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VARIATION OF INCORPORATION OF [^{3}H]OROTIC ACID INTO THE NUCLEOTIDE AND RNA FRACTIONS OF DIFFERENT PARTS OF THE SAME LIVER LOBE IN THE RAT

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Abstract—1. Anaesthetized rats were given [^{3}H]orotic acid either intraperitoneally or via a catheter into the hepatic artery with or without degradable starch microspheres.
2. The radioactivity in the acid soluble and RNA fractions of five pieces of the left lateral liver lobes was determined.
3. A variation of the distribution of the precursor into the different parts of the same liver lobe was shown.
4. This variation was most pronounced (3000–17,000 cpm/μg in the acid soluble fraction) when the precursor was administered via the artery and without microspheres.
5. The correlation between the radioactivity in the acid soluble and RNA fractions within each liver piece was 0.85, 0.90 and 0.75 in the three groups respectively.
6. It is suggested that the variation of the distribution depends on circulatory differences within the liver.

INTRODUCTION

For several years we have studied RNA metabolism in rat liver under different conditions, using labelled nucleotide and RNA precursors administered via different routes. (Yngner *et al.*, 1979; Christensson and Stenram, 1985; Teder *et al.*, 1985). However, we have noticed a variation in incorporation of the precursors in the acid soluble fraction and RNA in different parts of the liver. As differences in blood flow to various areas of human liver (Sherriff *et al.*, 1977) and pig liver (Persson *et al.*, 1986) have been reported, we wanted to investigate the possibility that similar differences in blood flow in rat liver might be expressed as an uneven distribution within the tissue of injected radioactive precursors. We also wanted to compare the effects of different administration routes on these possible variations. Thus radioactive labelling in the acid soluble fraction and RNA of five pieces of the left lateral liver lobe was measured after administration of [^{3}H]orotic acid either via the hepatic artery or intraperitoneally. We also analyzed the correlation between the radioactivity in the acid soluble fraction and RNA within each liver piece.

MATERIALS AND METHODS

Animals and liver sampling

15 male Wistar rats (Möllegaard Hansens Avlslaboratorium A/S Denmark) weighing about 200 g, were divided into three groups and anaesthetized with nitrous-oxide-halothane (Hoechst, FRG) (Christensson and Eriksson, 1985). [^{3}H]Orotic acid (New England Nuclear Co, Boston, Mass, U.S.A.) with a specific activity of 20 Ci/mmol, 1 mCi/ml, was given in doses of 0.25 mCi/100 g BW to each animal and administered as follows:

Group No. I (rats 1–5) intraperitoneally, injection time 5 sec.
Group No. II (rats 6–10) via a polyethylene catheter (Intramedic, PE 50, Clay-Adams, Parsippany, N.J., U.S.A.) in the hepatic artery together with 0.2 ml isotonic saline (Teder *et al.*, 1985). Injection time 1 min.
Group No. III (rats 11–15) via a catheter in the hepatic artery together with 0.2 ml amylase degradable starch microspheres (60 mg/ml, Spherex, batch 70635 Pharmacia AB, Sweden) (Teder *et al.*, 1985). Injection time 1 min.

After 20 min the left lateral lobe of the livers was excised. The lobes were rapidly divided into 5 pieces (Fig. 1) and these were frozen separately in liquid N_2 and stored at −80°C.

Chemical analyses

About 200 mg of each liver piece (for details see Fig. 1) was homogenized in a Colworth "Stomacher" (England) with 12 ml cold distilled water at 4°C. Double samples from each homogenate were analysed and the mean values used for calculations. The acid soluble fraction was extracted with cold perchloric acid and the RNA fraction with KOH at 37°C according to Munro and Fleck (1966). DNA was analysed by the technique of Scott *et al.* (1956) as modified by Hinrichs *et al.* (1964).

The radioactivity (cpm) of the fractions was measured in a Packard (Downers Grove, Ill., U.S.A.) Tri Carb 300 liquid scintillation system.

Calculations

The variation of incorporation within each liver lobe was determined by the range of the label in the acid soluble and RNA fractions respectively (Table 1). The covariation of

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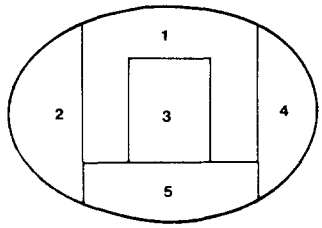

Fig. 1. Sections of the left lateral liver lobe. Pieces No. 2–5 weighed 250–300 mg, and 200 mg was taken from each for analysis. Piece No. 1, weighing 600–800 mg, was homogenized and part of the homogenate, corresponding to 200 mg of tissue, was analysed.

label (cpm/μg) in the two fractions of each liver piece was determined by plotting the RNA labels as functions of the labels of the acid soluble fractions (Fig. 3).

RESULTS

As shown by the large range of values of the radioactivities in the acid soluble and RNA fractions of each liver lobe, the distribution of [^{3}H]orotic acid into the different parts of the lobe was irregular (Table 1, Fig. 2). The variation was increased when the precursor was administered by a catheter into the hepatic artery, especially when the injection was made without microspheres. The irregularity was randomly distributed among the different parts of the liver lobe. The mean differences between double samples were 5% in the whole material.

The label of RNA in relation to that of the acid soluble fraction of each liver piece in each group of 5 rats is shown in the diagrams (Fig. 3). The correlation coefficients are 0.85 ad 0.90 in groups I and II respectively. In the third group, however, the conformity is weaker. The correlation coefficient is only 0.75 because of a poor incorporation of the ^{3}H-activity into RNA in two liver pieces.

DISCUSSION

Our experiment clearly shows that [^{3}H]orotic acid is incorporated into the acid soluble fraction and RNA to various extents in different parts of the same liver lobe in the rat, especially if the precursor is

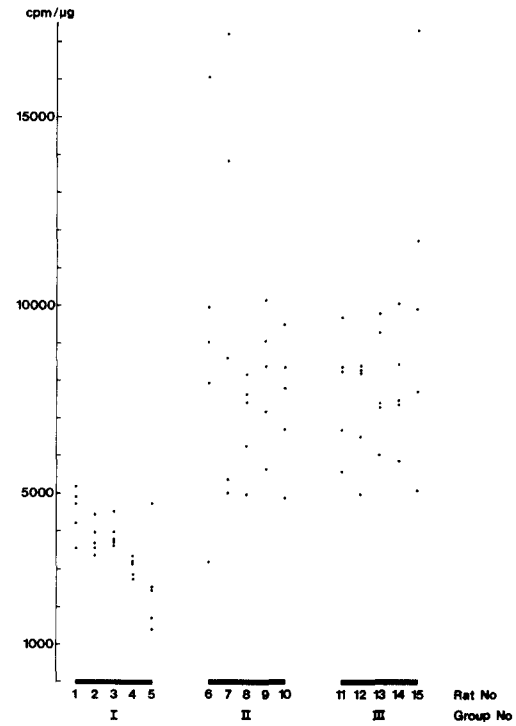

Fig. 2. Specific radioactivity in the acid soluble fraction of five pieces taken from the left lateral liver lobe of each rat in groups I–III (I: intraperitoneal injection; II: i.a. injection into the hepatic artery; III: i.a. injection with degradable starch microspheres into the hepatic artery.

injected directly into the circulation by way of the liver artery. This may probably be caused by a varying influx of [^{3}H]orotic acid into the tissue due to irregular blood flow to different liver areas and would be consistent with findings in human liver (Sherriff *et al.*, 1977) and pig liver (Persson *et al.*, 1986). On the other hand, several other investigations on the circulation of the entire liver have led to the conclusion that the total blood flow is quite homogeneous. In clearance tests of the liver it has been suggested that all areas receive well-mixed arterial and portal blood. Various compounds, administered by these different routes, have been equally accessible to the parenchy-

Table 1. Specific radioactivity in acid soluble fraction and RNA 20 min after administration of [^{3}H]orotic acid

Group	Rat	Acid soluble fraction (Mean cpm/μg ± SD)	Range	RNA fraction (Mean cpm/μg ± SD)	Range
I	1	4502 ± 653	3523–5165	137 ± 24	101–160
Intraperitoneal	2	3799 ± 418	3367–4434	124 ± 17	107–150
injection	3	3919 ± 357	3614–4515	111 ± 7	103–122
	4	3048 ± 248	2729–3197	92 ± 9	82–104
	5	2553 ± 1314	1371–4736	97 ± 47	50–187
II	6	9239 ± 4629	3173–16066	466 ± 248	157–837
Intraarterial	7	10023 ± 5364	5018–17222	401 ± 309	186–937
injection in	8	6883 ± 1283	4963–8166	235 ± 80	115–308
a hepatica	9	8088 ± 1745	5634–10159	357 ± 70	265–442
	10	7448 ± 1741	4890–9475	307 ± 67	208–375
III	11	7738 ± 1614	5566–9743	305 ± 52	241–377
Intraarterial	12	7302 ± 1496	4998–8411	274 ± 56	195–319
injection with	13	7988 ± 1554	6054–9836	363 ± 84	262–469
microspheres	14	7865 ± 1548	5899–10090	196 ± 121	45–326
in a hepatica	15	10382 ± 4626	5081–17342	403 ± 138	220–585

Mean values ± SD of five pieces of the left liver lobe and range of label in each lobe.

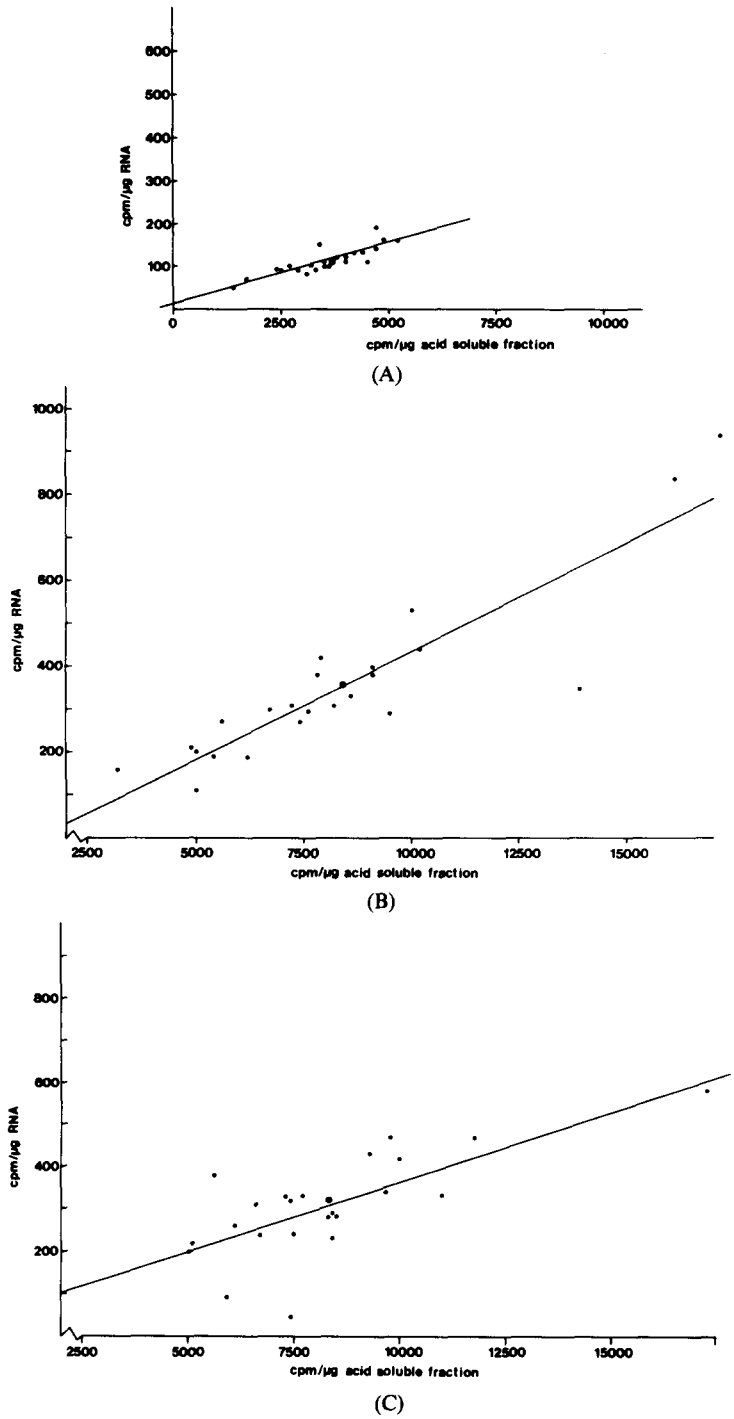

Fig. 3. Specific radioactivity of RNA in relation to acid soluble radioactivity in five pieces of the left lateral liver lobe of each rat in group I–III, 20 min after administration of [³H]orotic acid. A: I: intraperitoneal injection. Corr. coeff. = 0.85. B: II: i.a. injection into the hepatic artery. Corr. coeff. = 0.90. C: III: i.a. injection into the hepatic artery together with degradable starch microspheres. Corr. coeff. = 0.75.

mal cells (Lautt, 1985). For instance, taurocholate, given in either vessel to cats, has been equally effective in stimulating bile flow (Lautt and Daniels, 1983). Hepatic extraction of indocyanine green in cats has also been reported to indicate an even and well balanced blood supply to all parts of the liver (Lautt *et al.*, 1984). Similar findings for ^{85}Krypton clearance were reported in the dog (Blumgart *et al.*, 1977) and for hepatic blood flow distribution after microsphere injections in dogs and cats (Greenway and Oshiro, 1972).

The hepatic arterial buffer response is well described as regulating the total hepatic flow at a constant level (Lautt, 1985). Furthermore, studies on hepatic arterioles and sphincters (McCuskey, 1966) and on adrenergic and cholinergic receptors in liver

microcirculation (Koo *et al.*, 1976, 1977; Koo and Liang, 1977, 1979) tell nothing about any differences between larger liver areas. When indications of heterogeneous distribution of substances in the liver have been reported, most findings have been valid for very small units only, i.e. sinusoids (Teutsch, 1986), lobules (Morrison *et al.*, 1965), zones of the liver acinus (Rappaport and Schneiderman, 1976) or even different cell types within an acinus (Willson *et al.*, 1985). Variations between areas with sizes corresponding to our sections of the liver lobes indicate differences of blood flow to parts of the lobe that are considerably bigger than a lobulus or an acinus. The diameter of a lobulus is 0.7–2 mm (Maximow and Bloom, 1952), which would give an approximate weight of 0.5–3 mg.

Orotic acid, which is known to be taken up very rapidly by rat liver cells (Hurlbert and Potter, 1952) may function as a good indicator of heterogenous precursor incorporation especially after i.v. injection. The intermittent blocking of some of the resistance vessels by the degradable starch microspheres did not increase the variation in the uptake of the administered precursor. Metabolic alterations of the labelled precursor during blocking may be the reason for the decreased correlation between the radioactivity in the acid soluble fraction and RNA. After i.p. administration of the precursor, a successive uptake into the blood from the peritoneal cavity and systemic circulation may have equalized its distribution within the liver lobe, thus reducing the variation of incorporation.

When analysis of the incorporation of radioactive precursors are to be done in rat liver specimens, the possibility of local differences in the distribution of the injected substance should be taken into account. Homogenization of the whole liver, from which aliquots can then be taken for analyses, should reduce the range of the results.

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REFERENCES

Blumgart L. H., Harper A. M., Lieberman D. P. and Mathie R. T. (1977) Liver blood flow measurement with ^{85}Krypton clearance by portal venous and hepatic arterial routes of injection. *Br. J. Pharmacol.* **60,** 278.

Christensson P. I. and Eriksson G. (1985) Effects of six anaesthetic agents on UDP-glucuronic acid and other nucleotides in rat liver. *Acta anaesthesiol. scand.* **29,** 629–631.

Christensson P. I. and Stenram U. (1985) Protein deprivation: altered nucleotide profile and RNA synthesis in rat liver. *Nutr. Rep. Int.* **32**(1), 49–55.

Greenway C. V. and Oshiro G. J. (1972) Comparison of the effects of hepatic nerve stimulation on arterial flow, distribution of arterial and portal flows and blood content in the liver of anaesthetized cats and dogs. *J. Physiol.* **27,** 473–485.

Hinrichs H. R., Petersen R. O. and Baserga R. (1964) Incorporation of thymidine into DNA of mouse organs. *Arch. Pathol.* **78,** 245–253.

Koo A. and Liang I. Y. S. (1977) Blood flow in hepatic sinusoids in experimental hemorrhagic shock in the rat. *Microvasc. Res.* **13,** 315–325.

Koo A. and Liang I. Y. S. (1979) Stimulation and blockade of cholinergic receptors in terminal liver microcirculation in rats. *Am. J. Physiol.* **236**(6), E 728–732.

Koo A., Liang I. Y. S. and Cheng K. K. (1976) Hepatic sinusoidal responses to intraportal injection of phenylephrine and isoprenaline in the rat. *Clin. exp. Pharmacol. Physiol.* **3,** 391–395.

Koo A., Liang I. Y. S. and Cheng K. K. (1977) Adrenergic mechanisms in the hepatic microcirculation in the rat. *Exp. Physiol.* **62,** 199–208.

Lautt W. W. (1985) Mechanism and role of intrinsic regulation of hepatic arterial blood flow: hepatic arterial buffer response. *Am. J. Physiol.* **249**(12), G 549–556.

Lautt W. W. and Daniels T. R. (1983) Differential effect of taurochloric acid on hepatic arterial resistance vessels and bile flow. *Am. J. Physiol.* (7), G 366–369.

Lautt W. W., Legare D. J. and Daniels T. R. (1984) The comparative effect of administration of substances via the hepatic artery or portal vein on hepatic arterial resistance, liver blood volume and hepatic extraction in cats. *Hepatology* **4,** 927–932.

McCuskey R. S. (1966) A dynamic and static study of hepatic arterioles and hepatic sphincter. *Am. J. Anat.* **119,** 455–478.

Maximow A. and Bloom W. (1952) *Textbook of Histology*, 6th edn. Saunders, Philadelphia.

Morrison G. R., Karl I., Schwartz R. and Shank R. E. (1965) The quantitative histochemistry of the normal human liver lobule. *Lab. clin. Med.* **65,** 248–255.

Munro H. N. and Fleck A. (1966) Recent developments in the measurement of nucleic acids in biological materials. *Analyst* **91,** 71–88.

Persson B. G., Jeppsson B., Andersson L., Strand S. E. and Bengmark S. (1986) Arterial liver circulation after repeated transient dearterialisation of the liver in pigs. Accepted in *W. J. Surg.*

Rappaport A. M. and Schneiderman J. H. (1976) The function of the hepatic artery. In *Review of Physiology and Pharmacology*, Vol. 76, pp. 129–175. Springer Verlag.

Scott J. E., Fraccastoro A. P. and Taft E. B. (1956) Studies in histochemistry: I. Determination of nucleic acids in microgram amounts of tissue. *J. Histochem. Cytochem.* **4,** 1–10.

Sherriff S. B., Smart R. C. and Taylor I. (1977) Clinical study of liver blood flow in man measured by ^{133}Xe clearance after portal vein injection. *Gut* **18,** 1027–1031.

Teder H., Christensson P. I., Aronsen K. F., Lewan L., Ljungberg J. and Stenram U. (1985) Hepatic artery administration of degradable starch microspheres. *Res. exp. Med.* **185,** 405–414.

Teutsch H. F. (1986) A new sample isolated procedure for microchemical analysis of functional liver cell heterogenity. *J. Histochem. Cytochem.* **34**(2), 263–267.

Willson R. A., Liem H. H., Miyai K. and Muller-Eberhard U. (1985) Heterogenous distribution of drug metabolism in elutriated rat hepatocytes. *Biochem. Pharmacol.* **34**(9), 1463–1470.

Yngner T., Carlberg E., Lewan L. and Engelbrecht C. (1979) Interrelations between uptake of ^{14}C-orotic acid and labeling of UTP and RNA in rat and mouse after partial hepatectomy. *Hoppe-Seyler's Z. Physiol. Chem.* **360,** 1069–1074.