic regeneration rate was −10 per cent. However, it was

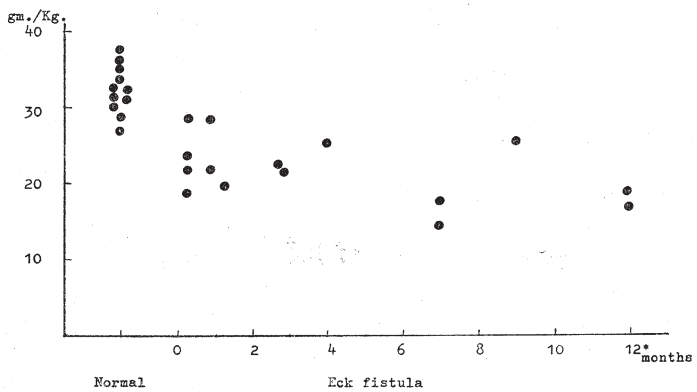

FIG. 3. Estimated weight of the liver in dogs with normal hepatic circulation and dogs with Eck fistula.

* Months after the shunt.

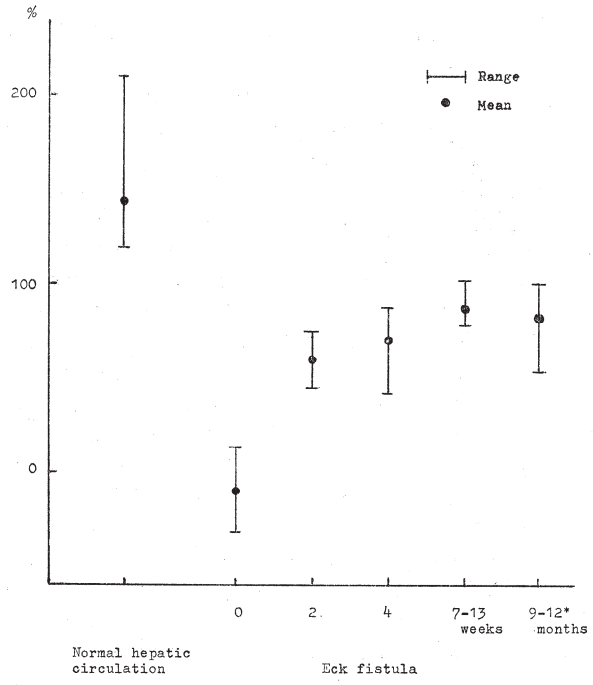

FIG. 4. Hepatic regeneration rate 2 months after partial hepatectomy in dogs with normal hepatic circulation and with Eck fistula.

* Interval between Eck fistula and partial hepatectomy.

revealed that dogs with delayed partial hepatectomy following the shunt achieved a considerable degree of hepatic restoration; their average hepatic regeneration rate being 60 per cent in the two dogs with partial hepatectomy 2 weeks after the shunt; 70 per cent in the two dogs with the hepatectomy 4 weeks after the shunt; 86 per cent in the three dogs with the hepatectomy 7 to 13 weeks after the shunt; 82 per cent in the three dogs with the hepatectomy 9 to 12 months after the shunt, respectively.

2. BSP clearance.

The BSP clearance was expressed as the BSP concentration retained in the peripheral blood 30 minutes after injecting 10 mg per kg of body weight of the dye and the results are presented in the left half of Table 2.

In dogs with normal hepatic circulation the average BSP concentration retained was 0.63 mg per dl before the hepatectomy, and at sacrifice it was reduced to 0.45 mg per dl. However, in the two dogs with simultaneous shunt and hepatectomy, excretion of BSP by the liver was severely impaired 2 months after the hepatectomy; the mean preoperative value was 0.68 mg per dl and the value at sacrifice 1.95 mg per dl. In the shunt and delayed hepa-

TABLE 2. BSP Clearance and Hepatic Blood Flow before and 2 Months after Partial Hepatectomy in Dogs with (1) Normal Hepatic Circulation and (2) Eck Fistula

No. of dogs	Interval between Eck fistula and partial hepatectomy	BSP clearance*		Hepatic blood flow		
		Before partial hepatectomy (mg/dl)	After partial hepatectomy (mg/dl)	Before partial hepatectomy (K min^{-1})	After partial hepatectomy (K min^{-1})	Per cent original blood flow (%)
(1) Normal hepatic circulation						
Lx 1	—	0.64	0.40	0.312	0.420	134
5	—	0.50	0.40	0.251	0.254	101
11	—	0.75	0.50	0.278	0.374	134
21	—	0.55	0.50			
22	—	0.70	0.45			
(2) Eck fistula						
20	0	0.60	1.30	0.279	0.097	35
25	0	0.75	2.60	0.308	0.141	46
10	2 weeks	1.25	1.05	0.169	0.144	85
19	2	1.30	2.30	0.178	0.139	78
9	4	1.55	1.60			
18	4	0.65	0.75	0.175	0.154	88
3	12	1.25	1.50	0.141	0.151	107
6	13	1.20	0.75	0.187	0.154	83
15	9 months	1.35	1.75			
24	12	1.30	1.25	0.165	0.209	127

* BSP clearance was expressed as the retained BSP concentrations at 30 min after injection.

tectomy dogs, all showed some impairment of BSP excretion before the hepatectomy, as compared with the dogs with normal hepatic circulation. It was found, however, that further impairment of this function after the hepatectomy was considerably milder than in the dogs receiving the simultaneous shunt and hepatectomy. The average retention at the end of 30 minutes in the two dogs with partial hepatectomy 2 weeks after the shunt, shifted from 1.28 to 1.67 mg per dl during the experimental period; in the two dogs with the hepatectomy 4 weeks after the shunt, it shifted from 1.10 to 1.18 mg per dl; in the two dogs with the hepatectomy 12 and 13 weeks after the shunt, it shifted from 1.23 to 1.13 mg per dl; and in the two dogs with the hepatectomy 9 and 12 months after the shunt it shifted from 1.32 to 1.50 mg per dl. These alterations are diagrammatically shown in Fig. 5.

3. Hepatic blood flow (HBF).

In the right half of Table 2 are listed the results of this study. All the dogs with normal hepatic circulation showed an increase in HBF at the time of sacrifice. However, in the two dogs with simultaneous shunt and hepatectomy, HBF was markedly reduced after the hepatectomy more than half of the original value; *i.e.*, in average, from 0.294 to 0.119 min^{-1}. Whereas, in the shunt and delayed hepatectomy groups, though all the animals manifested a considerable decrease in the blood flow already before the hepatectomy, it

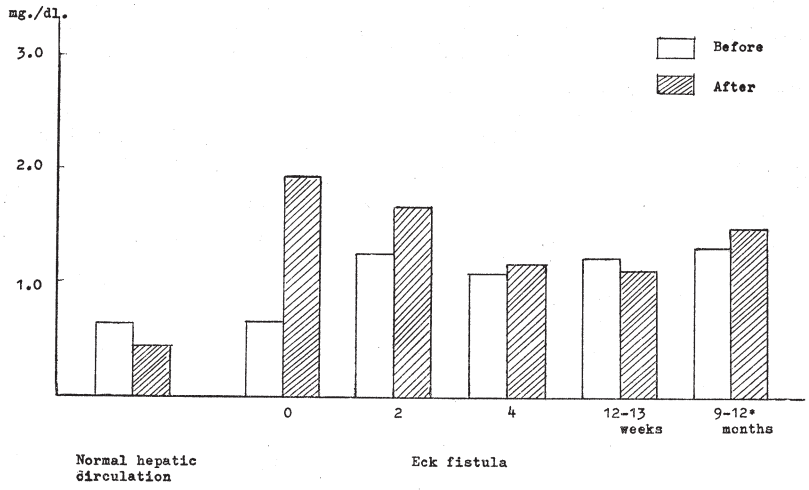

FIG. 5. BSP retention at 30 minutes after injection before and 2 months after partial hepatectomy in dogs with normal hepatic circulation and dogs with Eck fistula.

* Interval between Eck fistula and Partial hepatectomy.

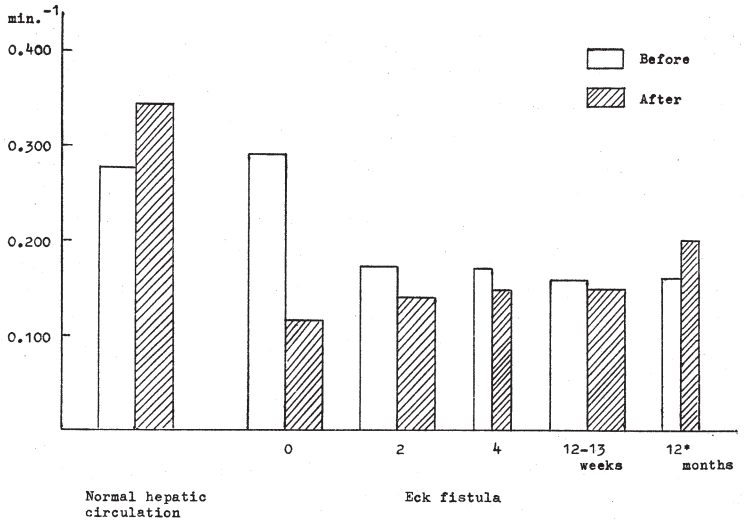

FIG. 6. Hepatic blood flow (K min^{-1}) before and 2 months after partial hepatectomy in dogs with normal hepatic circulation and dogs with Eck fistula.

* Interval between Eck fistula and partial hepatectomy.

was revealed that the posthepatectomy reduction in HBF became slight, as time elapsed after the shunt (Fig. 6).

Namely in the two dogs with partial hepatectomy 2 weeks after the shunt, the average HBF was reduced from 0.174 to 0.142 min^{-1} during the period; in the one with the hepatectomy 4 weeks after the shunt from 0.175 to 0.154 min^{-1}; in the two dogs with the hepatectomy 12 and 13 weeks after the shunt from 0.164 to 0.153 min^{-1}. The one dog with the hepatectomy 12 months after the shunt the blood flow increased from 0.165 to 0.209 min^{-1} at the time of sacrifice.

From the studies in BSP retention and HBF it was ascertained that these functions of the liver were impaired after creation of Eck fistula, but when partial hepatectomy was performed sometime after the shunt, the liver preserved well its function 2 months following the operation, although still being subnormal.

In order to compare restoration of hepatic blood flow with that of hepatic tissue mass, the residual liver at autopsy, expressed as per cent of the original liver mass, and the hepatic blood flow at the end of the experiment, expressed as per cent of the original blood flow, were chosen respectively from Tables I and II, and both figures were plotted in a correlation diagram with blood flow on the ordinate and with liver mass on the abscissa (Fig. 7). Except one with normal hepatic circulation (Lx-5) these figures showed a good correlation, and in the shunt group the coefficient of correlation "r" was calculated as

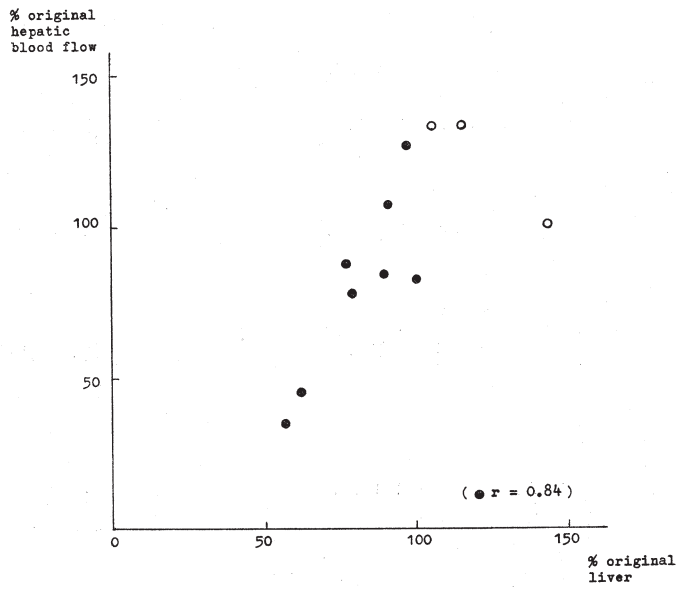

FIG. 7. Relationship between restoration of liver mass and that of hepatic blood flow.
o Normal hepatic circulation
● Eck fistula

TABLE 3. Concentrations of Nucleic Acids in Liver Tissue before and 2 Months after Partial Hepatectomy in Dogs with (1) Normal Hepatic Circulation and (2) Eck Fistula

(1) Normal hepatic circulation

No. of dog	Interval between Eck fistula and partial hepatectomy	RNA in liver tissue				DNA in liver tissue				Ratio: RNA/DNA	
		RNA-P μM/gm fresh tissue		Total amount of RNA μM in liver		DNA-P μM/gm fresh tissue		Total amount of DNA μM in liver		Before	After
		Before	After	Before	After	Before	After	Before	After		
Lx 1	—	20.5	21.9	5750	7170	5.7	5.8	1595	1900	3.6	3.8
5	—	17.3	20.1	4800	8170	5.1	6.4	1420	2600	3.4	3.1
11	—	19.5	21.9	7250	8650	4.3	4.4	1600	1740	4.5	5.0
21	—	21.0	21.9	6070	7150	5.4	6.3	1570	2060	3.9	3.6
22	—	21.2	22.6	6100	7110	5.8	5.7	1660	1800	3.7	3.9
(2) Eck fistula											
20	0	21.2	22.6	5070	2590	5.0	6.7	1340	1020	3.8	2.5
25	0	22.2	22.7	7870	5600	5.6	7.3	2200	1800	4.0	3.1
10	2 weeks	20.2	21.8	2380	2310	6.8	6.8	805	720	3.0	3.2
19	2	19.2	18.8	4570	3500	5.6	6.5	1340	1200	3.4	2.9
18	4	20.1	18.9	4310	3120	6.2	5.3	1330	880	3.2	3.5
3	12	21.6	22.8	4000	3880	5.9	6.1	1090	1040	3.7	3.7
6	13	21.4	21.2	2700	2720	5.0	5.9	630	760	4.3	3.6
15	9 months	23.0	19.0	3680	2520	6.3	4.9	1000	635	3.7	4.0
24	12	14.3	17.1	2860	3340	5.4	5.7	1080	1090	2.7	3.1

0.84 ($P<0.01$). Therefore it appears that regeneration of the liver, when portal blood deprived, occurs fairly in proportion to the restoration of hepatic blood flow after partial hepatectomy.

4. Concentrations of nucleic acids in the liver.

RNA and DNA in the liver were expressed by the amount of phosphorus (μM) per gm of fresh tissue and by the total amount of these two fractions in this organ, and the ratio, RNA-P/DNA-P, was calculated. The results are listed in the Talbe 3.

i) Concentration of RNA in liver tissue.

When concentrations of RNA-P in the dogs were compared in terms of per unit weight of fresh tissue, the figures in the non-shunt animals consistently increased 2 months after partial hepatectomy; the average concentrations changed from 19.9 to 21.7 μM per gm of fresh tissue. In the shunt groups, figures were varied, so that any definite characteristics could not be deduced from the obtained results (Fig. 8-2). The average RNA-P concentrations before and 2 months after the hepatectomy changed from 21.7 to 22.7 μM per gm of fresh tissue in the two dogs with simultaneous shunt and hepatectomy; from 19.7 to 20.3 μM per gm of fresh tissue in the two dogs with the hepatectomy 2 weeks after the shunt; from 20.1 to 18.9 μM per gm of fresh tissue in the one dog with the hepatectomy 4 weeks after the shunt; from 21.5 to 22.0 μM per gm of fresh tissue in the two dogs with the hepatectomy 12 and 13 weeks after the shunt; and from 18.7 to 18.2 μM per gm of fresh tissue in the two dogs with the hepatectomy 9 and 12 months after the shunt.

When total amounts of RNA-P in the liver were examined, however, significant changes were observed in the results, as in Fig. 8-1. Namely, the mean total amount of RNA-P in the liver of the normal dogs without shunt increased from 5994 to 7650 μM per liver at the time of sacrifice. Whereas, in the dogs with simultaneous shunt and hepatectomy, RNA-P decreased considerably during the period; the mean total amount of RNA-P was reduced from 6 470 to 4 090 μM per liver, *i.e.*, more than 30 per cent of the original value.

In the shunt and delayed hepatectomy dogs, though the prehepatectomy amounts of total RNA-P in the liver was significantly lower than the normal dogs without shunt, reduction in their figures during the test period was revealed to be less than that of dogs with simultaneous shunt and hepatectomy. Namely, the average total amount of RNA-P in the two dogs with the hepatectomy 2 weeks after the shunt changed from 3 480 to 2 900 μM per liver during the test period; in the one dog with the hepatectomy 4 weeks after the shunt, from 4 310 to 3 120 μM per liver; in the two dogs with the hepatectomy 12 and 13 weeks after the shunt, from 3 350 to 3 300 μM per liver; in the two dogs with the hepatectomy 9 and 12 months after the shunt, from 3 270 to 2 930 μM per liver, respectively.

ii) Concentration of DNA-P in liver tissue.

This experiment resulted in the following observations (Fig. 9-2). In the normal dogs with partial hepatectomy, the average amount of DNA-P in liver

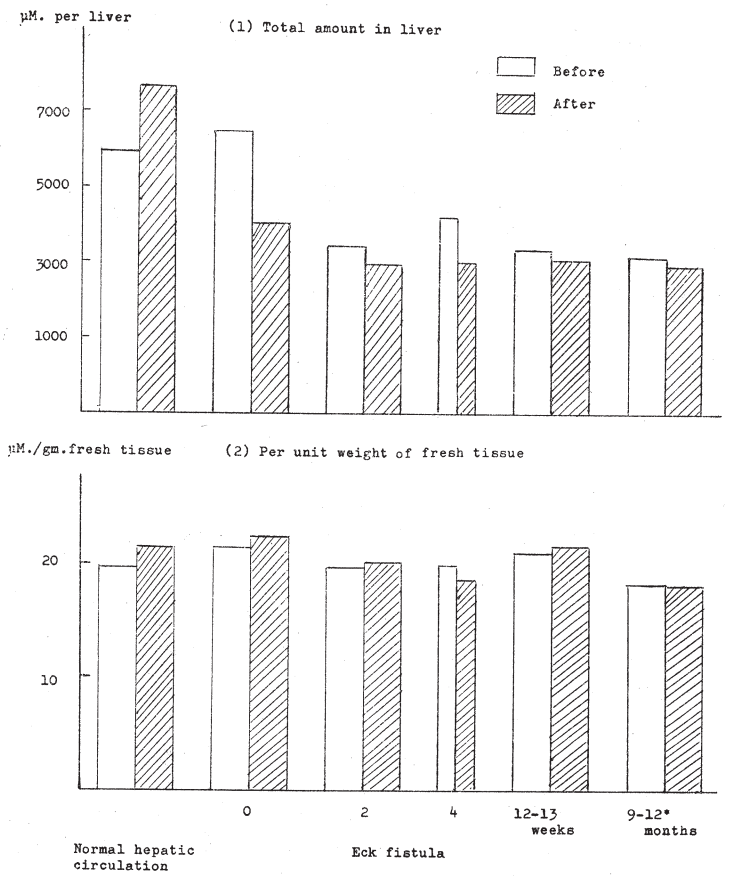

FIG. 8. RNA-P in liver before and 2 months after partial hepatectomy in dogs with normal hepatic circulation and dogs with Eck fistula.

* Interval detween Eck fistula and partial hepatectomy.

tissue was 5.2 μM per gm of fresh tissue and was increased to 5.7 μM per gm of fresh tissue at sacrifice. It was to be noted that in the two dogs with simultaneous shunt and hepatectomy, the averaged concentration of DNA-P changed from 5.3 to 7.0 μM per gm of fresh tissue during the test period. In the shunt and delayed hepatectomy dogs, though considerable variations were fonnd, a slight increasing trend in the amounts of DNA-P was observed prior to partial hepatectomy. The averaged amounts of DNA-P per gm of fresh tissue of the liver of these shunt and delayed hepatectomy dogs, before and 2 months after the hepatectomy, were as follows; 6.2 and 6.7 μM per gm of fresh tissue in the two dogs with the hepatectomy 2 weeks after the shunt; 6.2 and 5.3 μM per gm of fresh tissue in the one dog with the hepatectomy 4 weeks after the shunt; 5.5 and 6.0 μM per gm of fresh tissue in the two dogs

with the hepatectomy 12 and 13 weeks after the shunt; and 5.9 and 5.3 μM per gm of fresh tissue in the two dogs with the hepatectomy 9 and 12 months after the shunt.

When the total amount of DNA-P in the liver was calculated, hewever, remarkable differences were encountered among the groups of dogs. In the normal dogs with partial hepatectomy, the average total DNA-P in the liver shifted from 1 566 to 2 010 μM during the test period, and this was in proportion to the changes in the figures per gm of fresh tissue. Whereas, in the dogs with simultaneous shunt and hepatectomy the total amount of DNA-P in the liver decreased from 1 770 to 1 410 μM during the test period, contrary to the changes observed in the figures of this substance, expressed in terms of per gm of fresh tissue.

All of the shunt and delayed hepatectomy dogs were shown to be reduced in the amounts of the total DNA-P in the liver before partial hepatectomy, and, however, 2 months after the hepatectomy had been performed, these dogs

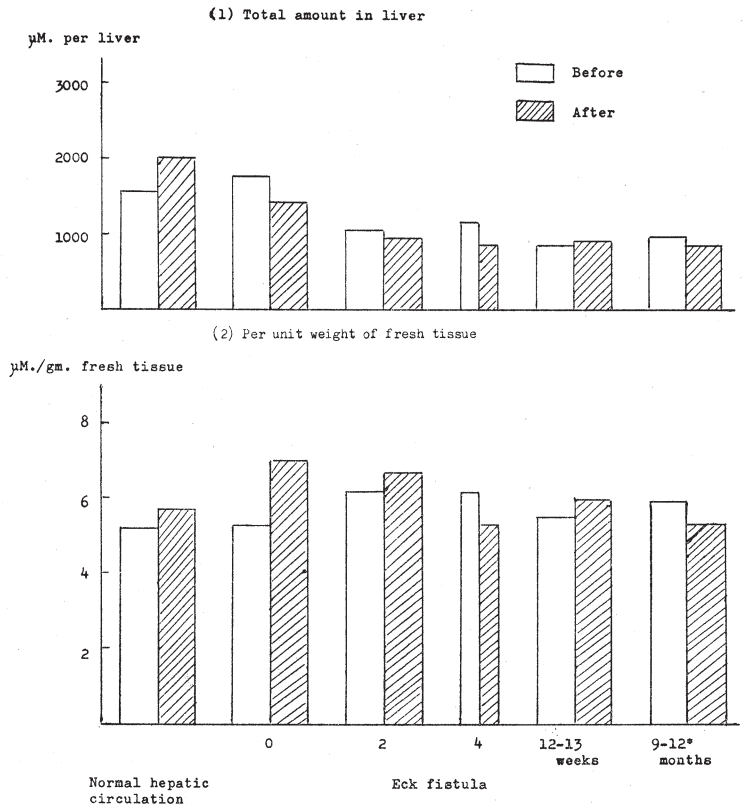

FIG. 9. DNA-P in liver before and 2 months after partial hepatectomy in dogs with normal hepatic circulation and dogs with Eck fistula.
* Interval between Eck fistula and partial hepatectomy.

underwent fairly little reductions in the amounts of total DNA, and maintained, except two dogs, almost equal amounts of this substance as compared to the prehepatectomy figures. The averaged amount of total DNA-P in the liver changed from 1 070 to 960 μM per liver in the two dogs with partial hepatectomy 2 weeks after the shunt; it changed from 1 330 to 880 μM per liver in the one dog with the hepatectomy 4 weeks after the shunt; it changed from 860 to 900 μM per liver in the two dogs with the hepatectomy 12 and 13 weeks after the shunt; and it changed from 1 040 to 860 μM per liver in the two dogs with the hepatectomy 9 and 12 months after the shunt. These changes are diagrammatically represented in Fig 9-1 and Fig. 9-2.

iii) Ratio of RNA/DNA in liver tissue.

From the preceeding two studies on RNA and DNA of the liver, the ratio of RNA/DNA was calculated by deviding RNA-P μM per gm of fresh tissue by DNA-P μM per gm of fresh tissue. The results are listed in the rightest two columns of the Table 3. In the five normal dogs with partial hepatectomy, the average ratios before and 2 months after the hepatectomy were equal, these being 3.8. While the dogs with simultaneous shunt and hepatectomy showed a considerable reduction in the average ratio, *i.e.*, from 3.9 to 2.8 during the experimental period, these dogs with the delayed partial hepatectomy following the shunt were revealed to undergo minor changes in the concerning figures during the period; namely, in the two dogs with the hepatectomy 2 weeks after the shunt, the mean ratio, RNA/DNA, shifted from 3.2 to 3.1; in the one dog with the hepatectomy 4 weeks after the shunt, it shifted from 3.0 to 3.5: in the two dogs with the hepatectomy 12 and 13 weeks after the shunt, it shifted from 4.0 to 3.7; in the two dogs with the hepatectomy 9 and 12 months after the shunt, it shifted from 3.2 to 3.6, respectively (Fig. 10).

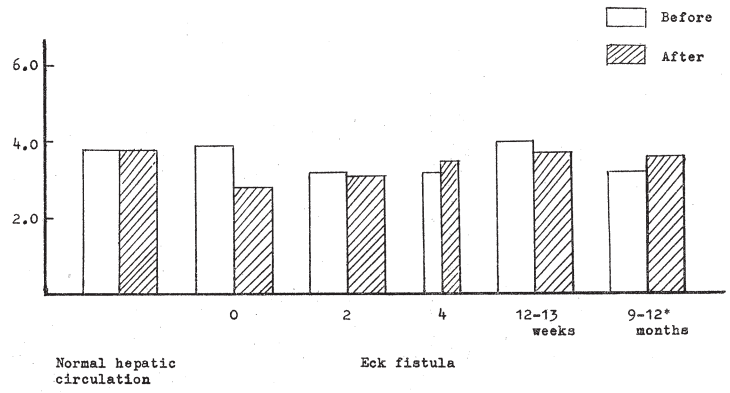

FIG. 10. Ratio, RNA/DNA in liver tissue of dogs with normal hepatic circulation and dogs with Eck fistula, before and 2 months after partial hepatectomy (mean figure).

* Interval between Eck fistula and partial hepatectomy.

TABLE 4. Incorporation of P^{32} into RNA and DNA of the Liver

	Hours after injection	DNA μM/gm fresh tissue	P^{32} incorp. into RNA per gm fresh tissue % administ. dose	P^{32} specific activity in RNA	DNA μM/gm fresh tissue	P^{32} incorp. into DNA per gm fresh tissue % administ. dose	P^{32} specific activity in DNA	RNA/DNA
Normal	2	22.4	0.0176	7.9×10^{-4}	5.2	0.00133	2.6×10^{-4}	4.3
	4		0.0209	9.3		0.00122	2.3	
	6		0.0202	9.0		0.00143	2.7	
	8		0.0253	11.3		0.00220	4.2	
Normal with partial hepatectomy	2	21.9	0.0091	4.2	5.8	0.00065	1.1	3.8
	4		0.0118	5.4		0.00110	1.9	
	6		0.0145	6.6		0.00182	3.1	
	8		0.0124	5.7		0.00110	1.9	
Eck fistula dog (2 weeks) without partial hepatectomy	2	21.2	0.0036	1.5	3.2	0.00000	0.0	6.6
	4		0.0046	2.2		0.00016	0.5	
	6		0.0091	4.3		0.00024	0.7	
	8		0.0051	2.4		0.00008	0.2	
Eck fistula dog (7 months) without partial hepatectomy	2	15.3	0.0036	2.4	3.6	0.00016	0.4	4.2
	4		0.0057	3.7		0.00087	2.4	
	6		0.0080	5.2		0.00079	2.2	
	8		0.0085	5.6		0.00048	1.3	
Eck fistula dog (13 weeks) with partial hepatectomy	2	21.2	0.0257	12.1	5.9	0.00070	1.2	3.6
	5		0.0318	15.1		0.00114	1.9	
	6		0.0298	14.0		0.00105	1.8	
	8		0.0292	13.8		0.00079	1.3	
Eck fistula dog (2 weeks) with partial hepatectomy	2	21.8	0.0342	15.7	6.8	0.00018	0.3	3.2
	4		0.0497	22.8		0.00114	1.7	
	$6\frac{1}{2}$		0.0444	20.2		0.00079	1.2	
	8		0.0412	18.9		0.00070	1.0	

5. Incorporation of P^{32} into RNA and DNA of the liver.

This study yielded the results, listed in Table 4 and in Fig. 11. In one dog, the second specimen was taken at 5 hours after the injection of P^{32} and in another dog, the third specimen was taken at 6.5 hours after the injection. The other specimens were taken just as described before. Fig. 11 represents the curves on which are plotted specific activities of P^{32} in RNA and DNA on every two hours during the eight hours after the injection.

As shown in Fig. 11-1, incorporation of P^{32} into RNA reached the highest level in the dogs with shunt and delayed partial hepatectomy, particularly in one dog, Lx-10, on which the latter operation was perfomed 2 weeks after the shunt, and the peaks of the curves of these two animals came earlier than the others. However, the dogs only with Eck fistula demonstrated the lowest rate of P^{32} turnover, particulary in the earlier shunt dog. The results of the normal dogs fell between the former two groups of dogs, and the one with

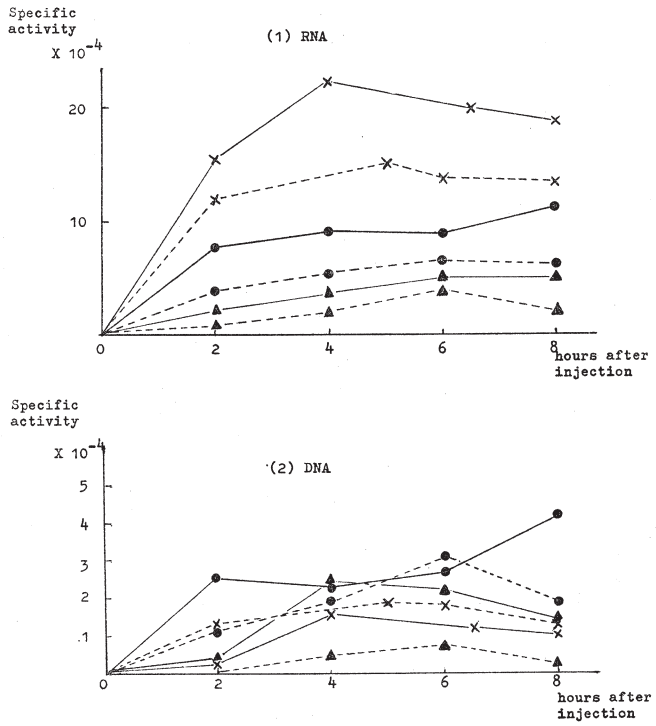

FIG. 11. Incorporation of P^{32} into RNA and DNA of the liver in various dogs.

●——● Normal dog
●- - - -● Normal dog with partial hepatectomy
▲——▲ Eck fistula dog (2 weeks)without partial hepatectomy
▲- - - -▲ Eck fistula dog (7 months) without partial hepatectomy
×——× Eck fistula dog (2 weeks) with partial hepatectomy
×- - - -× Eck fistula dog (13 weeks) with partial hepatectomy

the hepatectomy showed a lower turnover of P^{32} than the other without the hepatectomy.

Whereas, the rate of incorporation of P^{32} into DNA did not differ significantly among the animals studied, except one with earlier shunt, as shown in Fig. 11-2. It appears however that the shape of each DNA curve mostly resembles each of the RNA curves of the corresponding animals presented in the above figure.

6. Histologic studies.

The livers of the animals with and without Eck fistula were microscopically studied before and 2 months after partial hepatectomy. Since mitoses of parenchymal cells were very few in each specimen of the 2 months' late stage of regeneration, no specific consideration was taken concerning mitotic figures of the regenerating liver, and, accordingly, based on the criteria listed in Table 5, the specimens were examined.

The livers of the dogs with normal hepatic circulation demonstrated no significant alteration after partial hepatectomy, except the size of the parenchymal cells seemed to be enlarged and hypertrophic (Fig. 12-a and Fig. 12-b). However in dogs with simultaneous shunt and hepatectomy, structural deterioration of the liver 2 months after the operations became manifest. Namely the parenchymal cells were diffusely atrophic, and there were detected fat vacuoles, small foci of necrotic cells, or cells in degenerative process, in non-specific location (Fig. 12-c).

The liver of the animals in the shunt and delayed hepatectomy groups already manifested atrophic and degenerative changes in various degrees before partial hepatectomy, though some livers retained almost normal appearance. These degenerative changes did not substantially differ among the groups and

TABLE 5. Histologic Alterations of the Liver in Dogs with (1) Normal Hepatic Circulation and with (2) Eck Fistula, before and 2 Months after Partial Hepatectomy

	(1) Normal hepatic circulatoin		(2) Eck fistula									
			Simultaneous		2 weeks		4 weeks		12–13 weeks		9–12 months	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Congestion	−	−	−	−	−	−	−	−	−	−	−	−
Atrophy of parenchymal cells	−	−	−	+	+	+	++	+	+	+	+	+
Fatty accumulation	−	−	−	±	++	+	++	±	+	+	++	+
Degenerative changes	−	−	−	+	+	−	+	±	+	−	+	−
Cellular infiltration	−	−	−	−	−	−	−	−	+	+	−	−
Fibrosis	−	−	−	−	−	−	−	−	−	−	−	−
Hemorrhage	−	−	−	−	−	−	−	−	−	−	−	−

− none ± mild + moderate ++ severe

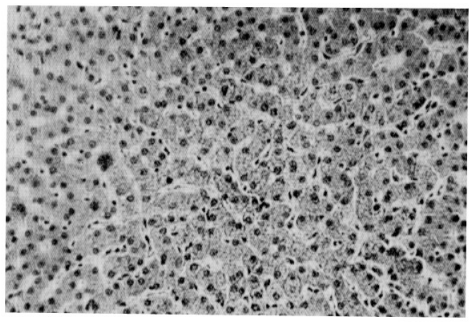

FIG. 12-a

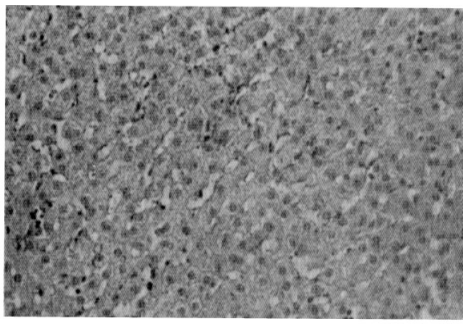

FIG. 12-b

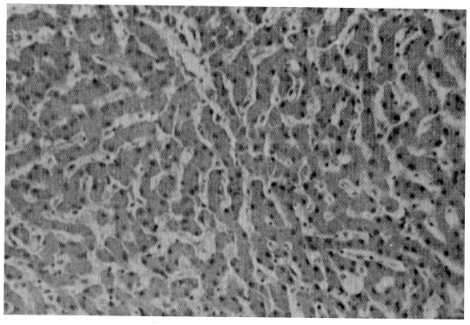

FIG. 12-c

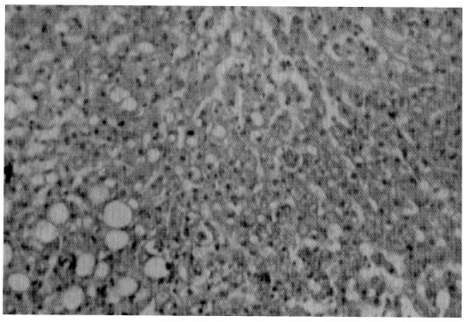

FIG. 12-d

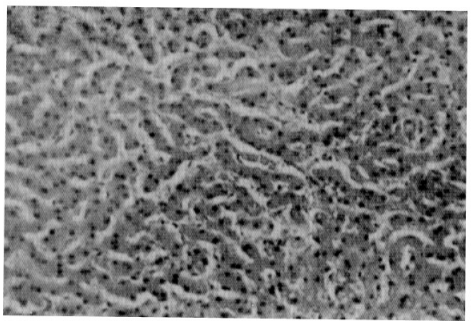

FIG. 12-e

FIG. 12. Representative microscopic sections from dogs with normal hepatic circulation and dogs with Eck fistula.

a) Normal liver before partial hepatectomy (Lx-11).
b) Normal, regenerating liver 2 months after partial hepatectomy (Lx-11).
c) Liver 2 months after partial hepatectomy in a dog with simultaneous shunt and partial hepatectomy. Note cellular atrophy (Lx-27).
d) Liver in a dog with Eck fistula existing for 12 months. Note diffuse fatty vacuoles and atrophy of parenchymal cells (Lx-28).
e) Liver of the dog of d), (Lx-28), 2 months after partial hepatectomy. Note diminution of fatty changes and improvement of cellular degeneration.

Hematoxylin-eosin. ×150.

no tendency of progressive deterioration along with the length of the period after the shunt was found. However, it was evident that all these abnormally appearing livers improved, to a certain extent, their histologic alterations during regeneration; namely, the parenchymal cell cords became evident; though these cells still remained small in size, or, in other words, atrophic; the cytoplasm became dense; the chromatin of nuclei increased; diffuse fatty metamorphosis was lessened or localized; and cells in a necrotic or degenerative process were scarecely encountered. These changes are representatively demonstrated in Figs. 12-d and -e; the histologic specimens of the livers before and 2 months after the hepatectomy in a dog, Lx-28, with the hepatectomy 12 months after the shunt.

DISCUSSION

Now it has long been known that the liver has a remarkable regenerative capacity,[75] and that when a part of this organ is ablated,[76] or this organ undergoes a massive necrosis in some pathologic process,[77] regeneration, or more precisely, compensatory hypertrophy of the hepatic remnant will ensue. Furthermore, this regenerative process of the liver is known to be modified by numerous factors; diet,[95~98] endocine deficiencies,[132,133] biliary obstruction,[78,79,131] cirrhosis and altered blood supply to the liver.[37~45] Above all, the effect of alteration in hepatic blood supply on liver regeneration has been the aim of studies performed by many investigators, since in recent years portacaval anastomosis, which diverts portal blood into the vena cava, has frequently been employed in patients with bleeding esophageal varices, and with ascites.

In 1920, Rous and Larimore,[78] observed that the lobes of the liver, when its draining portal branches were ligated, atorphies. In 1922, Mann and Magath[37] found, "accidentally" as Mann used the term,[79] that diversion of portal blood by Eck fistula in dogs caused a marked atrophy of the liver to about one half of its normal size, and that after removing approximately 70 per cent of the liver, the Eck fistula animals did not show any regeneration, or not to the extent to which it occurred with the portal circulation intact. Since these pioneer works, there have been performed a number of investigations, all of which resulted in an agreement that without portal blood the liver atrophies and fails to regenerate after partial hepatectomy.[38~41,45] Grindley and Bollman in 1952[40] reported the significance of venous filling of the liver for good regeneration to occur after partial hepatectomy. In the successive years, Child and associates,[43] and Fisher and associates[44] clearly demonstrated that portal blood per se is not essential for liver regeneration, but is required as blood, or in other words it is essential for the maintenance of hepatic blood flow in order to accomplish normal regeneration.

Contrary to those workers who have advocated an atorphy of the liver and the abscence of regenerative capacity of this organ in Eck fistula animals, there have been published a number of reports, stating that dogs with Eck fistula showed no symptoms, or had only minor symptoms, and maintained

apparently normal lives.[48) 49) 60~67)] Also in clinical practices, the facts which accord with the foregoing experiments have recently been accumulated by the studies on long term observations of the patients operated on with portacaval anastomosis, except those patients having severe hepatic impariment, detected prior to the shunt.[9) 10) 19) 22) 25) 26) 81) 82)] Furthermore it is well acknowledged that the regenerative nodules of liver cirrhosis have a marked regenerative capacity, though its portal blood supply is severely impaired, and that the hypertrophy of these nodules tends to further disturb hepatic circulation by compressing in- and out-let venous systems.[83)84)]

Likewise in experimental cirrhosis with CCl_4 in rats, the facts which are against the observations of Mann and associates[85)] and Cameron and Karunaratne,[86)] have been accumulated, indicating that the cirrhotic liver, even advanced, performed a well recovery from a partially-hepatectomized state.[87~90)]

On reviewing the literatures, therefore, it seems reasonable to consider that the true nature of the effect of diversion of portal blood on the liver is not yet fully clarified. In 1950, Williams[91)] pointed out that in those experiments of partial hepatectomy and the following hepatic regeneration in animals with altered portal circulation,[37~41)] adequate controls were not used to assess the effect of portal deprivation on the otherwise intact liver. In 1955, Weinbren[92)] claimed that in these experiments a comparison should not be made between liver tissue after simple partial hepatectomy and that after partial hepatectomy together with portal deprivation. He concluded, by using the rats with only ligation and diversion of a branch of the portal vein, as the controls, that deprivation of portal blood supply does not impair its ability to regenerate after partial hepatectomy, while this diversion of portal blood induced a considerable atrophy of the liver. His observation in rats accords with our results presented in this paper.

Our results indicate that the dogs with established Eck fistula still maintained hepatic regenerative activity after 40 per cent partial hepatectomy, although the livers of these dogs had already become small after the creation of the shunt, and at this time the reduction of the hepatic weight was almost 30 per cent, in average, in terms of per unit body weight. However, it was ascertained that when a partial hepatectomy performed immediately after creating an Eck fistula, the dogs thus operate failed to compensate the loss of the liver 2 months after the hepatectomy and the hepatic remnant was weighed almost equal to, or even under its estimated weight at the time of the hepatectomy. Therefore, if the regeneration rate was calculated, this resulted -10 per cent, in average, which means the liver weight at autopsy was -10 per cent less than the estimated weight of the hepatic remnant at the time of partial hepatectomy. At this moment, however, we can not simply conclude that no regeneration occurred in this group of dogs, as some workers reported.[43~45)]

On this occasion it is worth while to evaluate our results in regard to the factors which possibly affect the experiments of hepatic regeneration. Firstly, as to the extent of partial hepatectomy, Fishback wrote that the compensatory hypertrophy of the remnant liver was more rapid when larger amounts of

hepatic tissue were removed.[93] In the reported experiments in hepatic regeneration and Eck fistula, using dogs, 70 per cent,[37,42,98] 4/7,[43] 40 per cent[38,39,44] partial hepatectomies were performed. While the extent of removal varies in these experiments, the results are comparable, provided the control figures are obtained from the results of the hepatectomies on the normal livers. In addition, considering the large surgical trauma caused by extensive partial hepatectomy, 40 per cent resection like our study is considered appropriate to assess hepatic regeneration.

Secondly, the interval between the partial hepatectomy and the sacrifice should be discussed. According to Fishback,[93] the restoration of the normal canine liver was complete from 6 to 8 weeks after partial hepatectomy. However it was noted in previous experiments in dogs the intervals employed were very varied, or in some seemed too short to attain complete restoration. In two of the early reports of Mann and associates,[37,38] were made no definite statement concerning the intervals. Mann[39] in 1940 sacrificed the animals in from 15 to 46 days after the partial hepatectomy, but this study was done to determine the conditions of the liver at various periods of time after partial hepatectomy. In 1952, however, Grindley and Bollman,[42] who did portal deprivation by constricting the portal vein, took from 30 to 120 days after partial hepatectomy. Of 6 dogs with simple portal constriction which survived at least 30 days after the hepatectomy, 4 died, or moribund when killed. The dogs with portal constriction and the other combined operations survived the hepatectomy in good general condition and did excellent hepatic regeneration. Of these 16 dogs, 7 were killed after from 60 to 120 days, 4 were killed after from 31 to 35 days, and the remaining 5 were killed at uncertain periods. In the report, made by Child and associates,[43] one dog with Eck fistula and following partial hepatectomy died at 27th day after the latter operation, manifesting mild biliary cirrhosis. In their report this one poor dog constituted the Eck fistula group of the experiment. However, 8 dogs with portacaval transposition did well after the hepatectomy and were sacrificed from 35 to 60 days after the latter operation. Fisher and associates[44] in 1955 evaluated hepatic regeneration uniformly in 30 days period after partial hepatectomy. It can easily be speculated that in these improper conditions, Eck fistula animals might well have shown poor hepatic regeneration when parts of this organ were ablated.

As described by Islami and associates[88] and Rabinovici and Wiener[89] in the experiments with cirrhotic rats, the process of hepatic regeneration in Eck fistula dogs is assumed to lag behind that of dogs with portal circulation intact. Therefore it is considered that validity of the foregoing observations might be quenstioned in regard to the length of the interval between partial hepatectomy and sacrifice, during which hepatic regeneration had occurred. Therefore in our study the autopsy was made, unifromly in all dogs, 2 months after partial hepatectomy, and the dogs which had died before the scheduled date of autopsy were excluded from the results of the experiments.

Next, nutritional considerations. It has been ascertained that differences in the constituents of diet affect the rate of hepatic regeneration.[95~98] However,

no special consideration of diet has been taken in the works of previous investigators.[38) 39) 43) 44)] Therefore, the dogs in this experiment were not fed with any enriched food throughout the test period.

As stated before, rate of regeneration may vary, depending on the controls used. In this study the estimated weight of the hepatic remnant at the time of partial hepatectomy was used as the control for the calculation of the regeneration rate. Therefore, the controls of dogs with shunt and delayed hepatectomy were the small postshunt livers which indicated the effect of portal deprivation on the liver, and in this way the true amount of regenerated liver was obtained, as suggested by Williams[91)] and Weinbren.[92)] Accordingly the rate of regeneration thus calculated could sufficiently be appraised when compared to that of dogs with normal hepatic circulation. Recently, Fisher and associates[108)] performed 70 per cent partial hepatectomy in rats with Eck fistula existing 28 days prior to the former operation, and they observed 60.6 per cent of the removed liver had been restored. This figure accords with those obtained in our study.

However, in the group of dogs with simultaneous shunt and hepatectomy, the control used for the calculation of regeneration rate was the intact liver without the shunt, and the averaged regeneration rate was −10 per cent, which corresponds to those obtained by Child and associates[43)] and Fisher and associates.[44)] While they concluded on the basis of such figures that no regeneration occurred, yet it should be criticized as to whether the liver atrophied or its regeneration was reduced. From the studies mentioned above it is apparent that the control used in this particular group was inadequate. Since Mann[39)] observed approximately 50 per cent atrophy of the liver after creating Eck fistula, the following assumption may be possible that the hepatic remnant of these dogs would be one half of the original mass that had been estimated from the weight of the removed lobes. Accordingly if no regeneration would have occurred in these simultaneous shunt and hepatectomy animals after 40 per cent partial hepatectomy, the remaining liver should posses only 30 per cent of the original mass. As the figure, −10 per cent, obtained in this study indicates that the remaining liver at autopsy maintained 56 per cent of the weight of the original mass, the estimated gain of the liver would be 26 per cent. When the calculation would be made like other groups of this study, the regeneration rate should be 65 per cent. It is of interest to note that this figure is almost similar with the figures of the dogs with shunt and delayed hepatectomy.

The next item to be discussed is the interval between Eck fistula and partial hepatectomy. It is a fact beyond any doubt that the liver undergoes a marked, atrophic change after diversion of portal blood. Mann reported a decrease in size of approximately one half of the original weight,[39)] and Silen and associates[52)] observed the average liver weight per kg of body weight was reduced about 30 per cent, which accords with the figures obtained in this study.

However, contrary to the observations made by some investigators,[52) 55)] we failed to detect any progressive deterioration of hepatic function in Eck

fistula dogs before partial hepatectomy. In line with our observations Nakashio[62] described that Eck fistula dogs preserved hepatic function quite well, although in some cases being subnormal, and the condition of these dogs had been stable during the 120 days' following-up. Miyao[63] noticed a gradual improvement in the microscopic findings of the liver when dogs survived more than one month after the creation of Eck fistula. Mikami and associates[99~101] revealed, using electromicroscopic technics, that until 12th day after the shunt, some progressive, degenerating changes were manifest, but after 4 weeks after the shunt, signs of cell restoration began to appear. Tokumaru noted in his studies on serum protein and various hepatic function tests in Eck fistula dogs, that impairment of hepatic function as a whole tended to be recovered about 50 days after creation of the shunt.[118] Therefore, it can easily be assumed that when partial hepatectomy is performed on dogs with Eck fistula after the liver have tolerated the immediate deteriorating effect of the shunt, the results would be that the hepatic regeneration in these dogs is facilitated more than that of the dogs with simultaneous shunt and partial hepatectomy. The results of our present studies appear to have proved this assumption valid. Namely the mean regeneration rates in dogs of the earlier hepatectomy group, including dogs with simultaneous shunt and partial hepatectomy and dogs with the hepatectomy 2 weeks following the shunt, were lower than the dogs of the later hepatectomy group, including dogs with the hepatectomy more than 4 weeks after the shunt. Yet it is clear that those with simultaneous shunt and partial hepatectomy did most poorly in all respects during the test period.

These experimental evidences, together with those of others, indicate the hepatic regeneration in dogs with Eck fistula after partial hepatectomy, is influenced by the interval between the shunt and partial hepatectomy, not only because of the types of the control used, but as the interval between the shunt and the hepatectomy during which the function of the liver to accommodate the altered blood supply can be restored. However, since in this study the animals were followed up to 2 months after partial hepatectomy and hence this length of time might cover the period during which the effect of the shunt had persisted, we could not definitely indicate the time when this accommodation completed.

Furthermore it has been discussed that the effect of Eck fistula on the liver is greatly altered by the presence of hepatopetal collaterals which have developed after creating the shunt.[102~104] Recently Bollman and associates again emphasized the importance of collateral circulation in relation to survival and abscence of symptoms in Eck fistula animals.[105] They stated those dogs with Eck fistula which survived without manifesting Eck fistula syndrome were found their shunt closed, or portal collaterals entering the liver, or portosystemic collaterals present. Since the two stage operative procedures in this study would inevitably be followed by extensive adhesions between the liver and the other abdominal organs, it becomes nesessary to assess the results of the present study in view of whether or not the regeneration of the liver in Eck fistula dogs might be facilitated by the existing hepatopetal collaterals.

However, we consider the effect of these collateral circulation were insignificant in the evaluation of our results, because of the following two reasons.

The first is that for the Eck fistula group of this study were selected only those dogs in which the stoma between the portal vein and the inferior vena cava was identified patent at autopsy, and therefore, this uniform patency of the stoma is believed to minimize the development of hepatopetal collateral channels. Bollman stated in his extensive monograph on "The animal with an Eck fistula" that when this shunt was made after two stages occlusion of the portal vein, the dogs survived without symptoms, and that splenoportography done 6 months later, revealed the patent stoma and none of the injected medium entering the liver.[106] His observations might be interpreted in the way that when an Eck fistula is made in the presence of well established collaterals, the portal blood flows towards the anastomosis, namely in the hepatofugal direction, rather than hepatopetal, and therefore we consider an increase of portal blood by way of the collaterals after Eck fistula formation might be insignificant, provided that the anastomosis remains patent.

The second reason is that hepatic blood flow determined by the colloidal Au^{198} method was grealty reduced in the shunt animals compared to those with normal hepatic circulation, and that when the regeneration was examined two months later, this reduction was not altered. If a large amount of portal blood would regain the way into the liver via hepatopetal collatetals and would result in augmenting hepatic regeneration, the total hepatic blood flow must have been increased at the time of sacrifice. In this, however, since the colloidal Au^{198} method determines both arterial and portal blood flows, en bloc, here remains a possibility that the blood from the portal region might be included in the hepatic blood flow determined by this method. However, in veiw of the facts hitherto obtained by some workers, [107) 120~122)] it seems quite apparent that the arterial blood may contribute to the maintenance of the hepatic blood flow in these shunt and hepatectomy animals.

Now it is difficult to explain the existnece of some variability in the figures of hepatic regeneration both in normal and shunt groups. But this character of results of these experiments was also encountered in the findings of others.[43~45] At first we supposed that regeneration of the liver might be affected by some seasonal factor, but this was proved not plausible, since the lower figures were not restricted either in a series done from midspring to summer, or in those from early fall to winter.

From the preceding sections of discussions it will be justified that removal of parts of the liver in animals with Eck fistula is followed by a considerable degree of regeneration of the remnant tissue. It appears also that portal blood per se is not essential for the regeneration of the liver, and that restoration of hepatic tissue occurs in fairly close relationship with that of hepatic blood flow, and this is in keeping with that of others.[42~44]

In 1920, Rous and Larimore[78] observed that atrophy of the liver after partial ligation of the portal tributaries had been prevented to some extent by combined ligation of the draining bile ducts. On the ground of their findings, they stated that the nature of the atrophy following portal depriva-

tion was not of an absolute character, but of a conditional, depending on the types of the portal deprivation, and that the atrophy of the liver in animals with Eck fistula was of the latter character. Their idea seems to be suggestive of the possible occurrence of hepatic regeneration in the Eck fistula animals after partial hepatectomy.

In view of portal blood being the prime factor for hepatic regeneration, Mann[79] in 1944 postulated that the physiologic stimulus for the restoration of the liver originated in the tissues drained by the portal vein and was in amounts too small to be effective when this was diluted in the general circulation. Instead, we are of the opinion that if some stimuli would exist, although there remains a controversial problem concerning a humoral factor for hepatic regeneration,[108)126~130)] this would not be too small in effect.

Now, here arises a question how the regenerating liver, while its portal supply deprived, is functioning.

It was revealed that BSP retention at the time of sacrifice was improved in the regenerating canine liver with intact portal circulation. Whereas, in the shunt animals, particulary with the earlier partial hepatectomy, clearance of the dye tended to be retarded at the time of sacrifice but in the shunt animals with the later partial hepatectomy, the clearance was, though subnormal, well maintained, as compared with the prehepatectomy figures.

This impariment in the dye clearance may be ascribed either to decrease in blood flow,[52] or to intrinsic deterioration of hepatic function.[51] Yet, both factors are closely related, and hence we cannot say which factor is of prime importance.

It has been shown that Eck fistula formation reduces hepatic blood flow approximately 50 per cent of the normal value.[53~55] Our previous studies,[109] using the Au^{198} method, revealed that the hepatic blood flow in 6 normal dogs ranged from 0.251 to 0.312 min^{-1} with the mean 0.284 min^{-1}; in 5 dogs that had survived from 2 to 4 weeks after Eck fistula, the blood flow ranged from 0.126 to 0.178 min^{-1} with the mean 0.165 min^{-1}; in 8 dogs that had survived from 5 to 13 weeks after the shunt, it ranged from 0.133 to 0.187 min^{-1} with the mean 0.163 min^{-1}; and in 2 dogs that had survived 6 and 12 months after the shunt, it ranged from 0.136 to 0.165 min^{-1} with the mean 0.150 min^{-1}. The averaged reduction throughout the groups was calculated 44 per cent of the original volume. Restrepo's group[55] noted a permanent slow drop of the curve on which the postshunt figures were plotted in a series of dogs. On the contrary, Shinoda[117] reported a gradual increase of hepatic blood flow after having received a sharp drop at the time of shunting.

In spite of that the hepatic blood flow was severely reduced in all dogs with Eck fistula also in our experiments, after 2 months following 40 per cent partial hepatectomy, the blood flow of these dogs was well maintained, suffering only a slight reduction, particularly in the dogs with shunt and delayed hepatectomy. It is noted that dogs with simultaneous shunt and hepatectomy manifested the lowest figure, which may cause other functional derangements. Furthermore the comparison made in this study between restoration of hepatic tissue and that of hepatic blood flow revealed that these two factors were

closely related. Therefore, it is believed that these alterations in hepatic blood flow after partial hepatectomy also give support to the evidences that a certain degree of hepatic regeneration have occurred in Eck fistula animals. Recently, Rabinovici and Vardi, likewise, found that the CCl_4 cirrhotic liver in rats underwent 100 per cent increase of hepatic blood flow after 70 per cent partial hepatectomy.[110]

In 1944, Mann stated[79] that the restoration of hepatic tissue occurs primarily in order to maintain the portal pathways and the restoration of functioning hepatic tissue is secondary. Now that portal blood per se appears not primarily necessary for the regeneration of the liver, his statement might well be changed to "in order to maintain the arterial pathways". But, considering that the regeneration occurs immediately after parts of the liver are removed, or one such as nodular hyperplasia in cirrhosis occurs in the presence of circulatory derangements, it can be speculated that some functional necessity, if exists, might trigger these phenomena to occur.

It is well known that during the hepatic regeneration the concentrations of RNA and DNA, particulary the former, in the liver increase markedly in the early phase of this process.[112~114] In our study, however, the RNA concentrations, when expressed in terms of per unit weight of fresh tissue, did not differ greatly between the figures before and 2 months after partial hepatectomy. This is probably because the follow-up period of the experimental dogs was too long to detect an active phase of RNA metabolism which has been stimulated by partial hepatectomy, since Fishback wrote in his report on hepatic regeneration that two months after partial hepatectomy, a section of the regenerated liver could not be distinguished from that of the normal liver.

However, it is of interest to note that the total amounts of RNA in the livers of the dogs with Eck fistula decreased markedly, compared to those of the normal dogs, and nevertheless, 2 months after 40 per cent partial hepatectomy these amounts were restored nearly to the prehepatectomy levels. Considering the RNA concentration per unit weight of the liver was well preserved, this decrease in the total amounts of this substance in the shunt animals is essentially due to the reduction of hepatic tissue mass.

The results of analysis of DNA concentrations per unit weight of the liver showed that although some variations existed, this substance tends to have been increased in the shunt and earlier hepatectomy dogs, particulary in their posthepatectomy period. In the dogs with simultaneous shunt and hepatectomy, this increment was most marked, and, hence, this marked increase of DNA per unit weight of liver tissue is believed to have caused only a slight reduction of the total amounts of this substance in the liver after 40 per cent partial hepatectomy and its successive regeneration. Since DNA can be taken as a convenient reference substance indicating per one nucleus or per one cell,[115,116] these experimental evidences indicate that the averaged size of the regenerating liver cells in these dogs are smaller than that of the normal cells, and that this reduction in cellular size is assumed to be resulted from an atrophic change of this organ as observed in the histologic studies. The reduction in

the ratio of RNA/DNA observed in the shunt animals with the shunt and earlier partial hepatectomy can be ascribed to this small cellular size, indicating their function subnormal.

Yet, in the dogs with shunt and delayed partial hepatectomy, the DNA concentrations per unit weight of the liver before and 2 months after the hepatectomy were rather varied. but did not substantially differ from the figures in the dogs with normal hepatic circulation. This might be regarded as that the tissue constituents of these livers were similar with the normal hepatic tissue. But, on observing in the histologic specimens that the size of the hepatic cells in these shunt and delayed partial hepatectomy dogs was relatively smaller than that of the normal hepatic cells, it can be speculated that the figures representing the DNA concentration per unit weight of the liver may be affected by other tissue component, such as fat. Whereas, the total amounts of DNA in the liver in these dogs were well recovered almost to the prehepatectomy levels at the end of the test period. The figures in the total DNA indicate that a certain amount of hepatic tissue mass has been restored to increase the weight of the hepatectomized small liver nearly to the prehepatectomy weight.

It is noted that in the dogs with normal hepatic circulation the concentration of DNA per unit weight and the total amount of this substance were increased during the test perod. The former however was not marked, but the increase of the total amount of DNA was very prominent, exceeding the prehepatectomy level by almost 30 per cent of the original weight. This coincides with the striking increase of hepatic tissue mass 2 months after the partial hepatectomy.

At any rate, the present observation on the changes in DNA concentrations were made at the late stage of hepatic regeneration, and hence such peculiar changes as observed by others in the immediate posthepatectomy period[113) 114)] could not be detected.

Since early observation of G. H. Whipple and associates,[48) 49)] it has been known that Eck fistula animals manifest some impairment of protein metabolism. Tokumaru observed the soluble protein fraction of the canine liver was decreased greatly after creation of Eck fistula, and a concomitant reduction of serum protein occurred.[118)] Furthermore he noted the former began to be restored from 1 months after the operation, and the restoration of the latter lagged behind one month later, and the occurrence of the latter restoration was more gradual than the former. Anyhow, it is almost surprising that the Eck fistula liver with its limited blood supply can carry on so effectively its metabolic duties, even when partial hepatectomy was performed. For this vital activity of the liver, one may simply say that this can be attributed to a great "reserving" capacity of this organ.

However, our results in examining P^{32} incorporation into the hepatic RNA and DNA may give some clue as to the understanding of this problem. Namely, incorporation of P^{32} into the RNA of the regenerating liver of Eck fistula dogs was accelerated as compared with the normal liver, whereas incorporation of P^{32} in case of simple Eck fistula dogs remained subnormal.

The fact that the figures of the P^{32} incorporation in the shunt dogs with partial hepatectomy were higher than those without, may indicate the possibility that some stimulating effect caused by removing parts of the liver were working in the shunt dogs with the hepatectomy.

It was difficult to understand that the P^{32} incorporation in case of hepatectomized, normal dogs fell below the figures of non-hepatectomized, normal dogs. However, it can be speculated that this might be indicative of a supranormal character of the regenerated normal liver, *i.e.*, an excessive regeneration in which the function per unit amount of the hepatic tissue is inversely kept being subnormal. These interpretations of our findings in P^{32} incorporation becomes most plausible, when refered to the works of Munro and associates.[119] They noted an increased rate of P^{32} turnover into RNA of the liver when reduction in total amount of liver RNA had been caused by removal of protein from the diet, and this reduction was counterbalanced by the increased rate of turnover, so as to maintain the amount of RNA synthesized.

In line with these interpretations of the experimental results, if one could speculate further, the fact that the increase of P^{32} incorporation was enhanced more in the shunt and earlier hepatectomy dog than the one with shunt and later hepatectomy, might be to compensate the impaired function which is found more in the former than the latter.

Since in this study the other fractions, such as the acid-soluble and lipids fractions, obtained by the method of Schmidt and Thannhauser[73] were not examined, and moreover, the RNA per se obtained by this method contains phosphorus compounds other than nucleotides in almost 25 per cent,[124] the precise nature of these hyper- and hypo-functions in Eck fistula animals may be clarified by further studies.

On the other hand, from the studies on P^{32} incorporation into DNA of the liver we could not deduce any definite character of the metabolism among the dogs This might be due to the fact that the interval of examining the regeneration was too long to detect an active change of this substance which is known to exist in the earliest phase of regeneration.[115] In fact, Fisher and associates observed an increase of P^{32} incorporation into DNA of the liver in rats with Eck fistula, two days after 70 per cent partial hepatectomy.[108]

The histologic studies on the regenerating livers with and without the shunt could not yield any results which might further elucidate the complexity of the problems in Eck fistula and hepatic regeneration. Miyao[63] described however that the canine liver, when portal blood was deprived by the formation of Eck fistula, underwent marked parenchymal alterations, *e g.*, congestion, atrophy of cell cords, irregular size of cells, small foci of necrosis, etc., up to 2 weeks after the operation. He stated also that when the animals had survived more than 1 months following the shunt, these alterations tended to be improved, and after 6 months, they were hardly detected, except those of slight fatty accumulation and focal degeneration. Yet, his observations do not accord with ours in the point that we observed an atrophic appearance of the liver in dogs with Eck fistula which had remained stable for 12 months

or longer after the shunt.

It should be noted, however, that all the liver with portal blood deprived, except dogs with simultaneous shunt and partial hepatectomy, showed a conspicuous recovery in its morphologic appearance 2 months after partial hepatectomy, and this finding well agrees with those obtained in the other functional studies of this paper.

Now, when viewed from the clinical standpoint, the afore-mentioned experimental results seem to suggest that when a portacaval anastomosis is performed on such patients that their livers have accomodated to the long-existing portal obstruction and have acquired a compensatory increase of arterial blood flow, this operation can well be tolerated, and, accordingly, favorable postoperative results can be expected. Imanaga,[17)18)] has claimed that the patients with portal hypertension due to intrahepatic portal vein obstruction have good postoperative courses after end-to-side portacaval anastomosis. In these patients, he stated, the portal inflow to the liver has been blocked at the presinusoidal level for a long time, and the ratio, hepatic arterial versus portal inflows is reversed, while the total hepatic blood flow is kept in the normal range.

The results of the present study seem to provide some rationale in employing portacaval anastomosis as a treatment of choice for patients with bleeding esophageal varices due to intrahepatic portal vein obstruction.

SUMMARY AND CONCLUSION

In this report, the regeneration of the liver in dogs with Eck fistula was reevaluated by performing 40 per cent partial hepatectomy in dogs with normal hepatic circulation, with simultaneous shunt and partial hepatectomy, and with partial hepatectomy in various later intervals following the shunt. The results were as follows:

1. The liver in dogs with already preexisting Eck fistula regenerated considerably after partial hepatectomy, though portal deprivation consistently resulted in an atrophy of the liver. The regeneration rate in these dogs 2 months after partial hepatectomy ranged from 60 to 86 per cent in the mean figures. Effects of the differences in the intervals between the shunt and partial hepatectomy, ranging from 2 weeks to 12 months, were not marked, but a slight increasing trend in the mean regeneration rates was observed, as the interval became longer. Whereas, all the livers with normal hepatic circulation exceeded the original weight, the mean regeneration rate being 145 per cent.

2. When partial hepatectomy was performed at the same time with creation of Eck fistula, the remnant liver 2 months following these operations was almost the same in weight as the original remnant at partial hepatectomy, and the mean regeneration rate was −10 per cent. However if an assumption was attempted to employ a theoretically adequate control for these dogs, this figure was still suggestive of the possibility that some degree of regeneration had occurred in these canine livers.

3. Studies on BSP clearance and hepatic blood flow before and 2 months after partial hepatectomy revealed that in dogs with preexisting Eck fistula, these functions of the liver were recovered nearly to the prehepatectomy level after the hepatectomy, and that, however, in dogs with simultaneous shunt and partial hepatectomy, these functions were greatly depressed compared to the prehepatectomy levels. Whereas, in dogs with normal hepatic circulation, the posthepatectomy figures in these studies were found to be exceeding the original figures. It was noted that there was a close relationship between the restoration of tissue mass and that of blood flow in the shunt dogs.

4. The RNA concentration per unit weight of the liver tissue was not altered significantly by creation of Eck fistula and partial hepatectomy. The DNA concentration per unit weight of the liver tissue tended to be elevated in dogs with Eck fistula, suggesting the reduction in cellular size; namely an atrophic character of the liver in these dogs. The total amounts of RNA and DNA in the liver decreased remarkably in the shunt dogs, in proportion to the decrease in hepatic tissue mass, but were well maintained after partial hepatectomy.

5. The evidences obtained by examining P^{32} incorporation into the RNA of the liver seem to suggest that the decrease in the total amount of RNA in the liver of the shunt dogs was counterbalanced by an increased rate of metabolism which had been triggered by the process of regeneration. Studies on P^{32} incorporation into DNA failed to produce any definite results.

6. In microscopic specimens, the histologic appearance of the liver of the shunt animals was improved 2 months after partial hepatectomy, except those with simultaneous shunt and partial hepatectomy in which degenerative changes became manifest, like other shunt dogs, 2 months after the two simultaneous operations.

7. Through discussions on the factors influencing the results of the experiments of hepatic regeneration after partial hepatectomy, those foregoing evaluations of the experimental figures proved validated.

In conclusion, though Eck fistula always produces a considerable degree of atrophy of the liver, the results of this study indicate that the liver does not lose its regenerative activity in the presence of Eck fistula, and this has a certain clinical implication especially when a portacaval anastomosis is attempted.

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