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PORTACAVAL TRANSPOSITION AND SUBSEQUENT PARTIAL HEPATECTOMY IN THE RAT: EFFECTS ON LIVER ATROPHY, HYPERTROPHY AND REGENERATIVE HYPERPLASIA

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Summary.—Portacaval transposition diverts portal blood from the liver. It allows systemic venous blood to perfuse the portal bed.

Body weight and liver weight have been followed before and after portacaval transposition and control procedures in rats, and the DNA activity ratio studied in the liver of rats after partial hepatectomy in portacavally transposed animals.

The results suggest that the liver atrophy seen after portal diversion is a result of diversion of trophic substances in the portal blood rather than of a decrease in absolute liver flow.

Recovery of liver weight after partial hepatectomy in portacavally transposed animals occurs within the same time as in control animals, and the time course and magnitude of regenerative hyperplasia, as assessed by liver DNA activity ratio, is unimpaired.

PORTACAVAL SHUNT is followed by profound changes in liver size and function, but there has been difficulty in determining whether these changes are due to alterations in the total quantity of blood supplied by the portal vein to the liver or to diversion of specific constituents within the portal blood. In addition, the difficulty in separating the effects of diversion of portal blood from the changes consequent on resection of liver mass has been an obstacle in the investigation of liver regeneration in the portally deprived animal.

Recently it has been suggested that diversion of endogenous insulin is responsible for the atrophy after portal shunt (Starzl *et al.*, 1975) and intraportal exogenous insulin has been shown to ameliorate this atrophy (Starzl *et al.*, 1976). In addition, it is proposed that the contents of portal blood, and insulin in particular, may be of importance in the process of regenerative hyperplasia (Starzl *et al.*, 1975; Starzl *et al.*, 1976).

Portacaval transposition diverts portal blood from the liver through the cephalad inferior vena cava. Systemic venous blood from the caudal inferior vena cava perfuses the portal bed maintaining the dual liver blood supply and total hepatic blood flow (Child *et al.*, 1953; Heer, Silvius and Harper, 1960; Starzl *et al.*, 1962; Kreutzer and Schenk, 1971). Liver size and function after partial hepatectomy may thus be studied in the presence of adequate hepatic blood flow without direct perfusion of the liver by blood flowing from the intestines and pancreas.

Much experimental work related to liver regeneration has been done in the rat and the proliferative response which follows resection is well documented (Bucher, 1963). This study records the changes in the liver/body weight ratio which follow end-to-end portacaval transposition in the rat (Ryan, Benjamin and Blumgart, 1974). In addition, the regenerative hyperplastic response consequent upon partial hepatectomy is examined in rats subjected to portacaval transposition.

MATERIAL AND METHODS

1. *Animals.*—Male Sprague–Dawley rats weighing between 200–220 g were fed on a standard pellet diet (41 b Oxoid Ltd), allowed water *ad libitum* and housed in a constant temperature and controlled 12-h daylight environment.

2. *Surgical procedures.*—All surgical procedures were carried out under open ether anaesthesia through a midline ventral incision.

Portacaval transposition was performed by the method of Ryan *et al.* (1974). Immediately prior to portacaval transposition, a left adrenalectomy was performed since, in the rat, the left adrenal gland venous effluent drains *via* the left renal vein into the inferior vena cava. This was done because glucocorticoids have been shown to inhibit mitotic activity in the liver (Hyde and Davis, 1966) and following portacaval transposition, inferior vena cava blood drains directly to the liver.

Adrenalectomy was performed by simple excision after ligation of the adrenal vessels. In animals in which adrenalectomy alone was performed, the liver was not disturbed.

Sham portacaval transposition was performed by dissection of the vessels as for portacaval transposition and the portal vein and inferior vena cava were clamped for similar time periods.

Partial hepatectomy was carried out by the method of Higgins and Anderson (1931). In order to avoid variation in the mitotic activity due to diurnal rhythm (Jaffe, 1954), partial hepatectomy and control procedures were performed between the hours of 9 a.m. and 11 a.m.

The abdominal wound was closed with continuous catgut for the muscle layers and stainless steel clips to the skin.

3. *Relative liver weight.*—The animals were weighed before operation and at intervals thereafter. On killing, 3 or 6 weeks after the initial procedure, the abdomen was opened and the portal vein clamped. The liver was removed, blotted and weighed.

Liver weight was expressed as a percentage of body weight (relative liver weight). The normal value ($\pm$ s.d.) for this ratio was first obtained in a separate group of 5 animals and found to be $4{\cdot}23 \pm 0{\cdot}29\%$. Comparison of this ratio between groups was made by Student's t test incorporating a Fisher's F test of comparison of variance.

4. *Measurement of DNA synthesis.*—DNA synthesis was measured by the method of Weinbren and Woodward (1964) with a modification for the use of tritiated thymidine (Weinbren, Arden and Stirling, 1969). Each animal in each group received 100 μCi of tritiated thymidine (Radiochemical Centre, Amersham: specific activity 20,000 μCi/mmol) by injection into the jugular vein 1 h before killing.

Radioactivity in the DNA extracted from the liver was measured in a Packard Tricarb liquid scintillation counter and corrected to dpm by a linear quench calibration curve based on the channel ratio. The radioactivity of the DNA in dpm is expressed as a ratio of the total DNA estimated by measurement of its optical density in a SP 600 spectrophotometer at 260 nm wave length.

The DNA activity ratio was statistically analysed by the Mann-Whitney non-parametric test utilizing Wilcoxon's U statistic.

5. *Arrangement of experiments.*—Experiments are reported in two parts, utilizing the following groups of animals:

Group I (experimental group)
  Portacaval transposition + left adrenalectomy (PCT + A)
Group II (control group)
  Sham portacaval transposition + left adrenalectomy (Sham + A)
Group III (control group)
  Left adrenalectomy alone (Ad)
Group IV (control group)
(Experiment 2 only)
  Ether anaesthesia alone (Eth)

Experiment 1

In this experiment the relative liver weight was measured in animals after portacaval transposition plus left adrenalectomy and control procedures.

Fifteen of 16 animals survived PCT + A (Group I) and 3 weeks later one-third of the animals was killed, the anterior and left lateral lobes being removed as for partial hepatectomy, and weighed. The remnant of the liver was then removed and similarly weighed, and the total liver weight noted. A further one-third of the animals was subjected to partial hepatectomy but allowed to survive a further 3 weeks (6 weeks from the time of portacaval transposition). They were then killed and the liver remnant weighed. The remaining animals were not subjected to partial hepatectomy but allowed to survive 6 weeks and were then killed and the liver removed and weighed.

Fifteen rats (Group II) survived sham portacaval transposition + adrenalectomy, and 10 left adrenalectomy alone (Group III). The animals in Groups II and III were treated in an identical manner to those in Group I, one-third of the animals in each group being killed at 3 weeks, one-third receiving partial hepatectomy, and one-third being allowed to survive until the 6th postoperative week without partial hepatectomy.

Experiment 2

In this experiment, the DNA synthetic activity was measured at 8 time points during

the first 72 h following partial hepatectomy. Within each of the experimental groups I, II and III, and for each of the time points studied, batches of rats (4–7 rats per batch) were subjected to the initial operation (portacaval transposition or control operation) and were allowed to recover and survive for 3 weeks, at which time partial hepatectomy was performed. The animals were allowed to survive and a batch of animals was killed at each of the time points, 12, 18, 21, 24, 30, 36, 48 and 72 h after partial hepatectomy. Duplicate batches were assessed for the time intervals 18, 21 and 24 h in order to cover the known peak of DNA synthetic activity associated with hepatocyte replication (Bucher, 1963).

In addition, for each batch of animals at each time interval, 2 further control animals (Group IV—Eth) were used. These 2 animals were anaesthetized at the time of the initial operation and then 3 weeks later one animal was subjected to a further ether anaesthetic and one to sham partial hepatectomy, the liver being mobilized but no resection carried out.

Each animal in each group received 100 μCi of tritiated thymidine 1 h before killing by intravenous injection.

At sacrifice a 700–800-mg portion of the right lateral lobe was deep-frozen for later estimation of DNA synthetic activity.

RESULTS

Two hundred and fifty rats were used in the 2 experiments and 218 (87%) survived to be killed. In animals subjected to PCT + A (Group I), 78% survived and 87% of animals in Group II (Sham + A) survived. In those animal groups subjected to adrenalectomy alone (Group III) or ether anaesthesia (Group IV), survival was 96%.

Animals in all groups gained weight after the initial operative procedure (Table I). However, portacavally transposed animals did not gain as much weight as did control groups (PCT + A *vs* Sham + A, $P < 0{\cdot}001$).

TABLE I.—*Body Weight at Initial Procedure and at Partial Hepatectomy*

Group	No.	Initial body weight	Body weight at partial hepatectomy
I (PCT + A)	58	210·2±6·9	265·3±29·5
II (Sham + A)	68	210·2±6·7	289·5±26·9
III (Ad)	50	210·7±5·5	303·6±31·5
IV (Eth)	25	209·3±7·1	316·8±26·8

PCT + A *vs* Sham + A, $P < 0{\cdot}001$ at partial hepatectomy.

Following partial hepatectomy, the animals in all groups lost weight. This loss of weight was significant at 3 weeks after partial hepatectomy in Group I ($0{\cdot}05 > P > 0{\cdot}025$) and Group II ($0{\cdot}01 > P > 0{\cdot}005$) but not in Group III.

Experiment 1

Table II shows the changes in relative liver weight in the 6 weeks after portacaval transposition or control operation. A progressive reduction in the relative liver weight was found in all animals in all groups not submitted to partial hepatectomy but this reduction was significantly greater at 3 and at 6 weeks in animals subjected to PCT + A (Group I) as compared to sham operation (Group II) ($0{\cdot}005 > P > 0{\cdot}001$) at 3 and at 6 weeks).

In animals subjected to partial hepatectomy 3 weeks after the initial procedure and then immediately killed, it was found that the relative weight of the liver remnant (after partial hepatectomy) was not significantly different in any of the

TABLE II.—*Experiment 1—Relative Liver Weight*

	At 3 weeks after initial procedure		At 6 weeks after initial procedure	
Group	Total	Remnant after partial hepatectomy	No partial hepatectomy	Partial hepatectomy
I (PCT + A)	3·29±0·19	1·26±0·19 (5)	2·88±0·23 (5)	2·47±0·42* (5)
II (Sham + A)	3·96±0·32	1·45±0·17 (6)	3·64±0·13 (4)	3·47±0·37* (5)
III (Ad)	3·77±0·22	1·26±0·12 (4)	3·31±0·28 (3)	3·35±0·46* (3)

* No significant difference compared to non-hepatectomized group at 6 weeks ($P > 0{\cdot}05$).
Normal value of relative liver weight determined in 5 rats = 4·23 ± 0·29.
Figures in parentheses = no. of animals.

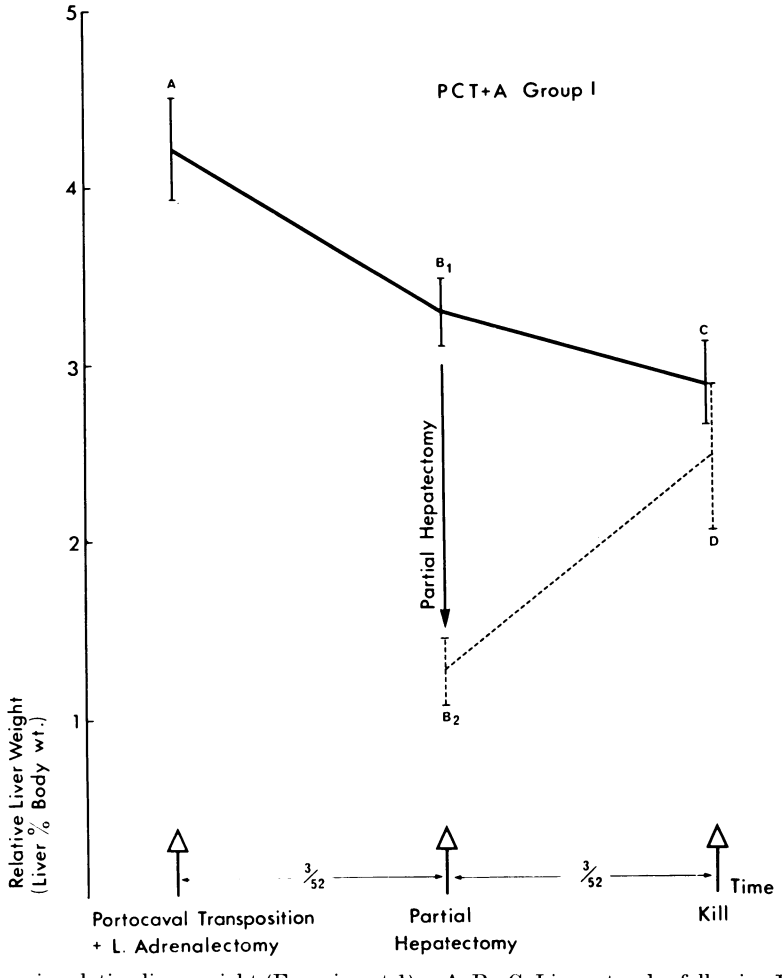

FIG.—Changes in relative liver weight (Experiment 1). A–B_1–C, Liver atrophy following PCT + A. B_2, Relative weight of liver remnant after partial hepatectomy. B_2–D, Gain in relative weight of remnant after partial hepatectomy in PCT + A animals. CD, $P > 0{\cdot}05$. No significant difference. Each point A, B, C and D represents the mean ± s.d. at time of killing for a separate group of 5 animals (see text).

groups. The relative liver weight 6 weeks after the initial procedure (3 weeks after partial hepatectomy) was significantly less in animals in Group I when compared to animals in Groups II or III, but it did not differ significantly from the expected relative liver weight found in non-hepatectomized animals of Group I 6 weeks after the initial portacaval transposition (Table II, Fig.). The changes in relative liver weight in animals of Group I (PCT + A) reveal that the atrophy consequent on a portacaval transposition results in a smaller liver 9 weeks after operation. However, in animals subjected to partial hepatectomy during this period, there is recovery to expected liver weight in the same time period as for control animals without portal diversion (Table II).

Experiment 2

Examination of the DNA activity ratio after partial hepatectomy in Groups I, II and III revealed a rise to peak DNA synthetic activity 21 to 24 h after partial

TABLE III.—*Experiment 2—DNA Activity Ratio after Partial Hepatectomy (P. H.) (Median Results)*

Group	12 h	18 h	21 h	24 h	30 h	36 h	48 h	72 h
I (PCT + A)	345 (5)	10461 (8)	23101 (10)	14592 (5)	4981 (4)	8116 (5)	3533* (3)	8540* (4)
II (Sham + A)	290 (5)	10590 (12)	21635 (8)	24079 (11)	11281 (5)	10547 (6)	12717 (6)	2778 (6)
III (Ad)	517 (3)	34096 (7)	32324 (8)	26495 (7)	8059 (4)	17689 (4)	11216 (4)	8797 (4)
IV (Eth)	354 (2)	286 (4)	378 (3)	548 (4)	354 (1)	214 (2)	323 (2)	450 (2)

* Significant difference when compared to Group II (Sham + A) ($P < 0{\cdot}05$).
Figures in parentheses = no. of animals killed.

hepatectomy, whereas in Group IV (ether controls, no partial hepatectomy) no such rise occurred (Table III). Statistical comparison between Group I (PCT + A) and Group II (Sham + A) at each time point from 12–36 h after partial hepatectomy revealed no statistical difference, although at time points 48 and 72 h, by which time the greater part of hepatocyte DNA synthetic activity is complete, there was a significant difference ($P < 0{\cdot}05$) detectable (Table III).

DISCUSSION

Ever since Mann (1944) suggested, on the basis of observations of changes in liver weight, that the regenerative response following partial hepatectomy was diminished by deviation of portal blood, there has been difficulty in deciding upon the role of the portal circulation in the control of liver regeneration. It has, however, been demonstrated that, although portacaval shunt or indeed portal venous ligation to a segment of liver results in atrophy of liver tissue, the capacity for a regenerative hyperplastic response following partial hepatectomy is retained (Weinbren, 1955; Fisher *et al.*, 1962; Weinbren *et al.*, 1972). Indeed, partial hepatectomy is not necessary for the initiation of a proliferative response, since the cellular atrophy which occurs after portal venous ligation represents a net loss of liver tissue, and this in itself may act as a stimulus sufficient to initiate a hyperplastic response (Weinbren *et al.*, 1975).

Child (1953) utilized the operation of portacaval transposition to explore liver regeneration in the dog and suggested that the operation allowed recovery of liver size after partial hepatectomy even though portal blood was entirely deviated. More recently, Lee *et al.* (1974) reported recovery of liver size after partial hepatectomy in portacavally transposed rats. However, their studies of regenerative hyperplasia relied on measurements of DNA synthesis made as late as the 7th day after partial hepatectomy and, since this is well beyond the peak of hepatocyte replication (Bucher, 1967), the results must be interpreted with caution.

Not only the weight of the liver but the weight of the animal changes after operation. Expression of liver weight as a reflection of atrophy or of recovery after partial hepatectomy must take this into account. The results presented confirm our earlier observations (Ryan *et al.*, 1974) that all animals in all groups regained weight after the initial operative intervention but that portacavally transposed animals did not gain as much weight as control groups. In addition, portacaval transposition is associated with a degree of liver atrophy reflected by a fall in relative liver weight, which was evident by the 3rd postoperative week and was also progressive, so that 6 weeks after operation there was a significant further fall in the relative liver weight. Nevertheless, in animals in which partial hepatectomy was carried out 3 weeks after portacaval transposition, there was a recovery by the 6th week to the liver

weight previously defined in animals with portacaval transposition but not subjected to partial hepatectomy (Fig.). This recovery occurred within the same time period as did recovery of liver weight in the control animals subjected to partial hepatectomy without previous portacaval transposition.

The relationship of the atrophy consequent on portacaval transposition to the possible alteration in blood flow is of interest. Liver blood flow has been demonstrated to be at least as great as normal after portacaval transposition in the dog (Heer *et al.*, 1960; Starzl *et al.*, 1962; Kreutzer and Schenk, 1971). More recently, we have studied liver blood flow before and after partial hepatectomy in the rat, using an ^{85}Krypton clearance technique (Rice *et al.*, 1976) and have also demonstrated that total nutritional blood flow to the liver remains the same before and after portacaval transposition (Ryan *et al.*, in preparation). Although a greater loss of relative liver weight occurs after portacaval shunt than after portacaval transposition (Ryan *et al.*, 1974), and this may be flow related, the atrophy that we have demonstrated after portacaval transposition does not appear to be associated with a fall in total liver blood flow. Our observations support the suggestion that liver atrophy is a consequence of deviation of factors within the portal blood rather than the result of a decrease in total flow alone (Starzl *et al.*, 1975; Starzl *et al.*, 1976). However, it is of particular interest that, despite the atrophic changes occurring in the liver of portacavally transposed animals, we found no change in the DNA activity ratio, either in timing or degree, in the first 36 h after partial hepatectomy. This observation suggests that regenerative hyperplasia following partial hepatectomy is not impaired by diversion of portal blood factors. Whether the large increase in blood flow per gram of liver tissue following partial hepatectomy in normal rats (Ryan *et al.*, unpublished) is concerned with the initiation or is permissive of the process of DNA synthesis remains to be seen.

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