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CITATION:

ISSUE DATE:
1968-07-01

URL:
http://hdl.handle.net/2433/207473

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Effects of Hepatic Periarterial Neurectomy upon Hepatic Blood Flow and Regeneration of the Liver after Partial Hepatectomy in the Dog

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Received for Publication April 24, 1968

INTRODUCTION

Manipulation of the hepatic nerves in surgical practice has rarely come into question, while it is anatomically well established that the liver has an abundant autonomic nervous supply. One reason seems that little is known concerning the roles of the hepatic nerves in liver physiology.

Recently, however, many investigations have called attention to the possibilities that nervous factors may play a part in precipitating some liver injuries.

Since a presumptive work of Calvert and Brody, it has been suggested that the characteristic changes of the liver after administration of carbon tetrachloride result from a massive sympathetic discharge through the innervation of the liver and the adrenal medulla, and that interruption of these nervous stimuli offers some relief of such toxicity.

In a clinical approach, Mallet-Guy has advocated peri-hepatic artery neurectomy as a surgical treatment of chronic hepatitis with promising results. On the basis of wide experimental works, he concludes that the effect of this procedure must be due to metabolic changes in the liver, especially in respect of lipids.

Another importance of this neurectomy consists in hemodynamic effects, as recently emphasized by McDermott and his associates: Ackroyd and Mito demonstrated an increase in hepatic blood flow following hepatic periarterial neurectomy, which showed a favorable effect on survival of the dogs with portacaval shunt; Hori and Austen noticed a deleterious effect of crush stimulation of the hepatic nerve upon the hepatic and renal circulations.

These observations have encouraged further clinical trials of this operation, though the true nature of the innervation of the liver still remains much to be discussed.

The above situation led to the present investigation, which was designed to make clear the possible influences of hepatic periarterial neurectomy upon hepatic blood flow, and in this connection, upon liver regeneration after partial hepatectomy. And a supporting anatomical study of the hepatic periarterial nervous plexus was added for better understandings of the consequence of this neurectomy.
ANATOMICAL AND HISTOLOGICAL OBSERVATIONS OF THE HEPATIC PERIARTERIAL NERVOUS PLEXUS

For the accurate interpretation of the proposed experiments, it is required to confirm which nervous component is to be involved in hepatic periarterial neurectomy.

The collected anatomical informations\textsuperscript{12-16} are summarized as follows:

The liver is supplied by the sympathetic and the parasympathetic nerves, which form the anterior and the posterior hepatic plexus near the hepatic portal. The former is composed of sympathetic postganglionic fibers arising from the left celiac ganglion, and joined by preganglionic rami of the left vagus branching in the vicinity of the cardia. It lies in close association with the hepatic artery. The latter consists of sympathetic fibers from the right celiac ganglion and a branch of the right vagus, which traverses the right celiac ganglion and gives preganglionic fibers to the plexus. It runs along the posterior aspect of the portal vein. The sympathetic and parasympathetic fibers of both plexus are distributed to the extrahepatic bile duct and within the liver. Innervation of the arteries is exclusively by the sympathetics.

HASEBE\textsuperscript{17} described another nervous supply with suprahepatic entrance along the hepatic vein.

Thus hepatic periarterial neurectomy, usually carried out within the extent of the common hepatic artery, is to involve the sympathetic postganglionic fibers from the celiac ganglia and possibly the preganglionic fibers from the right vagus (Fig. 1).

MATERIALS AND METHODS

Mongrel dogs were used. Sections for histologic studies were excised from various

Fig. 1 Diagrammatic representation of the innervation of the liver in the dog.

- preganglionic parasympathetic
- preganglionic sympathetic
- postganglionic sympathetic
parts of the liver after exsanguination and perfusion with 10% formalin. The following histological staining methods were employed in addition to gross anatomical observations:

2. Heidenhain-Werke's lithium hematoxylin stain in frozen sections for myelin sheaths.
3. Hematoxylin eosin stain.

Fluorescence Method: Small pieces of fresh tissues were dissected and rapidly frozen in isopentane cooled by liquid nitrogen. They were dried in vacuo at $-35^\circ C$ for 7 days, and treated with formaldehyde gas at $80^\circ C$ for 1 hour. During this treatment catecholamines condense with formaldehyde to fluorescent products. The preparations were embedded in paraffin, sectioned and mounted in Entellan (Merck) on non-fluorescent slides for fluorescence microscopy.

Denervation Study: Some experimental dogs were subjected to hepatic periarterial neurectomy. Two weeks after the operation they were sacrificed and treated as above mentioned.

RESULTS

Macroscopic observations in the dog operated on mostly assured the above anatomical informations. The common hepatic artery is accompanied by a dense meshwork of nerve bundles arising from the celiac ganglia. But it seems difficult to discriminate between the anterior and the posterior plexus because of a spatial relationship of the hepatic artery and the portal vein (Fig. 2).

Microscopic figures of the hepatic periarterial plexus showed those of unmyelinated nerves (Fig. 3). Lithium hematoxylin stain failed to find any contribution of myelinated fibers to the plexus.

Fluorescence study demonstrated a noradrenalin-inherent fluorescence in these nerve bundles (Fig. 4). It proves that the plexus contains sympathetic adrenergic fibers. The highly fluorescent terminal fibers of adrenergic neurons are rich in the superficial muscular
Fig. 4 Fluorescence photomicrograph of cross section of the hepatic artery and surrounding nervous bundles, showing a fluorescence due to intraneuronal noradrenalin.

Fig. 5 Fluorescence photomicrographs: (a) Cross section of the common hepatic artery. Note fluorescent adrenergic terminal varicosities in the outer layer of the media. (b) Intrahepatic arteriole with adventitial adrenergic terminal fibers.

Fig. 6 Fluorescence photomicrographs of cross section of the common hepatic artery (a), and a intrahepatic arteriole (b); two weeks after denervation. Compare with Fig. 5 (a, b).
EFFECTS OF HEPATIC PERIARTERIAL NEURECTOMY

layer of the proximal portion of the hepatic artery (Fig. 5 a), and in the adventitia of the intrahepatic arterioles (Fig. 5 b). They are sparsely distributed to the bile ducts and the portal vein.

Two weeks after denervation, the fluorescence of noradrenalin extensively disappeared distal to the neurectomy (Fig. 6 a, 6 b).

COMMENTS

Although Mallet-Guy states that hepatic pariarterial neurectomy is quite distinct from periarterial sympathectomy, the present study suggests that it involves exclusively sympathetic adrenergic fibers, if not extensive to the hepatic portal. It seems more reasonable to expect of this procedure vasomotor influences rather than metabolic ones.

II. CHANGES IN HEPATIC BLOOD FLOW AFTER HEPATIC PERIARTERIAL NEURECTOMY

Many inaccuracies in earlier studies on the vasomotor control of the liver circulation have been corrected by more recent investigations with improved techniques especially in direct flow measurement.

In the following experiments, a sine-wave electromagnetic flowmeter and a Doppler ultrasonic flowmeter were employed and proved sufficiently dependable.

MATERIALS AND METHODS

Operative procedure: Thirty fasting mongrel dogs were anesthetized with intravenous pentobarbital sodium, 5 mg. per kg., and laparotomized. The celiac axis was exposed with the duodenum reflected to the left. The nervous plexus accompanying the common hepatic artery was separated from the adventitia by gentle dissection. This maneuver occasionally resulted in mild to moderate vasospasm of the involved portion with decreased flow, but it usually dissolved within 10 to 30 min. to stable resting flow. Then the separated nerve bundles were divided; in a small number of cases, carbocaine block instead of dividing was tried.

Flow study: The common hepatic artery and the portal vein were fitted with the transducers of a sine-wave electromagnetic flowmeter (Medicon; Model FM-6R) and of a Doppler ultrasonic flowmeter (Ward; Model 1502-System) respectively for flow measurement.

Narrow catheters were inserted into the aorta just below the celiac axis through the femoral artery, and into the portal vein for pressure records.

Volume flow calibration of each transducer was examined by perfusing the excised samples of the corresponding vessels with heparinized canine blood at the appropriate pressure, correlating the perfused volume in cc. per min. with readings of the flowmeter connected to the vessels. But for convenience flow changes were described as a percentage of the resting baseline values, using each animal as his own control.

RESULTS

Changes in hepatic blood flow after hepatic periarterial neurectomy were considerably
inconstant among individual animals. Their responses were roughly divided into three groups.

1. In 13 of 25 experimental dogs, hepatic periarterial neurectomy resulted in immediate increase of hepatic arterial flow to 40 to 50 per cent above normal resting levels, with a gradual moderation eventually to 20 to 30 per cent above normals (Fig. 7a).

2. 8 of 25 animals showed little flow change after the neurectomy but insignificant fluctuations within 10 per cent above and below normals (Fig. 7b).

3. In remaining 4, hepatic arterial flow decreased to 40 to 70 per cent of resting levels with or without a transient initial increase, and within one hour recovered to normal to 30 per cent increase (Fig. 7c).

Portal vein flow was not significantly altered by the neurectomy.

Effects of carbocaine block in another 5 dogs were similar to those of the neurectomy, except that flow changes after local anesthesia were more mild and temporary than after dividing (Fig. 7d).

Augmented hepatic arterial flow was maintained during flow recording for several hours.

COMMENTS

There is much evidence to indicate that stimulation of the hepatic plexus causes a constriction of the intrahepatic vessels. But it is another question whether denervation enhances resting blood flow or not. That depends on tonic vasomotor activity. No direct evidence is available on this point.

Although ACKROYD et al. reported that hepatic periarterial neurectomy in normal dogs as well as in dogs with portacaval shunt produced an increase in total hepatic blood flow due to increased hepatic arterial flow, a partly contrary result was obtained by SMITH et al., who recognized augmented total hepatic blood flow following the same procedure in dogs with portacaval shunt, but not in normal dogs.

The present experiment demonstrated in more than half of the periarterial neurectomized dogs a moderate increase in hepatic arterial flow. In consideration of also the denervation study described in the preceding chapter, it is probable that the vasomotor innervation of the liver is excluded by such procedure. It can be assumed that the inconstancy in the present results represents a difference in tonic vasomotor activity among individuals, besides technical factors in flow measurement, operative procedure and anesthesia; what may account for some discrepancies in estimating the circulatory effect of this neurectomy.

The duration of the neurectomy-effect is maximal in four to six weeks according to the above authors. However, efforts failed to confirm such a statement, because of difficulties in performing a successive flow measurement exactly under the same conditions with the present operative method.

Detailed observations of the flow records suggested that to some extent autonomous vascular tonus took the place of the nervous regulation lost by neurectomy, as an abrupt change immediately after the procedure was mitigated with the lapse of time.

It is likely that these indefinite factors make it difficult to evaluate the net effect of hepatic periarterial neurectomy in its practice.
Fig. 7 (a) Hepatic blood flow following hepatic periaorterinal neurectomy.
Upper trace: Hepatic arterial flow.
Lower trace: Portal venous flow.

Fig. 7 (b)
Fig. 7 (c) Hepatic blood flow following hepatic periarterial neurectomy - Upper trace: Hepatic arterial flow. Lower trace: Portal venous flow.
III. CHANGES IN HEPATIC BLOOD FLOW AFTER PARTIAL HEPATECTOMY ASSOCIATED WITH HEPATIC PERIARTERIAL NEURECTOMY

As a preliminary to the study of liver regeneration, the following experiment was undertaken to determine whether changes in hepatic blood flow after partial hepatectomy could be modified by hepatic periarterial neurectomy.

MATERIALS AND METHODS

In normal and hepatic periarterial neurectomized dogs, the left upper and lower lobes were ligated at the pedicle and resected. A preliminary assessment using nine dogs showed that the resection of these two lobes represented 39.8 ± 3.2% per cent of total liver weight, namely 40 per cent hepatectomy.

Hepatic arterial and portal blood flow were measured by the electromagnetic and the ultrasonic flowmeters respectively as precedingly described. Care was taken lest the fitted flow probes should be disturbed by operative intervention.

RESULTS

Hepatic arterial flow underwent a consistent reduction of 30 to 40 per cent of normal levels with ligation and resection of the two lobes in normal dogs (Fig. 8).

No definite change was observed in portal vein flow except for a little increase in portal blood pressure.

Partial hepatectomy decreased also the increased and non-increased hepatic arterial flow following the neurectomy in the same proportion as shown in the normal dogs. Consequently, the flow reduction in instances with 20 to 30 per cent increase owing to the preceding neurectomy was 10 to 30 per cent of normal preoperative values (Fig. 9).

COMMENTS

As for the flow change after partial hepatectomy, only a few informations by means of estimated total hepatic blood flow are available.

The present study, in which hepatic arterial and portal vein flow were measured separately, revealed that the above hepatectomy resulted in a reduction of hepatic arterial flow approximately proportional to the extent of hepatectomy, without any recognizable change in portal vein flow. It implies that a part of the remnant liver is supplied by a part of arterial flow and more of portal blood, at least in the initial stage of liver regeneration. It is to serve in altering the flow patterns in the hepatic sinusoids, that once was considered of prime importance in the incitation of liver regeneration.

Hepatic periarterial neurectomy does not influence the flow change peculiar to partial hepatectomy.

It is supposed that the minor increase in hepatic arterial flow following neurectomy is of less significance in respect of total volume flow after partial hepatectomy, which reduces the arterial contribution to total hepatic blood flow.

* mean ± standard error
Fig. 8  Hepatic blood flow following 40% hepatectomy.
Upper trace: Hepatic arterial flow.
Lower trace: Portal venous flow.

Fig. 9  Hepatic blood flow following 40% hepatectomy, when preceded by hepatic periarterial neurectomy.
EFFECTS OF HEPATIC PERIARTERIAL NEURECTOMY

IV. REGENERATION OF THE LIVER AFTER PARTIAL HEPATECTOMY AND HEPATIC PERIARTERIAL NEURECTOMY

The possibility that hepatic periarterial neurectomy might affect liver regeneration after partial hepatectomy through its circulatory effects or otherwise, was examined in the following experiments.

MATERIALS AND METHODS

Healthy adult mongrel dogs weighing 7 to 15 kg. were used. They were divided at random into two groups: animals of Group I were subjected to 40 per cent hepatectomy; those of Group II to 40 per cent hepatectomy and hepatic periarterial neurectomy at one stage, in the same manners as precedingly stated. Each removed liver was blotted as free as possible from blood, and weighed.

At the intervals of 2, 14 and 28 days after operation, they were sacrificed and the remnant livers were wholly removed by dividing the hepatic vein, the hepatic artery and the portal vein in this order. Maximal liver exsanguination was attained in this manner. The removed livers were weighed after careful trimming, and tissue blocks for histology were placed in appropriate fixatives.

Postoperative recovery of each experimental dog was judged from its general activity and appetite, though no exact measurement of food consumption was made. Occasionally dogs which failed to eat were encountered. Most of them proved to have had such a complication as hemorrhage, peritonitis or intercurrent infection, and were excluded from the report.

Weight index of liver regeneration: The preoperative total liver weight was calculated by multiplying the removed liver weight with 2.5. Regeneration of the remnant livers was expressed (1) as per cent of the calculated preoperative liver weight, and (2) as per cent of the ratio of liver weight to body weight at operation with the formula:

\[
\frac{\text{Liver Wt.}\div \text{Body Wt. (at autopsy)}}{\text{Liver Wt.}\div \text{Body Wt. (at operation)}} \times 100
\]

Histologic Investigation: Preparations of the parenchymal tissue were stained with:
1. Hematoxylin-eosin in paraffin sections.
2. Sudan III in frozen sections for lipids.
3. Periodic acid-Schiff method in celloidin sections fixed in Carnoy’s solution for glycogen.

RESULTS

Restoration of the remnant liver mass was found to follow grossly the average regenerative process in the partially hepatectomized and hepatic periarterial neurectomized dogs as well as in the non-neurectomized controls. The macroscopic appearance of the livers at autopsy was similar to that of the normal regenerating liver.

But, a tendency was noted that the weight gain in the remnant livers of the neurectomized animals was somewhat greater than in those of the non-neurectomized (Fig. 10).
Fig. 10 Changes in liver weight and body weight after 40 per
cent hepatectomy and hepatic periarterial neurectomy.

At autopsy, the average liver weight in the non-neurectomized dogs was $87.8 \pm 2.6^*$
per cent and $95.8 \pm 5.6$ per cent of the preoperative values 2 and 4 weeks after surgery
respectively, while in the neurectomized it was $92.1 \pm 4.5$ per cent and $101.5 \pm 4.1$ per
cent at the same respective intervals (Table 1).

On the other hand, the body weight loss was less severe in the non-neurectomized
dogs than in the neurectomized, though the preoperative weight was achieved only in a
few instances of both groups (Fig. 10).

The body weight at autopsy was $93.2 \pm 3.2^*$ per cent and $93.5 \pm 2.5$ per cent in the
non-neurectomized animals; $88.1 \pm 3.8$ per cent and $87.6 \pm 2.1$ per cent in the neurectomized,
of the preoperative values in the postoperative courses of 2 and 4 weeks respectively (Table
1).

Corresponding to the weight loss, the neurectomized group tended to make a retarded

$^*$ mean \pm standard error
recovery from the procedure as compared with control. Consequently regeneration, as expressed in relation to the body weight, was estimated as follows (Table 1):

<table>
<thead>
<tr>
<th>No. of Dog</th>
<th>Body Wt. kg</th>
<th>Removed Liver Wt. gm.</th>
<th>Total Liver Wt. gm.</th>
<th>Body Wt. kg</th>
<th>% of Remnant Liv.</th>
<th>% of Total Liver</th>
<th>Remnant Liver Wt. gm.</th>
<th>Liv. Wt. gm.</th>
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<tr>
<td>2 weeks</td>
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<tr>
<td>2</td>
<td>11.4</td>
<td>111</td>
<td>213</td>
<td>7.5</td>
<td>94.7 ± 3.2%</td>
<td></td>
<td>119</td>
<td>297</td>
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<tr>
<td>3 weeks</td>
<td>10.7</td>
<td>125</td>
<td>313</td>
<td>9.0</td>
<td>91.7</td>
<td></td>
<td>230</td>
<td>313</td>
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<tr>
<td>2</td>
<td>9.6</td>
<td>113</td>
<td>283</td>
<td>10.0</td>
<td>81.3</td>
<td></td>
<td>230</td>
<td>313</td>
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<tr>
<td>3</td>
<td>10.4</td>
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<td>9.1</td>
<td>89.7</td>
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<td>230</td>
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<tr>
<td>4 weeks</td>
<td>8.0</td>
<td>85</td>
<td>213</td>
<td>7.5</td>
<td>94.8</td>
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<td>245</td>
<td>313</td>
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<td>4</td>
<td>9.3</td>
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<td>8.8</td>
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(*) mean ± standard error

However, the above differences between the experimental and control groups were short of statistical significance (p > 0.05).

There was no discriminative finding in histology of the regenerating livers of both groups with and without neurectomy, with no variations from what has been described.
as typical of the regenerating liver\textsuperscript{39-43}.

Two days after operation, irregularities in the lobular pattern, swelling of hepatic parenchymal cells and their nuclei and engorgement of sinusoids were noted (Fig. 11 a). At that time liver glycogen was scanty, and centrilobular fatty infiltration was observed. Within four weeks the structure of the regenerating liver approached to the normal histology (Fig. 11 b). Glycogen was abundant throughout each lobule, and fatty infiltration had receded.

(a) \hspace{2cm} (b)

\textbf{Fig. 11} Photomicrographs of regenerating liver after partial hepatectomy and hepatic periarterial neurectomy: two days (a), and four weeks (b) postoperatively.

\textbf{COMMENTS}

An enormous amount of efforts have been devoted to studies on stimulating and controlling factors in liver regeneration after partial hepatectomy\textsuperscript{44,45}.

Some investigators have emphasized that liver regeneration depends on the volume of portal blood\textsuperscript{36,38}. A recent concept of this question seems that portal blood is not indispensable to liver regeneration itself, even though the amount of available portal blood may determine the ultimate size of the regenerated liver\textsuperscript{46,47}.

Since hepatic periarterial neurectomy affects hepatic arterial flow, not portal vein flow according to the present study, its effects cannot be discussed in the same category with the above concept\textsuperscript{48}. This experiment failed to demonstrate any distinctive effect of the neurectomy on liver regeneration.

The flow change after the neurectomy appears to be of minor importance concerning liver regeneration after partial hepatectomy in otherwise normal dogs, though it is possible that it may offer a considerable benefit in instances with some disturbance of the portal circulation\textsuperscript{49,51}.

Possible metabolic effects of this procedure suggested by \textsc{Mallet-Guy}\textsuperscript{53} were not examined in the present paper.

\textsc{Clerici} et al. noticed a similarity of the histological features of the early regenerating liver to the liver pathology after administration of carbon tetrachloride such as fatty infiltration, glycogen loss and focal necrosis, and anticipated that these post-hepatectomy changes might be reduced by blocking the hepatic sympathetic nerves. This possibility,
EFFECTS OF HEPATIC PERIARTERIAL NEURECTOMY

However, was excluded by their own and other experimentations\(^\text{49-50}\).

It can be stated that in the absence of an unusual sympathetic discharge and in the presence of the normal portal circulation, hepatic periarterial neurectomy is of little or no therapeutic value in hepatic lesions.

**SUMMARY**

Hepatic periarterial neurectomy is a subject of current surgical interest. The present paper intended to elucidate some possible effects of this procedure upon hepatic blood flow and liver regeneration after partial hepatectomy in the dog, with better understandings of the nature of the hepatic periarterial nervous plexus.

1. Hepatic periarterial plexus is composed of sympathetic adrenergic fibers. They were distributed throughout the liver for the most part accompanied by the arterial branches.
2. Hepatic periarterial neurectomy produced a 20 to 30 per cent increase of hepatic arterial flow in approximately 60 per cent of the experimental animals, with no demonstrable change in portal vein flow.
3. 40 per cent hepatectomy resulted in a consistent reduction of 30 to 40 per cent of hepatic arterial flow, irrespective of the preceding neurectomy, without decrease in portal vein flow.
4. Regeneration of the liver after partial removal was not significantly influenced by hepatic periarterial neurectomy.

**ACKNOWLEDGEMENT**

The present author is indebted to Prof. Dr. Ichio Hojo for his invaluable advices and encouragement.

**REFERENCES**


(* in Japanese)
肝動脈圏神経切除の肝血行及び肝再生に及ぼす影響

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牧野尚彦

近年、肝臓外科における肝神経義の問題が関心を集めている。特に1947年、仏のMallet-Guyは肝動脈圏神経切除が肝炎の予防を助長せしめると述べ、この効果は、おそらく肝臓代謝の改善によるものと推定している。一方、McDermott等は、肝動脈圏神経義が肝血行に重大な影響を及ぼすことを認めた。神経切除により肝血流が増大する傾向を臨床的に応用する可能性を提起している。

しかし、肝神経義自体の生理的意義がなお明らかでないため、このような術式の効果に関しては、なお多くの議論が続いている。

この報告は、まず肝動脈圏神経義の構成を明らかにした上で、神経遮断の肝血行及び肝再生に及ぼす効果を観察し、次いで、これらの効果の肝部分切除後の肝再生に与える影響を検討せんとするものである。

【方法】実験は一部成犬を用い、手術はネブキターール静脈下に行なった。神経義の組織学的観察のために、受光光、酸性染色、酸性染色法を応用した。肝動脈及び門脈血流量測定には、各々電磁流量計及び酸反応血流計を使用した。肝部分切除は左上・下葉切除、或いは40％切除を行なう、肝再生率は術後4週までにつき、術前肝重量の百分比にて評価し、再生肝の組織学的観察を行った。

【結果】
1) 肝動脈圏神経義の構成要素
肝動脈圏神経義は殆ど交感神経義節後組織よりなり、その分布は肝動脈系に最も豊富にみられるた。同神経義切除後には、肝内における交感神経義素の広汎な脱著を認めた。

2) 肝動脈圏神経義切除後の肝血流量の変化
神経切断の影響は、各個体差が著しく、同一個体においては差がみられた。

3) 肝部分切除時の肝血流の変化
正常肝では40％肝切除時に、肝動脈血流量は術前30～40％の減少を示した。門脈血流量は著変なかった。即ち、残存肝の単位重量あたりの門脈血流量は増加するが、肝動脈血供給はほぼ術前に等しい。この変化は、肝動脈圏神経切除を併施しても基本的には異なるとみられた。

4) 肝動脈圏神経切除の肝部分切除後の肝再生に及ぼす影響
肝再生が上記の変化を圧迫的に影響される如き果実は得られなかった。肝動脈圏神経義切除後、肝血流量は著増し、術後4週までに、術前肝重量の百分比にて評価し、再生肝の組織学的観察を行った。

【要】肝動脈圏神経義の構成及び分布より、その主要な作用は、肝血管運動支配であることと推定されるが、その識別圏肝血液の増大させるか否かは同一に決定し難く、肝血管緊張支配の性格に対し検討を要する。

従来、門脈血流量の増大は肝再生を促進すると論じられているが、肝動脈圏神経義切除の効果が肝部分切除後の肝血流上昇に大きな役割を演ずると考え難しい。

肝神経遮断による血行上の影響とそれに肝代謝機能に関する影響の面については、別途検討の余地もあると思われる。