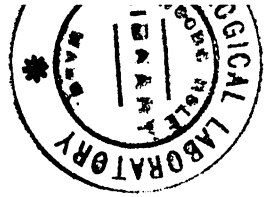
J. Physiol. (1957) 136, 287–293



THE MEASUREMENT OF LIVER BLOOD FLOW IN PARTIALLY HEPATECTOMIZED RATS

By B. BENACERRAF, D. BILBEY, G. BIOZZI, B. N. HALPERN and C. STIFFEL

From the Clinique médicale propédeutique de l'Hôpital Broussais, Association Claude Bernard et C.N.R.S., Paris

(*Received* 13 *November* 1956)

In a previous study (Benacerraf, Biozzi, Cuendet & Halpern, 1955) the phagocytic activity of the reticulo-endothelial system with regard to the regeneration of the liver in partially hepatectomized rats has been investigated by measuring the rate of clearance from the blood of carbon particles of known size (about 250 Å).

When 60–70% of the liver tissue is removed, the organ regenerates almost completely in the course of a week (Himsworth, 1947). Partial hepatectomy causes a decrease of phagocytic activity, but to a lesser degree than might be expected considering the reduced amount of liver tissue. The compensatory factor is presumably the increased blood flow through the remaining liver, caused by portal hypertension. It was felt from the data reported and from observations in the literature (Mann & Magath, 1922; Mann, 1940, 1943) that the increased blood flow through the remaining liver played a considerable role in the regeneration of the organ. However, measurement of blood flow could not be suitably made with the techniques already used and more adequate methods of measurement have been developed involving the injection of colloids labelled with radio-isotopes, which permit the use of very small doses of the injected medium (Dobson & Jones, 1952; Benacerraf, Biozzi, Halpern, Stiffel & Mouton, 1956).

When small enough doses of colloids which are phagocyted by the reticulo-endothelial system are injected intravenously, there is a critical dose below which the rate of clearance is maximal and independent of the quantity injected, and nearly all the injected material is contained in the Kupffer cells of the liver. The efficiency of clearance of the colloid by the liver is maximal in this dose range, and in the normal rat it is about 80–85% for chromium phosphate (^{32}P) and also for the complex human serum albumin labelled with ^{131}I (C.A.^{131}I).

In the present study the liver blood flow has been calculated both in normal rats and in partially hepatectomized rats from measurements of blood clearance of C.A.^{131}I. Maximal efficiency of clearance of this colloid was measured both in the normal rat and in the partially hepatectomized rat in the course of these experiments.

The results of the present study confirm that considerably greater liver blood flow occurs per unit weight of liver in the partially hepatectomized rat immediately following the operation than in the control rat, while after regeneration of the liver the values of blood flow in the two groups are comparable.

METHODS

Male white rats were used and the operative procedures, except where otherwise stated, were performed under light ether anaesthesia. Partial hepatectomy was carried out as described in a previous paper (Benacerraf *et al.* 1955). All the liver tissue supplied by the left branch of the portal vein, approximately 60–70% of the organ, was removed after ligation of this branch.

The phagocytic activity of the reticulo-endothelial system (R.E.S.) was investigated in ten control rats and eleven partially hepatectomized rats 4 hr, 16 hr and 5 days after operation, using a dose of 8 mg carbon/100 g body wt. Each animal was killed after experiment and the ratio of its body weight to the combined weights of its spleen and its available liver W/W_{ls} was calculated.

The phagocytic index, K, and the corrected index, α, have both been calculated (Biozzi, Benacerraf & Halpern, 1953; Halpern, Benacerraf, Biozzi & Stiffel, 1954). K, which varies with both the weight of the liver and spleen and the dose of carbon, was calculated for the 8 mg dose from the log. to the base 10 of the change in concentration of carbon in the blood with time.

The variation of K with the weight of the organs is related to the cube of W/W_{ls}. α is obtained by correcting K for this variation and, being an index of the phagocytic activity per unit wt. of liver and spleen combined, is more useful than is K in comparing the data from different rats.

The liver blood flow was determined from the clearance of serum albumin complex, tagged with ^{131}I (C.A.^{131}I), distributed in the general circulation by injection into the dorsal vein of the penis. To allow for the incomplete removal of C.A.^{131}I during a single passage through the liver, a correction factor was obtained by determining the efficiency of this removal. From the rate of clearance of C.A.^{131}I injected into the general circulation and the efficiency of its removal by the liver, together with the total blood volume, it is possible to calculate the liver blood flow. The calculation is made by employing the procedure introduced by Dobson & Jones (1952). The specific rate constant of colloid clearance (K_e) for the relevant dose range is multiplied by the total blood volume of the animal and the result corrected by the efficiency factor for clearance.

$$K_e = \frac{\ln C_1 - \ln C_2}{T_2 - T_1},$$

where C_1 and C_2 are the concentrations of C.A.^{131}I in the blood at times T_1 and T_2 respectively after its injection into the dorsal vein of the penis.

The three measurements required for determination of blood flow were made as follows:

(1) *Rate of clearance of* C.A.^{131}I *injected into the general circulation (determination of* K_e*).* It is important that the quantity of C.A.^{131}I injected is such as to cause its concentration in the blood to lie within the range required for maximal clearance. To attain this, a dose of 0·01–0·05 mg/100 g body wt. was injected into the dorsal vein of the penis. Measured blood samples, 0·025 or 0·05 ml., were obtained in a calibrated glass pipette from the retro-orbital venous plexus (Halpern & Pacaud, 1951). The blood samples were evenly spread on circles of filter paper, 2·5 cm in diameter, mounted on glass slides, and the β-radioactivity was measured directly with a Geiger–Müller counter. After the animal was killed 96–99% of the injected radioactivity could be found in the

liver. The γ-radioactivity of weighed samples of tissues was measured with a scintillation counter keeping the geometry constant.

(2) *The efficiency of clearance of* C.A.^{131}I *by the liver.* This was measured by injecting C.A.^{131}I directly into a mesenteric vein so that it would go directly through the liver before reaching the general circulation. Care had again to be taken that the quantity of C.A.^{131}I injected would give a concentration in portal blood within the range that permits maximal clearance. The quantity used was small (0·01 mg in 0·15 ml.). This was injected slowly (10–20 sec). The operation was done under local anaesthesia because of the report by Dobson & Jones (1952) that general anaesthesia modifies liver blood flow. The clearance of the C.A.^{131}I was measured by drawing blood samples of 0·1 ml. from the retro-orbital site and diluting them to 3 ml. with 0·1 % sodium carbonate solution; and, for increased sensitivity, the γ-radioactivity of the blood was measured with a well-type scintillation counter. The initial concentration of C.A.^{131}I in the blood indicated by extrapolation was compared with the concentration calculated for the same dose distributed in the blood volume of the animal. The ratio of these two concentrations is the required efficiency factor.

(3) *The total blood volume.* A value of 7·25 ml./100 g body wt. was accepted for control rats and also for partially hepatectomized rats 5 days after operation, when regeneration of liver is found to be complete. This value of 7·25 ml. is the volume found by extrapolating to zero time the clearance curves of carbon and of C.A.^{131}I for a large number of rats.

Individual blood volume values have been measured for partially hepatectomized rats investigated a few hours after operation. This was done because some of these animals had slightly lowered blood volume after operation.

The preparation, dosage and iodination of the human serum albumin complex is described in a previous publication (Benacerraf *et al.* 1956).

TABLE 1. Effect of partial hepatectomy on the phagocytic activity of the R.E.S. for carbon particles, measured by the phagocytic activity (indices K and α) for the dose of 8 mg of carbon/100 g body wt.

No. of rats	Time after operation	K (decimal log.)	W/W_{ls}	α
Control rats:				
10		0·026	22	6·6
Partially hepatectomized rats:				
4	4 hr	0·013	43	9·9
7	5 days	0·035	20·5	6·7

K = the phagocytic index, α = the corrected phagocytic index, W = total body weight of experimental animal and W_{ls} = weight of liver and spleen.

RESULTS

The data concerning the phagocytic activity of the reticulo-endothelial system for the dose of 8 mg of carbon per 100 g body weight are shown in Table 1. Four hours after partial hepatectomy the over-all activity measured by K is decreased, but not in proportion to the amount of liver tissue removed. The index α which measures the phagocytic activity per unit weight of hepato-splenic tissue is above normal owing to the increase of blood flow through the liver. The results obtained are identical with those reported previously (Benacerraf *et al.* 1955). Within 5 days after partial heptectomy the liver has recovered its normal size and the indices K and α have resumed their normal values.

The efficiency of clearance of C.A.^{131}I by the liver, in the range of concentration in the blood where clearance is maximal, is shown in Table 2 which includes results from both control rats and partially hepatectomized rats a few hours after operation. The efficiency of clearance in four control rats varies from 79–86 % with the mean value of 84 %. In rats subjected to partial hepatectomy and investigated a few hours later, the maximal efficiency of clearance is only 62·5 % (range 57–67 %). The reason for this difference is that the blood flow per unit weight is increased through the remaining liver which effects a decrease in the efficiency of colloid clearance, as shown by Brauer, Leong, McElroy & Holloway (1956).

Table 3 gives both the values of the minimal liver blood flow of normal rats before the efficiency correction has been made, and the values of corrected liver blood flow. Tables 4 and 5 present the values of liver blood flow in partially hepatectomized rats, 4 hr, 16 hr and 5 days after operation. The average normal liver blood flow of the normal intact rat, measured by the method described here, is 1·44 ml./g/min. A few hours after hepatectomy the average liver blood flow is 2·57 ml./g/min, nearly twice the normal. Five days later, when the liver has recovered its normal size, the liver blood flow has returned to normal values. Table 6 gives calculated data for an ideal rat of 100 g body wt.

TABLE 2. Maximal efficiency of portal clearance of C.A.^{131}I in the normal rat and in the partially hepatectomized rat

	Normal rat (%)	Partially hepatectomized rat 16 hr after operation
	79	65
	86	67
	86	61
	84	57
Mean	84	62·5

TABLE 3. Measurement of liver blood flow in normal rats by multiplying the specific rate of clearance of C.A.^{131}I (K_e) for a limited dose range (0·010–0·050 mg/100 g body wt.) by the blood volume of the animal and correcting for the efficiency of clearance

	Body wt. (g)	K_e (ln)	Liver wt. (g)	Minimal liver blood flow (ml./g/min)	Corrected liver blood flow: mean efficiency factor 84 % (ml./g/min)
	131	0·943	6·200	1·43	1·70
	166	0·655	7·900	0·99	1·18
	132	0·855	6·300	1·28	1·52
	154	0·690	7·100	1·08	1·29
	161	1·04	8·300	1·45	1·75
	171	0·545	6·500	1·03	1·23
Mean	152	0·788	7·05	1·21	1·44

C.A.^{131}I injected into dorsal vein of penis; blood volume considered in calculation of liver blood flow = 7·25 ml./100 g. (Corrected liver blood flow measured in 15 rats in dose range of C.A.^{131}I from 0·012 to 0·250 mg/100 g = 1·35 ± 0·24 ml./g/min; Benacerraf *et al.* 1956).

TABLE 4. Liver blood flow in partially hepatectomized rats

Body wt. (g)	K_e (ln)	Liver wt. (g)	Liver removed (g)	Blood volume (ml./100 g)	Minimum liver blood flow (ml./g/min)	Efficiency factor of clearance (%)	Corrected liver blood flow (ml./g/min)
After 4 hr:							
140	0·300	2·4	5·55	5·9	1·03	62·5	1·65
130	0·312	2·5	4·60	5·9	0·96	62·5	1·53
132	0·382	2·3	4·15	6·6	1·44	62·5	2·30
135	0·336	2·7	5·45	7·6	1·29	62·5	2·06
Mean 134	0·332	2·47	4·94	6·5	1·18	62·5	1·9
After 16 hr:							
142	0·360	2·4	3·5	7·25	1·54	65	2·37
180	0·308	3·1	4·05	7·25	1·30	67	1·94
130	0·457	1·9	3·05	7·25	2·25	61	3·70
113	0·272	1·7	3·25	7·25	1·31	57	2·30
Mean 141	0·349	2·27	3·46	7·25	1·6	62·5	2·57

For K_e C.A.^{131}I injected into dorsal vein of penis (0·05 mg/100 g body wt.); for efficiency factor, C.A.^{131}I injected into mesenteric vein (0·005 mg/100 g body wt.)

TABLE 5. Liver blood flow in partially hepatectomized rats after 5 days

Body weight (g)	K_e (ln)	Liver weight (g)	Liver removed (g)	Minimal liver blood flow (ml./g/min)	Corrected liver blood flow (mean efficiency factor 84%) (ml./g/min)
128	0·767	5·7	4·1	1·25	1·49
134	0·767	5·75	3·6	1·30	1·55
133	0·862	5·9	3·4	1·41	1·68
115	0·828	5·7	3·4	1·21	1·44
90	0·483	4·75	2·9	0·66	0·79
114	0·851	4·6	3·1	1·52	1·82
126	0·625	4·65	3·5	1·22	1·45
Mean 120	0·740	5·29	3·4	1·22	1·46

C.A.^{131}I 0·05 mg/100 g body wt. Blood volume used in calculations of liver blood flow: 7·25 ml./100 g.

TABLE 6. Data calculated for ideal rat of 100 g

	Intact rat	Partially hepatectomized rat. Time after operation		
		4 hr	16 hr	5 days
Blood volume (ml.)	7·25	6·5	—	—
Liver wt. (g)	4·6	1·85*	1·61	4·4
Liver blood flow:				
ml./g liver/min	1·44	1·9	2·57	1·45
ml./min	6·60	3·5	4·11	6·40
Percentage of body blood that passes through liver in each minute	91	54	56	88

* These figures differ because of small differences in liver weights and in quantities of liver removed at operation. There is no evidence of any significant change in liver weight during the first 16 hr after operation.

DISCUSSION

The measurement of liver blood flow at definite intervals after partial hepatectomy shows that the increased portal pressure results in a considerable increase in flow per gram of liver tissue and that this is associated with a decrease in the efficiency of clearance. These results are in complete agreement with the data reported by Brauer *et al.* (1956). These authors, investigating chromium phosphate clearance by the rat's liver perfused with blood, observed that the efficiency of clearance decreased with the increase of blood flow. The values reported by these authors for efficiency of clearance with respect to liver blood flow are identical with those obtained in our experiments *in vivo*. The data reported establish that, immediately following hepatectomy and up to the time that the liver has recovered its nearly normal size, the blood flow per gram of liver substance is considerably higher than normal. This also accounts for the increased phagocytic activity of the liver per unit of weight (α) following such operation. The results of these experiments emphasize further the role played by the liver blood flow in determining regeneration of this organ after partial hepatectomy.

SUMMARY

The removal of 60–70% of the liver of the male white rat has the following effects:

(1) Maximal efficiency of portal clearance of complex serum albumin ^{131}I falls from 84 to 62·5%.

(2) The total blood volume of a 100 g rat falls from 7·25 ml. to 6·5 ml.

(3) The total liver blood flow falls but the flow/g liver/min rises, thus: 1·44 ml. (normal); 1·9 ml. (4 hr after operation); 2·5 ml. (16 hr after operation).

(4) The proportion of the total blood volume passing through the available liver falls.

(5) After 5 days the liver has regenerated and normal values are restored.

(6) Values for an ideal rat of 100 g are tabulated.

REFERENCES

BENACERRAF, B., BIOZZI, G., CUENDET, A. & HALPERN, B. N. (1955). Influence of portal blood flow and of partial hepatectomy on the granulopectic activity of the reticulo-endothelial system. *J. Physiol.* **128**, 1–8.

BENACERRAF, B., BIOZZI, G., HALPERN, B. N., STIFFEL, C. & MOUTON, D. (1956). A study of the phagocytic activity of the R.E.S. towards heat-denatured human serum albumin and the application of this method to the measure of liver blood flow. *Brit. J. exp. Path.* (in the Press).

BIOZZI, G., BENACERRAF, B. & HALPERN, B. N. (1953). Quantitative study of the granulopectic activity of the reticulo-endothelial system. II. A study of the kinetics of the granulopectic activity of the R.E.S. in relation to the dose of carbon injected. Relationship between the weight of the organs and their activity. *Brit. J. exp. Path.* **34**, 441–457.

BRAUER, R. W., LEONG, G. F., MCELROY, R. F. & HOLLOWAY, R. J. (1956). Circulatory pathways in the rat liver revealed by ^{32}P chromic phosphate colloid uptake in the isolated perfused liver preparation. *Amer. J. Physiol.* **184**, 593–598.

DOBSON, F. L. & JONES, H. B. (1952). The behaviour of intravenously injected particular material. *Acta med. scand.* **144**, Suppl. 273, 1–71.

HALPERN, B. N. & PACAUD, A. (1951). Technique de prélèvement d'échantillons de sang chez les petits animaux de laboratoire par ponction du plexus ophthalmique. *C.R. Soc. Biol., Paris*, **145**, 1465–1466.

HALPERN, B. N., BENACERRAF, B., BIOZZI, G. & STIFFEL, C. (1954). Facteurs régissant la fonction phagocytaire du S.R.E. *Rev. Hémat.* **9**, 621.

HIMSWORTH, H. P. (1947). *Lectures on the Liver and its Diseases.* Ch. 3. Oxford University Press.

MANN, F. C. (1940). The portal circulation and restoration of the liver after partial removal. *Surgery*, **8**, 225–238.

MANN, F. C. (1943). Gastro-intestinal tract and the liver. *J. Amer. med. Ass.* **121**, 720–723.

MANN, F. C. & MAGATH, T. B. (1922). The production of chronic liver insufficiency. *Amer. J. Physiol.* **59**, 485–486.