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METHODS OF THE ASSESSMENT OF THE EFFECT OF DRUGS ON LIVER BLOOD FLOW IN MAN

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The liver is the central metabolic organ of the body and therefore the volume and the composition of the blood perfusing the liver are undoubtedly major determinants of hepatocellular function. In the past the amount of liver blood flow and its regulation was mainly investigated by physiologists and most of the work was done in animals (Bradley, 1963; Greenway & Stark, 1971). In recent years clinicians and pharmacologists have paid more attention to the problem of liver blood flow measurements as it was found that drug metabolism can be influenced by changes in liver blood flow under certain experimental conditions (Ohnhaus, Thorgeirsson, Davies & Breckenridge, 1971; Branch, Shand, Wilkinson & Nies, 1974). Based on these findings a physiological approach to hepatic drug clearance has been developed which seems very useful in predicting changes in drug kinetics produced by variations in the biological determinants of drug disposition (Wilkinson, 1975; Wilkinson & Shand, 1975). In addition, as surgeons started to treat portal hypertension occurring in liver disease such as cirrhosis by portocaval or similar anastomoses, liver blood flow was assessed with other parameters of liver function to select appropriate patients for surgery (Bircher, Blankart, Halpern, Häcki, Laissue & Preisig, 1973). These facts have increased tremendously the interest of clinical pharmacologists and clinicians in the methods for liver blood flow measurement and their limitations in order to investigate changes in drug metabolism under certain clinical and experimental conditions and the clinical outcome of liver disease following different operating procedures.

Anatomy and physiology

Some basic principles of anatomy and physiology which are necessary for the understanding of this review will be presented. More information about the anatomy of the liver and the physiology and the regulation of liver blood flow are given extensively elsewhere (Grayson & Mendel, 1965; Greenway & Stark, 1971). Under physiological conditions a dual blood supply to the liver occurs, one by the hepatic artery and one by the portal vein, whereby the hepatic artery provides the main source of arterial blood for liver. On the other hand, the portal vein receives the venous blood from the main splanchnic organs stomach, spleen, pancreas and intestine. Both the hepatic artery and the portal vein are drained in special capillaries of the liver called sinusoids and afterwards into the hepatic veins. In addition, arteriolovenular communications as capillary connections exist between the hepatic artery and portal vein and therefore a shunt of the blood stream off the sinusoidal capillaries can occur. Some of the problems in the measurement of liver blood flow arise by this dual circulation and the shunts between both flowing systems.

The average value of liver blood flow in animals and man measured by a variety of methods is about 100 ml min^{-1} g^{-1} liver for total liver blood flow which represents about 25% of the cardiac output. Thereby, the liver receives 25 to 30% of its blood supply from the hepatic artery, while 70 to 75% is provided by the portal vein.

Methods for measuring liver blood flow

Based on the dual circulation of the liver the quantitative evaluation of liver circulation has proved extremely difficult and is enhanced by differences arising from different animal species. Regardless of the species, surgical procedures of some kind are usually necessary and continuous observation over an extended period needs excellent experimental skill. With all methods available further limitations arise in liver blood flow measurements in man; these will be discussed mainly in the present article. All the major methods used can be divided into direct and indirect measurements of liver blood flow and are given in Table 1.

1. Direct methods

The mechanical stromuhr, the venous outflow method, the bubble flowmeter and the rotameter are the historical ancestors of the electromagnetic flowmeter which is nowadays extensively used. The transillumination method (Wakim & Mann, 1942; Grafflin, 1947; Knisely, Bloch & Warner, 1948; Seneviratne, 1949) is based on a microscopic observation of the

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transilluminated liver edge by high magnification in order to study the blood stream within the sinusoids and the movements of material from the blood into the parenchymal cells and bile canaliculi. However, the conditions under which this observation must be made are unphysiological and limit the extent of conclusions that can be made.

In contrast, the heat exchange method and the perfusion experiments have still value for certain experiments. In man only the electromagnetic flowmeter and the heat exchange method have been used under certain experimental conditions.

a) Electromagnetic flowmeter

The electromagnetic flowmeter (Kolin, 1936; Wetterer, 1937) depends on the principle that the movement of a conductor at right angles to the lines of force of a magnetic field induces an electrical potential. The conductor is the stream of blood passing between the poles of a magnet. The induced current is led off by electrodes placed across the conduit of the stream. The current is proportional to the velocity of the stream and its polarity is determined by the direction of the stream.

The probe containing the magnet and the electrodes can be attached to the hepatic artery and portal vein. In general the advantages of the electromagnetic flowmeter are its use on large and small vessels, on cannulated and uncannulated vessels, in chronic and acute experiments. Detailed phasic changes in the velocity, volume and the pattern of forward and backflow can be separately recorded. It has a linear calibration curve with a high frequency response. On the other hand, the problems in the use of electromagnetic flowmeters include the error in baseline stability, which is mainly based on the electrical characteristics of the model used, the stability of the zero offset and the sensitivity of flowprobes in long term *in vivo* experiments (Wyatt, 1966; Sellers & Dobson, 1968). Only extensive calibration experiments before, during and after the experiment solve these problems. In man electromagnetic flowmeters can be used during acute surgery (Schenk, McDonald, McDonald & Drapanas, 1962) and therefore this method is limited only to a few human experiments including all the problems of changed liver blood flow during anaesthesia (Levy, Palazzi, Nardi & Bunker, 1961; Pichlmayr, 1969). Despite the availability of an accurate method in the measurement of liver blood flow by which also a separation of arterial and venous inflow into the liver would be possible, this method is only of limited value in man.

Table 1 Methods of liver blood flow measurements

1. *Direct methods*
   - Mechanical stromuhr
   - Measurement of venous outflow
   - Bubble flowmeter
   - Rotameter
   - Electromagnetic flowmeter
   - Transillumination technique
   - Heat exchange method
   - Perfusion of the hepatic vessels
2. *Indirect methods*
   - Clearance technique
   - Single injection method
   - Fractional distribution method
   - Inert gas outwash technique

b) Heat exchange method

The heat exchange method (Gibbs, 1933) is based on the principle of heating by a constant current, a thermocouple which is implanted into the liver and cooled by the blood flow. Increased blood flow cools the relatively hot thermocouple, decreased flow permits it to warm up. The results are expressed in thernal conductivity ($Cal \cdot cm^{-1} \cdot s^{-1} \, °C^{-1}$) and all changes are expressed in changes in conductivity. There is a linear correlation in the physiological range of blood flow between thermal conductivity and blood flow measured in experimental animals (Grayson & Johnson, 1953). Two different methods using this principle are available. The principle of internal calorimetry (Grayson, 1952) uses a cyclic current for heating of the thermocouple in order to avoid external local heat production (Grayson & Mendel, 1954), while a constant current is supplied to the thermocouple utilizing the method of Hensel, Ruef & Golenhofen (1954). However, this measurement is semiquantitative and only changes in total liver blood flow can be measured, but the advantage is that measurements can be made over a longer period of time. This method was usually designed for animal experiments and was used in rats for long-term investigations (Ohnhaus, Emons & Breckenridge, 1970) but was also applied in man, whereby the thermocouple was implanted into the liver through a biopsy needle using a subcostal transcutaneous approach and changes after insulin and glucose administration were measured (Grayson & Kinnear, 1962). Therefore this thermocouple could be inserted into the liver for a shorter period of time by transcutaneous puncture, during operation or by peritoneoscopy; using the latter methods the liver could be seen and the thermocouple implanted under the view of the investigator.

Another approach utilizing the principle of a heated thermocouple and designing a special flowprobe was performed by Demling & Gromotka (1959). The site of measurement was the capillary region of the mucous membrane of the sigmoid colon as its blood drains into the portal vein system. The authors assumed that there was a clinically usable relationship between the blood flow in this capillary region

and the portal vein volume per unit time. This method was used in testing the effect of different physiological stimuli, pharmacological substances and for following the external application of heat or cold over the external part of the liver and between the lying and standing position (Demling & Gromotka, 1959). However, this modified method was never very widely used in liver blood flow measurement.

In summary, some limitations exist in using the heat exchange method and these restrict its application to man. It is semiquantitative with different baseline conductivities depending upon the position of the thermocouple, whereby a thermocouple sited in a bigger vessel gives a higher baseline conductivity than in a low flow area (Grayson & Johnson, 1953). The calibration of the flowprobe has to be done in dead liver or gelatine before and after the experiment in the same position as during the experiment. Formation of connective tissue can occur but this is rare and is soon realized during the daily measurements by a rapid decrease of thermal conductivity to the range obtained in a dead liver (Ohnhaus *et al.*, 1970).

2. Indirect measurements

While the direct measurements of liver blood flow usually need extensive operation, indirect measurements can be used without opening the abdomen and are applicable to man but most of them estimate total liver blood flow and a separation between hepatic arterial and portal venous inflow is not possible.

a) Clearance technique

The clearance method is based on Fick's principle by which organ flow can be measured according to the following formula

$$HBF = \frac{I}{C_A - C_{HV}}$$

whereby I is the indicator infusion rate, C_A the arterial concentration and C_{HV} the hepatic vein concentration of the indicator.

As indicator, a constant infusion of dyes like bromsulfphthalein, rose bengal and indocyanine green is performed (Bradley, Inglefinger, Bradley & Curry, 1945; Simpson, Ezrow & Sapirstein, 1954; Ketterer, Weigland & Rappaport, 1960) and blood sampling in a peripheral artery and the hepatic vein are necessary for the colorimetric estimation and calculation according to Fick's principle. The hepatic extraction ratio obtained by the catheterization of the hepatic vein is an important part of the estimation of hepatic blood flow. The problem of the clearance techniques using dyes is the extrahepatic loss which is smallest using indocyanine green as indicator even in patients suffering from hepatic cirrhosis (Preisig, Rankin, Sweeting & Bradley, 1966; Reemtsma, Hollinger, de Graff & Creech, 1960). The necessity of hepatic vein catheterization, which is possible in about 90% of patients under fluoroscopic control (Reynolds, Redeker & Geller, 1957) makes this method an invasive investigation. The blood samples obtained from the hepatic vein are influenced by the position of the catheter. Evidence has been presented, that the depth of the catheter influences the concentration of the sample (Sapirstein & Reininger, 1956). The deeper the catheter and the closer to wedging the lower the values obtained. Furthermore the accuracy is questioned if the extration ratio is less than 10–15%, ratios which are not uncommon in liver disease (Bradley, Ingelfinger & Bradley, 1952). In addition, there is indication of enterohepatic recirculation mainly when bromsulfphthalein is used (Lorber, Oppenheimer, Shay, Lynch & Siplet, 1953). However, this method using indocyanine green as an indicator is a widely performed investigation in man but it must be remembered that this technique has its limitations in disease, is an invasive investigation and only gives reliable results if the catheter in the hepatic vein is in the right position.

As the uses of indocyanine green dye, bromsulfphthalein, or other substances cleared by the liver are too invasive for routine use, a simpler approach was performed using a drug as an indicator based on the perfusion limited model suggested by Wilkinson & Shand (1975). If a drug is completely absorbed from the intestine, metabolism after oral administration is first order and there is no extrahepatic elimination, then hepatic blood flow can be calculated from the dose of this drug administered divided by the difference in the areas under the concentration v time curves obtained following intravenous and oral administration according to the following formula:

$$HBF = \frac{D}{AUC_{i.v.} - AUC_o}$$

where D is the drug dose, $AUC_{i.v.}$ the area under the concentration v time curve of systemic blood following intravenous and AUC_o the area following oral administration. This relationship implies that the AUC_o after an oral dose can be used to estimate the hepatic venous concentrations after systemic administration of the same dose. This approach has been used in man with imipramine and nortriptyline (Gram & Christiansen, 1975; Gram & Overo, 1975), but the dose was given once orally and once intravenously on two separate occasions. Therefore, if a drug is available in a radiolabelled form, the cold drug could be given by one route and the radioactive administered simultaneously by the other route. The advantage of simultaneous administration of the marker drug by both routes is that day to day variations will be avoided and equivalent kinetics of hepatic drug removal will be assured. A simultaneous administration was used with propranolol to estimate

hepatic flow (Nies, Shand & Wilkinson, 1976) and values of about 1.6 l/min were calculated. However, if portocaval anastomoses are present this method will give falsely high values for liver blood flow because the calculated value will include depatic blood flow plus shunt flow. If hepatic blood flow is known or estimated independently by another method, shunt flow can actually be calculated.

b) The single injection method

Some of the problems arising from the clearance technique were overcome by the single injection method. As indicators, colloidal substances taken up by the Kupfer-cells of the liver such as P^{32} labelled chromic phosphate, radioactive gold Au^{198} or I^{131}-human serum albumin are injected rapidly (Dobson & Jones, 1952; Vetter, Falkner & Neumayr, 1954; Caesar, Shaldon, Chiandussi, Guevara & Sherlock, 1961). The uptake of these substances is estimated by venous sampling in a peripheral vein or by external counting using a scintillation collimator or a gamma-camera. When the measurement of the disappearance of these radioactive substances, after intravenous injection, over the forehead, the calves and the uptake over the liver was made, the same disappearance rate constants were found (Vetter *et al.*, 1954; Torrance & Gowenlock, 1962; Ohnhaus & Locher, 1975). Therefore both curves can be used for measuring the elimination rate constant using the calculation of the colloidal clearance according to the following formula:

$$HBF = K_e \cdot BV$$

whereby K_e is the elimination rate constant of the colloid or 'colloidal disappearance constant' and BV the blood volume which has to be additionally estimated using Evans blue or another indicator for the measurement of plasma volume corrected by the haematocrit. Usually no hepatic venous catheterization is necessary, as extraction ratios of about 90% were found (Dobson, Warner, Finney & Johnstone, 1953) and therefore the calculation of the colloidal clearance is a reasonable approximation of liver blood flow. However, some incomplete extraction by an extrasplanchnic removal of the material, particularly in the bone marrow or possibly elsewhere might be likely (Vetter *et al.*, 1954). Differences in extraction can be observed having a nonuniform particle size of the colloidal substance, whereby mainly the small particles show a recirculation (Dobson & Jones, 1952). The toxicity of the material, the radioactivity which is usually not very extensive and the presence of counting equipment have to be taken into account as disadvantages of this method. In addition, in cirrhotic patients the hepatic extraction of these substances varies greatly from one patient to another (Huet, Marleau, Lavoie & Vialett, 1976). This decrease in hepatic extraction is mainly due to factors such as changes in the liver microcirculation. Thus decreased hepatic extraction can be related to part of the portal blood bypassing the sinusoids (intrahepatic porto-hepatic shunts) or to sinusoidal changes responsible for ineffective phagocytosis.

c) Fractional distribution method

The fractionation of the cardiac output to the different organs of the body including the liver is based on the intravenous injection of a radioactive substances like $K^{42}Cl$, $Rb^{86}Cl$ or I^{131}-antipyrine which are homogenously distributed in the body and taken up by the organs (Sapirstein, 1956; 1958). However, this method has only been useful in animals because the different tissues have to be removed and counted in a scintillation counter. Utilizing radioactive microspheres tagged with different isotopes like chromium, caesium, strontium and iodine in a size of 7–50 μ obstructing the capillary vessels of the organs investigated have the same limitation but some other difficulties such as mixing at the injection site and in the left ventricle of the heart (Wagner, Rhodes, Sasaki & Ryan, 1969) have to be taken into account.

d) Inert gas washout technique

Finally the inert gas washout technique provides a possibility of measuring liver blood flow. Inert radioactive gases like ^{85}krypton (Tobias, Jones, Lawrence & Hamilton, 1949) or ^{133}xenon (Conn, 1955) can be injected in a supplementary vessel, hepatic artery or portal vein and the washout curve over the liver is recorded by external counting using a scintillation detector or gamma camera (Rees, Redding & Ashfield, 1964; Birtch, Casey & Zakheim, 1967; Darle, 1970). In man the hepatic vessels must be taken as injection site for the measurement of liver blood flow, in patients undergoing abdominal surgery. Sometimes, an umbilical vein can be cannulated with a catheter inserted and used for injection of the inert gas for several measurements over a period of days (Künzli & Fridrich, 1972). Using this approach an operative procedure is still necessary. Therefore, the indirect application of the tracer by inhalation (Schmitz-Feuerhake, Huchzermeyer & Reblin, 1975; Dietze, Wicklmayr, Czempiel, Henftling, Hepp & Mehnert, 1975) provides some further advantage for liver blood flow measurements in man. 5 mCi ^{133}xenon in 5 l oxygen are inhaled from a spirometer in a closed system over a period of 5 min and the total radioactivity is recorded over the liver by a scintillation detector. After 5 min the spirometer is removed and the xenon washout curve is recorded over a period of 15 min. In addition, the exhalation of ^{133}xenon from the lungs is measured by an external scintillation detector as it was found that the exhalation curve represents the concentration in an arterial vessel (Veall

& Mallett, 1966). The curves obtained by external recording over the liver area are complex and can be difficult to analyse as most contain several compartments. Therefore for the calculation of liver blood flow a compartment analysis has to be done. As the true intrahepatic clearance is monoexponential (Darle, 1970) the first fast part of the washout curve recorded over the liver is used for calculation of liver blood flow according to the following formula,

$$HBF = K_e \cdot \lambda \text{ (ml min}^{-1} \text{ g}^{-1} \text{ liver)}$$

whereby K_e is the fast elimination rate constant of the washout curve and λ the partition coefficient of xenon between liver and blood having a value 0.74 (Conn, 1961). Taking the multiexponential washout curve recorded over liver and using a special computer program (de Valois, Smith & Peperkamp, 1970) for analysis, mean liver blood flow values of 40 ml min^{-1} 100 g^{-1} liver were found (Ohnhaus, Ramos & Noelpp, 1978). These values were reproducable under defined baseline conditions even in the same volunteer at different times. Therefore, the xenon washout curve obtained by external recording over the liver represents liver blood flow even regarding the fact that the values calculated are too low compared to other methods. These differences are based on an accumulation of xenon in some part of the gut which clears it very slowly (Strandell, Erwald, Kulling, Lundbergh, Marions & Weichel, 1973). This reservoir of xenon alters the shape of the hepatic washout curve and will appear as a slow component influencing the fast part of the curve. To overcome these problems Schmitz-Feuerhake *et al.* (1975) measured the exhalation of xenon which represents the arterial concentration, the washout from spleen and liver and calculated liver blood flow by an analogue computer model using a three-compartment model. Another approach was to measure the xenon washout curve by a gamma-camera and record the activity over the lungs and the total abdomen on a magnetic tape using a Digital PDP 11/40 computer system (Ohnhaus *et al.*, 1978). In addition, 1 mCi ^{99m}-Technetium-sulfur colloid was injected, which was used to mark the position of the liver and for the estimation of liver size according to the method of Walk (1960). The activity recorded over the lungs represented the arterial part of liver blood flow. The splanchnic part was obtained by subtracting lung and liver areas from the total gamma-camera field. As the hepatic artery contributes 30% and the portal vein 70% of total liver blood flow the arterial and splanchnic washout curve was corrected for this percentage and sensitivity per scintillation cell of the gamma-camera and a corrected curve was calculated. Using both methods a mean liver blood flow of 94 ± 20 ml min^{-1} 100 g^{-1} liver was found, a value which is in good agreement with other methods.

The inert gas washout inhalation technique has the advantage of being non invasive and applicable several times to the same patient and even to volunteers as the radioactivity administered is comparably low. However, a scintillation counting system or a gamma-camera with a sophisticated computer system is necessary. A further problem is the partition coefficient of xenon which might be influenced by the fat content of the liver, by administration of certain drugs and by liver disease. The problem of increased fat content can be solved by a double isotope measurement for indirect determination of fat, (Kitani, Ladefoged, Winkler & Tygstrup, 1970) and following the administration of different drugs like antipyrine and phenobarbitone and in experimental cirrhosis no changes in partition coefficient were found (Ohnhaus, unpublished results). Therefore, this non invasive technique seems promising in the future for the measurement of liver blood flow in man.

Conclusion

All methods discussed in the present review have their advantages, disadvantages and limitations, but in designing experiments to assess liver blood flow in man the choice of the appropriate technique will play an important role in the achievement of reasonable results under different experimental conditions.

References

BIRCHER, J., BLANKART, R., HALPERN, A., HAECKI, W., LAISSUE, J. & PREISIG, R. (1973). Criteria for assessment of functional impairment in patients with cirrhosis of the liver. *Eur. J. clin. Invest.*, **3**, 72–85.

BIRTCH, A.G., CASEY, B.H. & ZAKHEIM, R.M. (1967). Hepatic blood flow measured by the krypton-85-clearance technique. *Surgery*, **62**, 174–180.

BRADLEY, S.E. (1963). The hepatic circulation. In *Handbook of Physiology, Circulation II.* pp. 1387–1438. Washington D.C.

BRADLEY, S.E., INGELFINGER, F.J. & BRADLEY, G.P. (1952). Hepatic circulation in cirrhosis of the liver. *Circulation*, **5**, 419–429.

BRADLEY, S.E., INGELFINGER, F.J., BRADLEY, G.P. & CURRY, J.J. (1945). The estimation of hepatic blood flow in man. *J. clin. Invest.*, **24**, 890–897.

BRANCH, R.A., SHAND, D.G., WILKINSON, G.R. & NIES, A.S. (1974). Increased clearance of antipyrine and d-propranolol after phenobarbital treatment in the monkey. Relative contributions of enzyme induction and increased hepatic blood flow. *J. clin. Invest.*, **53**, 1101–1107.

CAESAR, J., SHALDON, S., CHIANDUSSI, L., GUEVARA, L. & SHERLOCK, S. (1961). The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. *Clin. Sci.*, **21**, 43–57.

CONN, H.L. (1955). Measurement of organ flow without blood sampling. *J. clin. Invest.*, **34**, 916–917.

CONN, H.L. (1961). Equilibrium distribution of radioxenon in tissue: xenon-hemoglobin association curve. *J. appl. Physiol.*, **16**, 1065–1070.

DARLE, N. (1970). Xenon^{133} clearance and liver blood flow. An experimental study in the cat. *Acta Chir. Scand.* (Suppl.), **407**, 1–64.

DEMLING, L. & GROMOTKA, R. (1959). The influence of physiologic irritations on enteroportal blood flow in man. Experimental investigations with a bloodless calorimetric method. In *Proceedings World Congress in Gastroenterology*, pp. 539–541. Baltimore: The Williams & Wilkins Co.

DEMLING, L. GROMOTKA, R. (1960). Ueber den Einfluss von heissen Kataplasmen und Bettruhe auf die Durchblutung der menschlichen Leber in therapeutischer Sicht. *Klin. Wschr.*, **12**, 599–602.

DE VALOIS, J.C., SMITH, J. & PEPERKAMP, J.P.C. (1970). A computer program for the determination of cerebral blood flow using the Kr-85 OR Xe-133 intra-arterial injection method. *Bio-Med. Computing*, **1**, 49–60.

DIETZE, G., WICKLMAYR, M., CZEMPIEL, H., HENFTLING, H.G., HEPP, K.D. & MEHNERT, H. (1975). Zur Analyse der lebervenössen ^{133}Xenon-Clearance nach Zufuhr des Gases durch Inhalation. *Klin. Wschr.*, **53**, 639–640.

DOBSON, E.L. & JONES, H.B. (1952). The behavior of intravenously injected particulate material: Its rate of disappearance from the blood stream as a measure of liver blood flow. *Acta med. scand.*, **144**, suppl. 273., 1–71.

DOBSON, E.L., WARNER, G.F., FINNEY, C.R. & JOHNSTONE, M.E. (1953). The measurement of liver circulation by means of the colloid disappearance rate: I. Liver blood flow in normal young men. *Circulation*, **7**, 690–695.

GIBBS, F.A. (1933). Thermoelectric blood flow recorder in form of a needle. *Proc. Soc. exp. Biol. Med.*, **31**, 141–146.

GRAFFLIN, A.L. (1947). The excretion of fluorescein by the liver under normal and abnormal conditions observed *in vivo* with the fluorescence microscope. *Am. J. Anat.*, **81**, 63–116.

GRAM, L.F. & CHRISTIANSEN, J. (1975). First pass metabolism of imipramine in man. *Clin. Pharmac. Ther.*, **17**, 555–563.

GRAM, L.F. & OVERO, K.F. (1975). First pass metabolism of nortriptyline in man. *Clin. Pharmac. Ther.*, **18**, 305–314.

GRAYSON, J. (1952). Internal calorimetry in the determination of thermal conductivity and blood flow. *J. Physiol.*, **118**, 54–72.

GRAYSON, J. & JOHNSON, D.H. (1953). The effect of adrenaline and noradrenaline on the liver blood flow. *J. Physiol.*, **120**, 73–94.

GRAYSON, J. & KINNEAR, T. (1962). Observations on temperature, blood flow and heat production in the human liver in relation to environment and to glucose and insulin administration. *Clin. Sci.*, **22**, 125–140.

GRAYSON, J. & MENDEL, D. (1954). The role of the spleen and the hepatic artery in the regulation of liver blood flow. *J. Physiol.*, **136**, 60–79.

GRAYSON, J. & MENDEL, D. (1965). *Physiology of the splanchnic circulation*. London: Edward Arnold Ltd.

GREENWAY, C.V. & STARK, R.D. (1971). Hepatic vascular bed. *Physiol. Rev.*, **51**, 23–65.

HENSEL, H., RUEF, J. & GOLENHOFEN, K. (1954). Fortlaufende Registrierung der Muskeldurchblutung am Menschen mit einer Calorimetersonde. *Pflügers Arch.*, **259**, 267–280.

HUET, P.-M., MARLEAU, D., LAVOIE, P. & VIALLET, A. (1976). Extraction of ^{125}I-albumin microaggregates from portal blood. An index of functional portal blood supply in cirrhotics. *Gastroenterology*, **70**, 74–81.

KETTERER, S.G., WEIGLAND, B.D. & RAPPAPORT, E. (1960). Hepatic uptake and biliary excretion of indocyanine green and its use in estimation of hepatic blood flow in dogs. *Am. J. Physiol.*, **199**, 481–484.

KITANI, K., LADEFOGED, J., WINKLER, K. & TYGSTRUP, N. (1970). Determination of blood flow and lipid content of the liver by the simultaneous use of 85-Kr and 133-Xe in patients and isolated pig livers with portal perfusion. *J. nucl. Med.*, **11**, 335–341.

KNISELY, M.H., BLOCH, E.H. & WARNER, L. (1948). Selective phagocytosis. I. Kgl. Danske Videnskab Selskab. *Biol. Skrifter*, **4**, 1–93.

KOLIN, A. (1936). An electromagnetic flowmeter. Principle of the method and its application to blood flow measurements. *Proc. Soc. exp. Biol. Med.*, **35**, 43–56.

KÜNZLI, H.F. & FRIDRICH, R. (1972). Zur Bestimmung der relativen portalen Leberdurchblutung mit Xenon-133 nach Kanülierung der Umbilikalvene. *Dtsch. med. Wschr.*, **97**, 1159–1161.

LEVY, M.L., PALAZZI, H.M., NARDI, G.L. & BUNKER, J.P. (1961). Hepatic blood flow variations during surgical anesthesia in man measured by radioactive colloid. *Surg. Gynec. Obstet.*, **112**, 289–294.

LORBER, S.H., OPPENHEIMER, M.J., SHAY, H., LYNCH, P. & SIPLET, H. (1953). Enterohepatic circulation of bromsulphalein: intraduodenal, intraportal and intravenous dye administration in dogs. *Am. J. Physiol.*, **173**, 259–264.

NIES, A.S., SHAND, D.G. & WILKINSON, G.R. (1976). Altered hepatic blood flow and drug disposition. *Clin. Pharmacokin.*, **1**, 135–155.

OHNHAUS, E.E., EMONS, E. & BRECKENRIDGE, A. (1970). Long term liver blood flow measurements in rats under physiological conditions. *Pflügers Arch.*, **323**, 182–186.

OHNHAUS, E.E. & LOCHER, J.Th. (1975). Liver blood flow and blood volume following chronic phenobarbitone administration. *Eur. J. Pharmac.*, **31**, 161–165.

OHNHAUS, E.E., RAMOS, R. & NEOLPP, U.B. (1978). Eine Modifizierung der ^{133}-Xenon-Inhalationsmethode zur Messung der Leberdurchblutung am Menschen. In *Nuklearmedizin*. Stuttgart-New York: Schattauer Verlag. In press.

OHNHAUS, E.E., THORGEIRSSON, S.S., DAVIES, D.S. & BRECKENRIDGE, A. (1971). Changes in liver blood flow during enzyme induction. *Biochem. Pharmac.*, **20**, 2561–2570.

PICHLMAYR, I. (1969). Leberdurchblutung, Herzminutenvolumen und andere Kreislaufgrössen unter Einwirkung verschiedener Narkosemittel beim Hund. *Z. Kreisl. Forsch.*, **58**, 759–773.

PREISIG, R., RANKIN, J., SWEETING, J. & BRADLEY, S.E. (1966). Hepatic haemodynamics during viral hepatitis in man. *Circulation*, **34**, 188–197.

REEMTSMA, K., HOLLINGER, G.C., DE GRAFF, A.C. &

CREECH, O. (1960). The estimation of hepatic blood flow using indocyanine green. *Surg. Gynec. Obstet.*, **110**, 353–356.

REES, J.R., REDDING, V.J. & ASHFIELD, R. (1964). Hepatic blood flow measurement with xenon 133. Evidence for separate hepatic-arterial and portal-venous pathways. *Lancet*, **ii**, 562–563.

REYNOLDS, T.B., REDEKER, A.G. & GELLER, H.M. (1957). Wedged hepatic venous pressure. *Am. J. Med.*, **22**, 341–350.

SAPIRSTEIN, L.A. (1956). Fractionation of the cardiac output of rats with isotopic potassium. *Circulation Res.*, **4**, 689–692.

SAPIRSTEIN, L.A. (1958). Regional blood flow by fractional distribution of indicators. *Am. J. Physiol.*, **193**, 161–168.

SAPIRSTEIN, L.A. & REININGER, E.J. (1956). Catheter induced error in hepatic venous sampling. *Circulation Res.*, **4**, 493–498.

SCHENK, W.G., McDONALD, J.C., McDONALD, K. & DRAPANAS, T. (1962). Direct measurement of hepatic blood flow in surgical patients: With related observations on hepatic blood flow dynamics in experimental animals. *Ann. Surgery*, **156**, 463–471.

SCHMITZ-FEUERHAKE, I., HUCHZERMEYER, H. & REBLIN, T. (1975). Determination of the specific blood flow of the liver by inhalation of radioactive rare gases. *Acta Hepato-Gastroenterol.*, **22**, 150–158.

SELLERS, A.F. & DOBSON, A. (1968). Some applications and limitations of electromagnetic blood flow measurements in chronic animal preparations. *Gastroenterology*, **52**, 375–379.

SENEVIRATNE, R.D. (1949). Physiological and pathological responses in the blood vessels of the liver. *Quart. J. exp. Physiol.*, **35**, 77–110.

SIMPSON, A.M., ERZOW, L. & SAPIRSTEIN, L.A. (1954). Measurement of plasma volume with rose bengal. *Am. J. Physiol.*, **177**, 319–324.

STRANDELL, T., ERWALD, R., KULLING, K.G., LUNDBERGH, P., MARIONS, O. & WIECHEL, K.L. (1973). Measurement of dual hepatic blood flow in awake patients. *J. appl. Physiol.*, **35**, 755–761.

TOBIAS, C.A., JONES, H.B., LAWRENCE, J.H. & HAMILTON, J.G. (1949). The uptake and elimination of krypton and other inert gases by the human body. *J. clin. Invest.*, **28**, 1375–1385.

TORRANCE, H.B. & GOWENLOCK, A.H. (1962). Radioactive colloidal clearance techniques to measure liver blood flow in man. *Clin. Sci.*, **32**, 413–423.

VEALL, N. & MALLETT, B.L. (1966). Regional cerebral blood flow determination by ^{133}Xe inhalation and external recording: the effect of arterial recirculation. *Clin. Sci.*, **30**, 353–369.

VETTER, H., FALKNER, R. & NEUMAYR, A. (1954). The disappearance rate of colloidal radiogold from the circulation and its application to the estimation of liver blood flow in normal and cirrhotic subjects. *J. clin. Invest.*, **33**, 1594–1602.

WAGNER, H.N., RHODES, B.A., SASAKI, Y. & RYAN, J.P. (1969). Studies of the circulation with radioactive microspheres. *Invest. Radiol.*, **4**, 374–386.

WAKIM, K.G. & MANN, F.C. (1942). The intraheptatic circulation of blood. *Anat. Record*, **82**, 233–253.

WALK, L. (1960). Roentgenologic determination of the liver volume. *Acta Radiol.*, **55**, 49–56.

WETTERER, E. (1937). Eine neue Methode zur Registrierung der Blutströmungsgeschwindigkeit am uneröffneten Gefäss. *Ztschr. Biol.*, **98**, 26–36.

WILKINSON, G.R. (1975). Pharmacokinetics of drug disposition: Haemodynamic considerations. *Ann. Rev. Pharmac.*, **15**, 11–27.

WILKINSON, G.R. & SHAND, D.G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmac. Ther.*, **18**, 377–390.

WORTHINGTON, G., SCHENK, J.R., MCDONALD, J.C., MCDONALD, K. & DRAPANAS, T. (1962). Direct measurement of hepatic blood flow in surgical patients: With related observations on hepatic flow dynamics in experimental animals. *Ann. Surg.*, **156**, 463–471.

WYATT, D.G. (1966). Baseline errors in cuff electromagnetic flowmeters. *Med. biol. Engng.*, **4**, 17–45.