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Dolichols and proliferating systems

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The results obtained on dolichol metabolism, in two *in vivo* model systems, the developing rat liver and the regenerating rat liver, which provide different timing and interplay of proliferation and differentiation processes, have been reported. The regenerating liver presents a marked increase of both synthesis and content of dolichol, a decreased cholesterol/dolichol ratio, unchanged synthesis and content of dolichyl phosphate, or dolichol-kinase and dolichyl phosphate-phosphatase activities; no significantly modified distribution of dolichol homologs, with respect to the control. Total content of dolichols is growing during perinatal development. At fetal stages only short chain dolichols are detectable, while the content of dolichyl phosphate is very low and the activity of dolichyl phosphate-phosphatase is high.

The study of the role of liver in dolichol supply to the body in the partially hepatectomized rat shows an increased content of dolichol in the blood; blood dolichol is essentially provided by the release from liver and dolichol traffic in the blood is mediated by multiple carriers.

The importance of dolichols in the cell physiology and the role exerted by the liver of vertebrates in the production and interconversion of dolichol compounds has been well established [1].

However, it is still debated whether the liver is responsible for dolichol distribution to other organs or tissues [2] and whether its function is modified during proliferation or differentiation processes.

Little information on dolichol metabolism in physiologically proliferating systems is available.

Our interest has been focused on two *in vivo* model systems: the developing rat liver and the regenerating rat liver, characterized by different cell strategy and providing different timing and interplay of proliferative and differentiative processes.

Actually both systems represent models of well controlled proliferation with a cell program strictly regulated and coordinated by mitogenic and comitogenic factors as shown by the pattern of oncogene product activation or by the growth factors involved [3].

Perinatal liver is a classical model of proliferation and differentiation; it is especially used to study the ontogenic development of cell functions. During development the hepatocytes pass from a low state of differentiation with a high growth rate, typical of fetal and neonatal age, to a highly differentiated state and a progressive lowering of the growth rate, until the quiescent state of adult life. Hypertrophy and hyperplasia prevail in fetal life, hyperplasia in postnatal life.

Regeneration of liver involves tremendous proliferation of the hepatic residual lobes after

[1] Abbreviations: 2-3H-MVA, 2-[^{3}H]mevalonic acid; HMGCoA, 3-hydroxy-3-methylglutaryl CoA; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

70% resection of the liver (partial hepatectomy); this proliferative activity stops at the complete recovery of the original mass. Therefore, adult cells, blocked in G_0 phase of the cell cycle, are stimulated by partial hepatectomy to enter synchronously into the G_1 to go through the cell cycle until S phase and mitosis (16 h and 24 h after surgery, respectively).

Because of good synchronization, the regenerating liver is especially used to study events occurring in specific phases of the cell cycle; it is also employed to investigate the effect of hepatic and extrahepatic factors affecting the onset and the end of proliferation; moreover it can serve as an excellent basis for comparison with uncontrolled proliferation.

Taking into account the important work of authoritative groups in this field (only to mention some: Andrew Kandutsch in Bar Harbor, Frank Hemming in Nottingham, William Lennarz in Baltimore, Gustav Dallner in Stockholm), we supposed that in these proliferating systems the requirement and production of dolichol for building new structures should undergo change.

First, we started the study in these systems following some aspects of dolichol metabolism and, in particular, analysing synthesis and content of dolichols, the activity of the enzymes regulating the dolichol/dolichyl phosphate ratio and the distribution of isoprenologs. More recently we used the partially hepatectomized animal as an experimental tool to study the role of liver in dolichol distribution to the body. In particular, the plasma content and the distribution of dolichol isoprenologs, the association with different classes of lipoprotein and the release by isolated and perfused liver have been measured.

MATERIALS AND METHODS

Partial hepatectomy was performed on male Sprague Dawley rats according to the procedure of Higgins & Henderson [4]. Sham operated animals were used as controls.

Foetuses were delivered by rapid hysterectomy of pregnant Sprague-Dawley rats, anesthetized intraperitoneally with Farmotal (20 mg/100 g of body weight) and the livers immediately removed and cooled on ice. Embryonal age was established by the appearance of the vaginal plug and confirmed by foetal weight and length.

The isolation and perfusion of the liver have been performed according to Kvetina & Guaitani [5].

Plasma lipoprotein classes were isolated and separated according to Havel *et al.* [6].

The 2-3H-MVA incorporation into dolichols liver slices was performed according to Dull *et al.* [7].

The extraction and separation of dolichol and dolichyl phosphate from the liver were performed according to the procedures of Tavares *et al.* [8]; the extraction and separation of dolichols from the blood following the methods reported by Pullarkat *et al.* [9]. Content and chain length distribution of dolichol were analyzed by high performance liquid chromatography (HPLC), on a Perkin Elmer apparatus with an ODS-C-18 reversed phase column using methanol:isopropanol (20:80, v/v) as solvent system. Flow rate was 1 ml/min; the A_{210} variations were measured. Dolichols were eluted as a family of peaks corresponding to 16, 17, 18, 19, 20, 21 isoprene units. The recovery measured using dolichol-22 as internal standard, was equal to 80–85%. The sensitivity of the method allowed to measure at least 1.7 ng of total dolichol.

Liver homogenates were assayed for dolichyl-phosphate phosphatase essentially as described by Rip *et al.* [10].

RESULTS AND DISCUSSION

During the ***perinatal development*** (Fig. 1A, B, C) a progressive increase of dolichol and dolichyl phosphate content was observed, with a minimal content of dolichyl phosphate at prenatal age; at this developmental stage only shorter-chain dolichols were detectable [11], while high activity of dolichyl phosphate phosphatase was measurable (data not shown). These results deserve some consideration.

The progressive increase of dolichol content observed during rat liver development could be fairly consistent with the growth requirement of the hepatocytes. Interestingly, this increase was not always related to the HMGCoA reductase activity and cholesterol synthesis, both presenting a sharp decrease at birth [12]. The very low content of dolichyl phosphate in

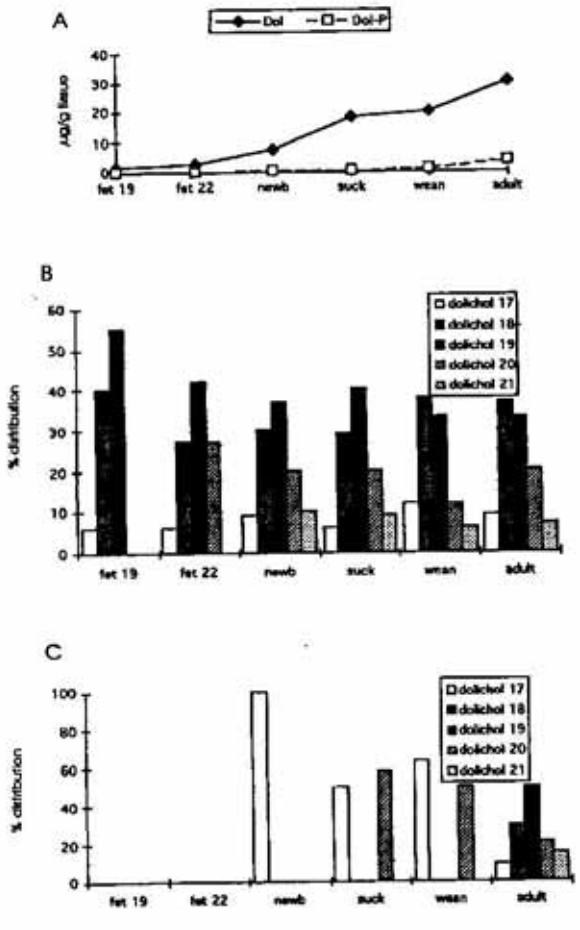

Fig. 1. Dolichol and dolichyl phosphate in developing rat liver.
A, Dolichol and dolichyl-P content; B, distribution of dolichol isoprenols (%); C, distribution of dolichyl-P isoprenols (%). Abbreviations: fet, fetal age (days); dol, dolichol; dol-P, dolichyl-P; new, newborn; suck, suckling; wean, weaning.

fetal liver is particularly intriguing and seems to indicate some differences in the role of these isoprenoid compounds at this stage of development. This is strongly supported by the minor effect exerted by exogenous dolichol on glycoprotein biosynthesis (Marino M., manuscript in preparation). The low content of dolichyl phosphate could be explained by hypothesizing that the free alcohol and its phosphorylated form derive from two distinct biosynthetic pathways. The higher activity of phosphatase measured at this stage is consistent with a higher proportion of dol-P being dephosphorylated, but it does not allow us to discriminate which pathway has been followed. The almost exclusive presence of shorter chain dolichols at fetal stage suggests a stepwise activation of prenyltransferase, which delays the long chain formation to a later stage of development; this probably occurs in order to prevent the strong destabilizing effect exerted by the long chain dolichols on the membrane bilayer [13].

The *regenerating liver* presented (Fig. 2 A, B, C) a marked increase of both synthesis and content of dolichol, but no change in dolichyl phosphate, nor significant modification of isoprenolog distribution [14]; dolichol kinase and dolichyl phosphate phosphatase activity was not changed (data not shown) [15].

The enhanced synthesis and content of dolichol is not always related to modifications of the cholesterogenesis pattern: in fact, during the first cell cycle after partial hepatectomy, a constantly high activity of HMGCoA reductase is accompanied by a fluctuating synthesis of cholesterol from labelled precursors [16]. Therefore the Chol/Dol ratio is low, for example, during the S phase (data not shown).

An increased dolichol content in plasma as well as in the liver was observed in partially hepatectomized animals (16 and 24 h after surgery) (Fig. 2 A). Its transport in the blood by lipoproteins was also changed, with an increased LDL and a decreased HDL involvement (Fig. 2 D). The plasma presented a distribution of dolichols at various chain lengths different from that detectable in the liver. This difference was further enhanced during liver regeneration (Fig. 2 C).

On the other hand, the perfused liver from partially hepatectomized animals released a significantly higher amount of dolichol into the perfusate; the dolichol secreted by perfused liver exhibited a distribution of isoprenologs more similar to that observable in the blood than to that in the liver (Fig. 3 A, B).

These data taken together show that during the proliferative process that follows partial hepatectomy there is a comparable increase of dolichol content in liver and plasma, along with an enhanced capacity of the regenerating perfused liver to release this compound. These findings appear to be peculiar to liver regeneration, and limited to dolichol, since blood cholesterol does not change. The very low concentration of dolichol in blood makes it hard to define the increase detectable in blood during liver regeneration as a simple discharge by the liver, while the short life-time in the blood suggests an uptake by other organs or by the liver

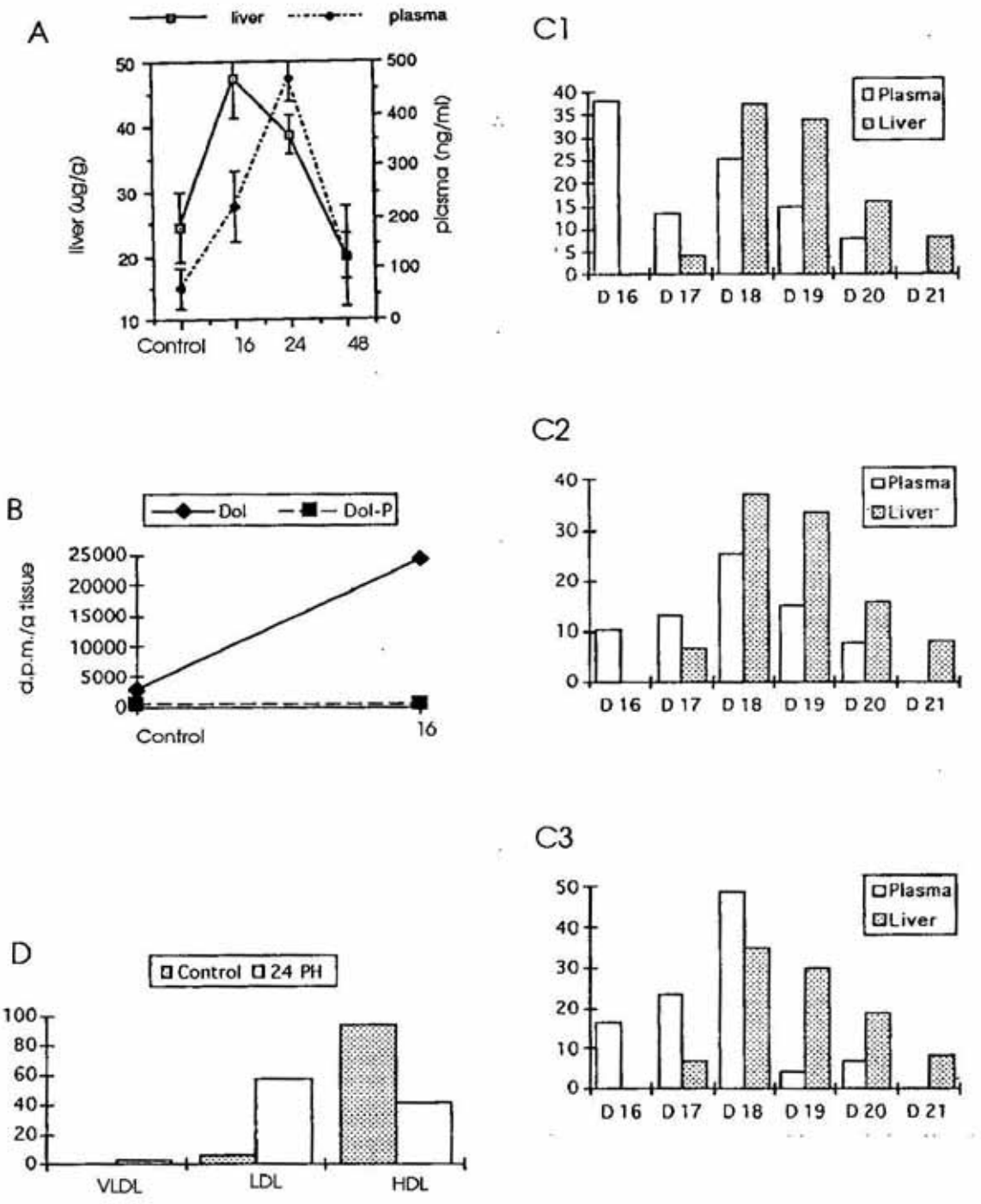

Fig. 2. Changes occuring during rat liver regeneration.
Time after partial hepatectomy (PH) is given in hours. A, Dolichol content in liver and plasma; B, incorporation of 2-[^{3}H]mevalonate into dolichol and dolichyl-P by liver slices; C, distribution of isoprenologs (%), 1, controls; 2, 16 PH; 3, 24 PH; D, distribution of dolichol in lipoproteins isolated from plasma, 24 h after partial hepatectomy (%).

itself. During the regenerative process multiple carriers are involved in the transport of dolichol in the blood, whereas in control rats dolichol was associated with HDL, as observed also by Elmberger *et al.* [17].

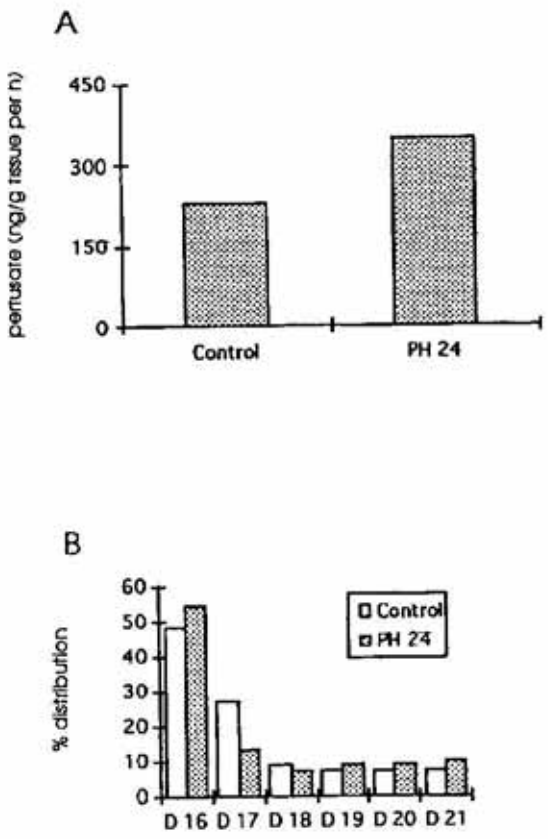

Fig. 3. Dolichol content (A) and isoprenolog distribution (B) in rat liver perfusate after partial hepatectomy.
For abbreviations see legend to Fig. 2.

CONCLUSIONS

We have identified an enhanced synthesis and content of dolichol in proliferating liver and an increased content in the blood; the blood dolichol is essentially provided by release from liver and both its content and composition are modulated by the liver and other organs in accordance with specific physiological needs; dolichol traffic in the blood is mediated by multiple carriers; moreover, an ontogenic stepwise activation of prenyltransferases may be involved. On the other hand, these observations on proliferating systems raise many questions on the role of dolichols in the proliferative or ontogenic process that deserve to be answered; for example the dependence of dolichol synthesis on the fluctuation of HMGCoA reductase activity; the relationships between quantitative modification of dolichol compounds and metabolic behaviour in certain physiological states; the ontogenesis and the physiological role of single isoprenologs in the liver.

REFERENCES

1. Dallner, G. & Chojnacki, T. (1988) The biological role of dolichol. *Biochem. J.* **251**, 1–9.
2. Elmberger, P.G., Engfeldt, P. & Dallner, G. (1988) Presence of dolichol and its derivatives in human blood. *J. Lipid Res.* **29**, 1651–1662.
3. Bucher, N.L.R. (1991) Liver regeneration: an overview. *J. Gastr. Hepatol.* **6**, 611–624.
4. Higgins, G.L. & Henderson, R.M. (1931) Experimental pathology of liver. Restoration of liver of white rats following partial surgical removal. *Arch. Pathol.* **12**, 186–202.
5. Kvetina, J. & Guaitani, A. (1969) A versatile method for the *in vitro* perfusion of isolated organs of rats and mice with particular reference to liver. *Pharmacol.* **2**, 65–81.
6. Havel, R.J., Eder, H.A. & Bragdon, J.H. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**, 1343–1350.
7. Dull, B.J., Mc Carthy, R.D. & Kilara, A. (1983) The modulating effect of an inhibitor of cholesterogenesis present in bovine milk upon the synthesis of cholesterol, dolichol and ubiquinon. *Atherosclerosis* **49**, 231–239.
8. Tavares, A., Coolbear, T. & Hemming, F.W. (1981) Increased dolichol and dolichyl phosphate mediated glycosylation in rats fed cholesterol. *Arch. Biochem. Biophys.* **207**, 427–436.
9. Pullarkat, R.K., Reha, H. & Pullarkat, P.S. (1984) Age-associated increase of free dolichol levels in mice. *Biochim. Biophys. Acta* **793**, 494–498.
10. Rip, J.W., Rupar, C.A., Chandhary, N. & Carrol, K.K. (1981) Localization of a dolichyl phosphate phosphatase in plasma membranes of rat liver. *J. Biol. Chem.* **256**, 1929–1934.
11. Marino, M., Girelli, A.M., Leoni, S. & Trentalance, A. (1990) Variations of hepatic dolichols during rat development. *Biochim. Biophys. Acta* **1047**, 192–194.
12. Leoni, S., Spagnuolo, S., Conti Devirgiliis, L., Dini, L., Mangiantini, M.T. & Trentalance, A. (1984) Cholesterogenesis and related enzymes in isolated rat hepatocytes during pre- and postnatal life. *J. Cell. Physiol.* **118**, 62–66.

13. Valtersson, C., van Duyng, A., Verkleij, A.J., Chojnacki, T., de Cruiff, B. & Dallner, G. (1985) The influence of dolichol, dolichol esters and dolichyl phosphate on polymorphism and fluidity in model membrane. *J. Biol. Chem.* **260**, 2742–2747.

14. Marino, M., Bruscalupi, G., Spagnuolo, S., Leoni, S., Mangiantini, M.T., Trentalance, A. & Hemming, F.W. (1986) Enhanced production of dolichol, but not dolichyl phosphate, in the earliest stages of rat liver regeneration. *Bioscience Reports* **6**, 409–413.

15. Marino, M., Trentalance, A. & Hemming, F.W. (1987) Changes in the biosynthesis of dolichol in regenerating rat liver. *Chemica Scripta* **27**, 51–54.

16. Trentalance, A., Leoni, S., Mangiantini, M.T., Spagnuolo, S., Feingold, K., Hughes-Fulford, M., Siperstein, M.D., Cooper, A.D. & Erickson, S. (1984) Regulation of 3-hydroxy-3-methyl glutaryl CoA reductase and cholesterol synthesis and esterification during the first cell cycle of liver regeneration. *Biochim. Biophys. Acta* **794**, 142–151.

17. Elmberger, P.G., Kalen, A., Brunk, U.T. & Dallner, G. (1989) Discharge of newly synthesized dolichol and ubiquinone with lipoproteins to rat perfusate and to the bile. *Lipids* **24**, 919–930.