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DETERMINATION OF THE HEPATIC BLOOD FLOW IN MAN BY SIMULTANEOUS USE OF FIVE TEST SUBSTANCES MEASURED IN TWO PARTS OF THE LIVER

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The method of Bradley, Ingelfinger, Bradley & Curry (1945) for determination of the hepatic blood flow can only be evaluated by comparison with other indirect methods, as direct determination of the hepatic blood flow is impossible in man. In the present study simultaneous indirect hepatic flow measurements by five test substances have been compared. Furthermore simultaneous sampling from two hepatic venous catheters has been employed to assess to uniformity of the extraction of these substances.

MATERIAL AND METHODS

Eight patients have been examined. One patient (No. 1) had a moderate cirrhosis of the liver without fluid retention. The remaining seven patients had no circulatory or hepatic disease. They were premedicated with 200 mg of phenobarbitone and were examined more than 12 hours after a meal.

Arterial blood was sampled from a small catheter in the femoral artery. Two cardiac catheters (Cournand No. 8) were placed in the hepatic veins *via* a medial and lateral antecubital vein. In case No. 7 the catheters were placed in a right and left hepatic vein, in the remaining cases both catheters were placed in hepatic veins of the right lobe of the liver, one cranially and the other caudally. The tip of the catheter was advanced to the wedged position and then withdrawn sufficiently to obtain a free flow of blood during suction from the catheter. The distance between the tip of the two catheters was about 5—10 cm as observed on the X-ray screen.

Intravenous infusion of the substances mentioned below was given by calibrated syringes driven by one motor. At the start of the infusion initial doses were given of all substances except of galactose (Bromsulfalein 145 mg, Indocyanine Green 4.6 mg, Rose Bengal-I-131 10 microcuries and ethanol 265 mg, on the average). After 60 minutes six samples were taken simultaneously from the artery and the two hepatic catheters at intervals of five minutes, each sampling lasted about 30 seconds. In one case (No. 1) only two samples, and in one (No. 8) four samples were taken. The amount of blood withdrawn was about 300 ml, and the same amount of saline was given.

Bromsulfalein (Sulfobromophtalein, Hynson, Westcott & Dunning) and Indocyanine Green was determining according to Winkler & Tygstrup (1960 a, b). The total radioactive dose of Rose Bengal-I-131 was in the first four cases about 100 microcuries, whereas the remaining cases received about 40 microcuries (carrier substance 0.25 mg or 0.10 mg, respectively). It was measured in a shielded well-scintillation detector tor (Tracerlab RLD-3 with a 1 7/8″ x 2″ (Tl) crystal) in connection with a gammaspectrometer (Tracerlab Research Line Equipment). The statistical error of the counting was between 1 and 4 per cent. Ethanol was measured by the alcohol dehydrogenase method (Larsen, 1959) and galactose was analysed by the method of Tygstrup, Winkler, Lund & Engell (1954).

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Table I. *Data of the patients and the amounts of the five substances infused.*

Case No.	Sex	Age	Height cm	Weight kg	Hematocrit (per cent)	Amount infused/min. Bromsulfalein mg	Indocyanine Green mg	Rose Bengal-I-131 counts×10⁻³	Galactose mg	Ethanol mg
1	♂	59	173	85	35	3.22	0.27	2265	211	40.0
2	♂	23	182	81	48	4.65	0.27	3040	223	38.7
3	♂	52	164	67	35	3.42	0.32	2260	185	33.0
4	♂	51	160	51	58	3.73	0.28	1575	267	—
5	♂	40	188	100	44	5.50	0.51	2470	366	55.9
6	♂	19	183	80	41	5.10	0.61	2775	305	52.1
7	♀	49	159	49	40	5.76	0.67	2775	269	33.5
8	♀	17	171	56	38	5.20	0.69	2105	287	31.1
					Average	4.60	0.45	2410	265	40.6

The data of the patients and the amounts infused are shown in Table I.

Three different methods for caulculation of the hepatic blood flow were employed.

I. The method of Bradley, where the arterio-hepatic venous difference is determined as the difference of the average of two successive arterial and venous concentrations. Using galactose and ethanol, no correction for changing arterial concentration was applied. The urinary elimination of galactose was disregarded (Tygstrup & Winkler 1958).

II. The average change in arterial concentration during the experiment was determined by rectilinear regression. The amount eliminated was corrected with this value, multiplied by the volume of distribution, based on the body weight (5 per cent for the three dyes, 15 per cent for galactose and 55 per cent for ethanol). The actually measured arterio-venous differences were used for calculation of a value for the blood flow for each sampling period. This method was used for a correlation study of the changes in the flow during the experiment.

III. As method II except that the average arterio-venous difference during the experiment was employed for determination of the average hepatic blood flow.

Table II. *Average values for the hepatic blood flow determined by five substances (Bradley's method).*

Case	Catheter position	Hepatic blood flow (ml/min) Brom-sulfalein	Indocyanine Green	Rose-Bengal-I-131	Galactose	Ethanol
1	I	2040	680*	2100	—	1900
	II	1900	1600*	1570	—	1720
2	I	2430	2890	3100	2390	2510*
	II	2420	2830	5270	2600	3590*
3	I	2180	1540	2290	2000	1820
	II	2780	3260	2320	2250	2190
4	I	1960*	1430	1390	1450	—
	II	1480*	1320	1460	1510	—
5	I	1470	1350	1740	1780	2240
	II	1460	1190	1660	1680	2220
6	I	1260	1460	1880	1580	1790
	II	1280	1460	1700	1800	1750
7	I	1440	1130	1640	1450	2230
	II	1230	1010	1390	1730	2310
8	I	1410	1220	1950	1470	1370
	II	1100	1230	1470	1430	1400
Average		1740	1600	2060	1790	2070
S. D.* (%)		12	31	36	7	15

* calculated by regarding the determinations in the two positions of the catheter as duplicates.

o the average hepatic venous concentrations in the two positions are significantly different (5 per cent limit, t-test).

Conventional statistical methods have been used. The 5 per cent limit has been taken as indicating statistical significance.

RESULTS

Table II shows the hepatic blood flow calculated by Bradley's method from the five substances and in each catheter position. The mean values of all determinations by each

Table III. *Averages of arterial concentration and extraction of the five substances.*

Case No.	Arterial concentration						Arterio-hepatovenous extraction (per cent)				
	BSP	ICG	RB	GAL	ET		BSP	ICG	RB	GAL	ET
1	1.68*	2.19	1934	—	58.4	I	13.7	25.1	8.4	—	43.2
						II	16.7	11.9	10.4	—	50.2
2	0.73	0.81	1088	152*	22.4	I	50.6	25.9	16.5	81.6*	88.4
						II	54.8	29.6	13.0	71.7*	46.0
3	2.37*	1.81	1537	161	34.0	I	9.2	24.9	9.6	65.8	80.0
						II	7.2	13.8	13.1	58.4	80.6
4	1.00	1.10	962	346	—	I	45.0	42.7	32.5	67.3	—
						II	60.0*	46.4*	25.2	65.3	—
5	1.25*	1.74*	1018	271*	31.6	I	53.6	34.5	24.7	93.0*	96.2
						II	53.6	35.6	25.8	98.5*	97.5
6	2.10	1.30	1142*	294	38.4	I	33.3	50.0	21.5	77.6	90.1
						II	33.3*	49.2	24.8	68.4	92.4
7	1.61	1.49	1332	237	21.9	I	39.8	60.4	21.0	92.8	81.3
						II	46.6	66.4	22.1	78.1	79.0
8	1.66	0.98	1142	266	27.3	I	39.2	90.8	14.2	85.3	99.6
						II	44.6	88.8	19.6	90.2	97.4
Average	1.55	1.43	1266	247	33.4		37.6	43.5	18.9	78.1	80.1

* changing significantly during the experimental period.

BSP: Bromsulfalein (mg/100 ml).
RB: Rose Bengal-I-131 (counts/min).
ET: Ethanol (mg/l).
I, II: catheter positions.
ICG: Indocyanine Green (Mcg/ml).
GAL: Galactose (mg/l).

method are also given. Indocyanine Green gave the lowest value, those with bromsulfalein and galactose were 10 per cent and those with Rose Bengal-I-131 and ethanol were 30 per cent higher. The mean value of Indocyanine Green was significantly smaller than that of Rose Bengal-I-131 and ethanol.

The hepatic flow in different parts of the liver

The simultaneous measurements from two catheters can be regarded as duplicate determinations of the flow, and the difference between the mean flow in the two positions of the catheters thus expresses the error of the flow determination arising from the varriability of the arteriohepatic venous difference of the test substance. It is seen that this error, expressed as the coefficient of variation, varies much from one substance to another, being smallest for galactose (7 per cent) and largest for Rose Bengal-I-131 (36 per cent). The variance of this difference for bromsulfalein is not significantly different from that of galactose, while those of the other substances are.

The difference between the mean hepatic venous concentrations in the two positions

Table IV. *Statistical data from cases Nos. 1—6.*

	Brom-sulfalein	Indo-cyanine Green	Rose Bengal-I-131	Galactose	Ethanol
CASES WITHOUT SIGNIFICANT CHANGES					
Average change/minute in					
arterial concentration[1]	−0.0095	0.007	5	0.9	−0.13
hepatic blood flow (ml/min)	0.9	7.4	−35.5	−2.0	1.9
Average variation (S. D.) in					
arterial concentration	0.023	0.098	111	17.6	3.96
hepatic venous concentration[2]	0.059	0.127	51	14.4	1.81
Average variation (S. D per cent) of the hepatic blood flow in each experiment					
Method I (actual)	16	52	41	9	11
Method II (actual)	18	26	50	12	15
Method III (estimated)[3]	7	35	75	14	28
ALL CASES					
Hepatic blood flow[4] calculated by					
Method I	1890	1640	2310	1900	1950
Method II	1940	1500	2300	1520	1730
Method III	1880	1480	1930	1550	1570

[1] Same units as Table III.
[2] Regarding the concentration in the two hepatic catheters as duplicates.
[3] Estimated from the averages of change in arterial concentration, volume of distribution, amount infused and arterio-venous difference, and the average S.D. of the arterial concentration (Hald 1955).
[4] Method I: Bradley's method.
— II: method using an average correction for changing arterial concentration.
— III: as method II but using the average arterio-venous differences during the experiment.

of the catheters in the liver was calculated. Statistically different figures, resulting in significant differences in the flow, were found in three experiments, *i.e.* with Indocyanine Green in case No. 1, with ethanol in case No. 2 and with bromsulfalein in case No. 4. This was not caused by a markedly low variation of the hepatic venous concentration in these patients.

The arterio-hepatic venous difference

In Table III further experimental details are given, *i.e.* the mean arterial concentrations and the mean arterio-hepatic venous differences in per cent of the arterial concentration ("the extraction percentage"). The latter varied with the different substances from 20—80 per cent. In some cases continuous changes in the arterial concentration took place. The significance of the changes was evaluated from regression coefficients (with time as independent variable), and those which are statistically significant are marked in the table. Determined in a similar way, significant changes in the extraction during the experiment were found in two

cases in one catheter position, *i.e.* case No. 4 (with bromsulfalein and Indocyanine Green) and in case No. 6 (with bromsulfalein). In these cases the arterial concentration did not change, but such a change can account for the decreasing extraction found with galactose in two cases (Nos. 2 and 5).

The variation in the hepatic flow during the experiment

Since fluctuations of the hepatic blood flow might occur during the experiment, the flow values were recalculated by method II which tends to cancel random changes in the arterial concentration and to emphasize variations in the arterio-hepatic venous difference. From each experiment a correlation coefficient between two methods was calculated and significant positive correlation was found in four of 59 instances, *i.e.* between bromsulfalein and Indocyanine Green ($r = 0.83$) in case No. 2, catheter II, and between bromsulfalein and Rose Bengal-I-131 ($r = 0.91$), between bromsulfalein and galactose ($r = 0.97$), and between Rose Bengal-I-131 and galactose ($r = 0.97$), in case No. 6, catheter I.

From this and the following statistical considerations, cases Nos. 7 and 8 were omitted because only 2 and 4 determinations of the arterio-venous difference were available. In Table IV the average variation in the flow calculated by method II has been compared with that of method I, and it is seen that from a statistical point of view the methods are not different in this respect. The table furthermore gives the average changes in the arterial concentration and the hepatic blood flow during the experimental period in the cases where no significant change occurred. From the same cases the standard deviations of the arterial concentration and the hepatic venous concentration have been determined. The standard deviation of the arterial concentration is somewhat greater, but of the same order of magnitude as the known analytical error, and generally the concentration of the hepatic veins varies no more than in the artery. Taking the standard deviation of the arterial concentration as the maximal analytical error, its influence on the variation of the hepatic blood blow can be estimated by using the averages for amounts infused, volume of distribution and arterio-venous difference (Hald 1955). It is seen from the table that most of the variation in the flow values during the experiments

Table V. *Pressures and oxygen saturation in the two hepatic veins.*

Case No.	Catheter position	Oxygen saturation percentage*	Hepatic venous pressures (mm Hg)**	
			wedged	free
1	I	–	15	3
	II	–	20	5
2	I	83	10	8
	II	79	11	8
3	I	67	9	8
	II	66	9	7
4	I	56	–	–
	II	56	–	–
5	I	61	9	7
	II	60	9	8
6	I	69	9	7
	II	69	–	–
7	I	80	7	3
	II	78	7	3
8	I	73	–	–
	II	70	–	–

* Brinkman hemoreflector.
** Tybjaerg Hansen condensor manometer.

can be accounted for the analytical error in these cases. In Table IV, finally, the values for the flow obtained by method III are given. When they are compared with those of method II, only the values with Rose Bengal-I-131 differ significantly ($p < 0.02$).

Table V demonstrates that no difference in oxygen saturation and free and wedged hepativ venous pressures can be found in the two catheter positions.

DISCUSSION

In determinations of the hepatic blood flow by the indirect FICK-method, two important assumptions are made: a) the test substance is not eliminated outside the splanchnic area, and b) a blood sample from a single hepatic vein is representative of the mixed hepatic venous outflow. Direct measurement of the extrasplanchnic removal of a substance which is mainly removed by the liver is difficult under physiologic conditions, because the arterio-venous difference in other organs is usually unmeasurable, particularly if the perfusion rate is high as, *e.g.*, in the kidney. The opposite approach is to calculate the hepatic uptake from the arterio-venous difference of the substance and the hepatic blood flow and subtract this value from the total body elimination, but this requires a reliable reference substance with no extrasplanchnic removal. Therefore, in man the only possible method is to compare the values for the hepatic blood flow obtained by several test substances, as significantly lower flow corresponds to a lower extrahepatic elimination.

This method is employed in the present study where Indocyanine Green gives significantly smaller values than Rose Bengal-I-131 and ethanol. On the other hand, the lack of significance between the values for Indocyanine Green, bromsulfalein and galactose does not prove that their extrahepatic elimination is identical, as small differences may be concealed by the analytical error. From the present experiments it must be concluded that from the point of view of extrasplanchnic elimination, the methods using Indocyanine Green, bromsulfalein or galactose are equally well suited for hepatic blood flow determination.

It should, however, be noted that the experimental conditions used here in some respects are abnormal. When several substances are administered simultaneously, mutual effects on their elimination are inavoidable. Competitive inhibition tends to give smaller and thus more unreliable determinations of the arterio-venous differences and possibly an increase in the relative importance of the extrasplanchnic removal. Indocyanine Green and Rose Bengal-I-131 probably are inhibited by bromsulfalein (Leevy, Mendenhall, Lesko & Howard 1962, Mendeloff, Kramer, Ingelfinger & Bradley 1949) (*cfr.* Table III) and the extraction of galactose may be affected by ethanol (Tygstrup & Lundquist 1962). Furthermore, the analytical error was not always as small as otherwise possible because the amount of blood taken was kept at a minimum. The importance of this question is stressed by the finding that an essential part of the variation found in the hepatic blood flow could be attributed to the analytical error. Finally it is possible that the metabolic load imposed on the liver by the various test substances may directly cause some of the observed differences, as discussed below.

The question whether a hepatic venous sample represents mixed venous blood in man can only be answered in terms of the variation from one sampling site to another. By moving the hepatic venous catheter into different hepatic veins, Bradley (1950), in eight individuals, found changes in the extraction of bromsulfalein from 1—26 per cent, and this was the motivation for the expression *estimated* hepatic blood flow. In dogs only small differences in the extraction of bromsulfalein have been found (Gilmore 1958, Werner & Horvath 1952, Heinemann, Smythe & Marks 1953, Shoemaker 1960) by simultaneous sampling from the right and left lobes of the liver. By this method no significant differences were found in man by Munkner, Schambye & Tarding (1958). The differences in extraction have been ascribed to arteficial phenomena as regurgitation of blood from the caval vein and changes in the flow due to the presence of the tip of the catheter in the hepatic venous radicles (Sapirstein & Reiniger 1956, Brauer, Shill & Krebs 1959, Shoemaker, Steenburg, Smith & Moore 1961) but this "catheter-induced error" has only been demonstrated in animals.

The fundamental problem raised by the observed variations from one part of the liver to another is how local changes in the perfusion in the liver is related to changes in hepatic extraction and *vice versa*. A substance is ideally suited for blood flow determination if its elimination is proportional to the perfusion. Then the fraction of the hepatic blood flow perfusing the area from which the hepatic venous sample is taken equals the fraction of the hepatic elimination removed in the area, and it is immaterial if the sample is taken from a richly or a poorly perfused area. On the other hand, if local differences in extraction do occur, it is not known if the area with low extraction has a relatively high perfusion or low extraction. No precise information of the effect of changes in flow on extraction is available. Regarding bromsulfalein the relationship seems to be rather complicated. High perfusion rates resulted in a decreased efficiency in extraction in the experiments of Brauer, Pessotti & Pizzolato (1951) ("imperfect utilization at high throughput rates") and in patients with a porto-caval anastomosis the low hepatic blood flow results in a high extraction (Bradley, Smythe, Fitzpatric & Blakemore 1953).

Simultaneous determinations of the extraction of several substances in two areas of the liver were performed with the intention of elucidating this question. It was conjectured that variations caused by changes in the elimination might not have the same effect on all the substances which are eliminated by different mechanisms, in contrast to the effect of variations in the flow. It appeared that a considerable variation took place during an experiment, and in the majority of the cases the flows determined by the different substances were not correlated. Thus the variations are probably not caused by a common factor, *viz.* the flow, and consequently they must be considered as random, whatever their cause may be. The methods for hepatic blood flow determinations may be evaluated on the basis of this random variation, as shown in Table II where it is estimated from the difference between the values in the two catheter positions. It is seen that the substances with a high extraction have a relatively small variation. This agrees with the demands on the ideal test

substance. The elimination of this substance should only depend on the flow, which is the case when the extraction is 100 per cent.

Recognition of the random nature of the variations in the arterio-hepatic venous difference during an experiment calls for a revision of the method for calculation of the hepatic blood flow in cases where no *a priori* reasons for changes in the hepatic flow exist. In this case the average arterio-hepatic venous difference throughout the experiment should be employed. This furthermore has the advantage that a systematic error is avoided. When the average flow is calculated from single flow values, determined by the conventional method, one may get too high figures when the extraction is small, because the flow, other things equal, is a hyperbolic function of the arterio-venous difference. Thus, by an equal number of positive and negative deviations from the true arterio-venous difference (caused by the analytical error) the negative deviations — resulting in large flow — will dominate. It is furthermore suggested to use an average correction for changes in the arterial concentration which, in Bradley's method, is determined by the change from one sample to another, since a great part of the variation in the arterial concentration apparently depends on the analytical error. In the present material no great differences in the absolute values or the variations are found with the various methods of calculation, and the advantage of the method suggested is chiefly theoretical (except regarding Rose Bengal-I-131), but it may be of practical importance in cases with a small extraction, as *e.g.* in liver diseases.

While the present material does not demonstrate that significant changes in the flow or the extraction in different parts of the liver are a common phenomenon, such changes may occur. They were found in about 5 per cent of the cases (if the measurement in a single hepatic catheter with one substance is regarded as a determination of the hepatic flow). They may either appear as a difference between the flow values from the two catheters seen constantly during the experiment, or as a continuous change in the arterio-venous difference in one part of the liver. As identical changes were not found simultaneously with all substances, they are presumably caused by changes in the local extraction of the substance in question and, if only a single substance were employed, it would be registered as changes in the flow. The cause of such changes which have also been demonstrated in other materials (Winkler & Tygstrup 1965) is obscure, but they may be related to an alternating metabolic activity in different parts of the liver. Such experiments, where no obvious reason for changes in flow exists, should probably be discarded.

From the considerations given above it follows that among the available methods for determination of the hepatic blood flow in man, the one should be preferred which 1) gives the smallest flow values *i.e.* has the smallest extrahepatic elimination 2) is uniformly extracted in the liver and 3) has the highest extraction. None of the substances employed here fulfills all the requirements and therefore no definite choice of method can be given from the present studies, and the ideal substance still has to be sought for.

SUMMARY

In eight subjects the hepatic blood flow has been determined by five test substances, using simultaneous sampling of hepatic venous blood from two parts of the liver. The following conclusions are reached.

The extrahepatic elimination is smallest employing Indocyanine Green and becomes of rising importance in the following order: bromsulfalein, galactose, Rose Bengal, and ethanol.

The error of the flow methods, as judged from the difference between the two catheters, is smallest for galactose and bromsulfalein (about 10 per cent) and about 25 per cent in the remaining cases.

A considerable variation in the flow values is found during the experiments, but usually no correlation was observed between the different methods, indicating that changes in the flow were not the main cause. The analytical error contributes much to the observed variation. A method for calculation of the flow is suggested, whereby influence of this variation is reduced. Its principle is to use an average correction for changing arterial concentration and the average arterio-hepatic venous difference.

In about five per cent of the determinations, changes in the flow or a constant difference in the flow were seen in the two parts of the liver. They are considered to be due to local changes in extraction and not to local changes in perfusion.

From these findings the requirements of an ideal substance for hepatic flow determination are discussed.

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