Acta Biochimica Polonica

Vol. 41 No. 3/1994

QUARTERLY

Minireview

Disturbances in lipid metabolism in human hepatocellular carcinomas

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Key words: human hepatocellular carcinomas, lipid metabolism, mevalonate pathway

Surgical biopsies from healthy livers and from highly differentiated human hepatocellular carcinomas (HC) were used for analysis of lipid levels, enzyme activities and radioactive labelling patterns. Homogenates from autopsy samples — also including hepatomas of a low degree of differentiation (LC) — were used to study the lipid composition. In hepatoma (HC) homogenates, when compared to healthy livers, there was a ninefold decrease in the levels of the free dolichol fraction, a 50% decrease in the dolichyl ester fraction, while the dolichyl phosphate level in homogenates was practically unalterd. The ubiquinone-10 concentration in homogenates (HC) showed a 50% decrease, whereas the cholesterol level showed a more than 100% increased concentration in all hepatomas examined. The total phospholipid level in homogenates showed a minor decrease, (10%) in hepatomas (HC). The cholesterol concentration in the hepatoma (HC) microsomal fraction showed an increase of about 50%, whereas the total phospholipid level showed a 30% decrease in comparison to control. The dolichol level in hepatoma (HC) microsomes showed a 30% decrease, whereas the dolichyl phosphate level was approximately doubled. When controls were compared with hepatomas of a high degree of differentiation, the relative amount of polyisoprenols with different length and the degree of saturation of polyisoprenols showed minor alterations in all fractions examined. However hepatomas of a low degree of differentiation, exhibited a clear increase of shorter polyisoprenols and clear increase in the α-unsaturated polyisoprenol fraction. The fatty acid composition of individual phospholipids in hepatoma (HC) homogenates showed no major differences when compared with controls.

However, the microsomal fraction from the highly differentiated hepatomas demonstrated in comparison to control, a decrease in the relative amount of the long chain polyunsaturated derivatives and an increase in the percentage of 18:0 fatty acids.

In hepatomas (HC) the incorporation of the radioactive precursors ([^{3}H]mevalonic acid) into both dolichyl phosphate and dolichol showed an approx. 95% decrease when compared with control. The labelling of cholesterol and ubiquinone-10 showed an almost 100% increase and a 50% decrease, respectively, in hepatomas (HC). In hepatoma (HC) microsomal fractions the HMG-CoA reductase activity increased about 80% while the dolichol kinase activity increased over 40%. The dolichol monophosphatase activity in hepatoma (HC) microsomes was practically unaltered (increases of 8%). When labelled nucleotides were used in the glycosylation studies in hepatomas (HC), the incorporation into the dolichyl phos-

[1]Abbreviations: FPP, farnesyl pyrophosphate; HC, highly differentiated hepatocellular carcinoma; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LC, low degree differentiated hepatocellular carcinoma; LDL, low density lipoprotein.

phate monosaccharides and the total proteins showed a minor increase in the UDP-glucose fraction as was also the case when GDP-mannose was used. The results for the hepatomas indicate a major disturbance in the mevalonate pathway especially in the metabolism of polyisoprenols. The interpretation of the various results are discussed.

A number of experiments mostly on animals and cell cultures demonstrate that there are alterations in lipid levels and distrubances in membrane glycosylation in several neoplastic tissues [1, 2]. It has been suggested that alterations in lipid composition are responsible in part for some of the altered properties of hepatoma membranes [3, 4]. The ratio between cholesterol, phospholipids, fatty acids, ubiquinone, and polyisoprenols are all considered to influence the properties like membrane stability and fluidity [3, 5–7]. All tissues and membranes contain polyisoprenoid compounds [8–10]. The phosphorylated dolichol serves as an obligatory intermediate in the synthesis of N-linked glycoproteins, where its level is suggested to be rate limiting under certain conditions [10–14].

Earlier reports have demonstrated a high cholesterol level both in tissues [1] and sera [15] from individuals with hepatomas. Several scientific groups have also described specific distrubances in sterol metabolism in rat hepatomas and partly explained their result, i.e. the high cholesterol levels, by the lack of feed-back inhibition of the HMG-CoA reductase enzyme by LDL cholesterol [16]. In addition to HMG-CoA-reductase, other enzymes such as squalene synthetase are also suggested to be regulated by LDL cholesterol [17]. It has been suggested that under normal conditions inhibition of squalene synthetase leads to a decrease in cholesterol synthesis with a following accumulation of farnesyl pyrophosphate (FPP) and a flow of metabolites towards ubiquinone [18]. These and other results have shown that HMG-CoA reductase determines the availability of FPP for further metabolism to the other endproducts of the mevalonate pathway and that the flow of FPP into the sterol branch is determined by the activity of squalene synthetase. The purpose of this investigation was to analyse and compare lipid metabolism in carcinoma tissue and control.

There are few studies on human hepatoma material which have been focused to the mevalonate pathway, and especially the metabolism and levels of polyisoprenols in relation to other lipids.

MATERIAL AND METHODS

The methods in these investigations used were based on techniques which had been developed on animal liver tissues [19]. The procedures were modified for analyses on human tissues which contains a much higher lipid concentration than the animals studied [20, 21].

Samples were collected from healthy livers (controls) and from hepatocellular carcinomas (hepatomas) of various degrees of differentiation. However, the study focused on hepatomas with a high degree of differentiation which showed a morphological resemblance to the control. The patients involved were in the age of 45–65. The majority of the tissue samples were analysed immediately after surgery whereas a portion of the material was rapidly frozen to –70°C and stored until used. In case of the autopsy tissues, samples were collected about 22 h after death and used only for measurements of certain lipid levels.

All samples were subjected to histological examination and care was taken to exclude necrotic, hemorrhagic and fibrotic areas.

The tissues were homogenized and a portion was used for subcellular preparations or used in slice experiments. The investigations on enzyme activities, the analyses of the glycosylation and the radioactive incorporation studies were made on tissues from fresh human highly differentiated hepatomas and fresh healthy liver tissue. A part of the material was examined by electron microscopy. Marker enzyme activities were determined as previously described [22, 23], and was found to be identical between control and hepatomas with a high degree of differentiation. Lipid values were expressed on a wet weight or protein basis. The lipids, i.e. cholesterol, total phospholipids with fatty acids, ubiquinone-10, total polyisoprenols (both α-unsaturated and α-saturated polyprenols), dolichyl esters (with fatty acids) and dolichyl phosphates, both in homogenates and in microsomal fractions, were extracted and isolated according to methods described earlier

[19]. The concentrations of the polyisoprenols and ubiquinone were correlated to the initially added standards which in turn had been calibrated against weighed amounts of standards [24]. Measurements of protein were determined using the biuret procedure [25]. Lipid mixtures were dissolved in 10 ml of Aqualuma Plus and the radioactivity was determined by scintillation counting. In the case of the incorporation studies using [^{3}H]mevalonic acid, incubations and extraction procedures followed earlier methods [19, 26, 27].

Beside the HMG-CoA reductase activities, other enzymes such as dolichol kinase and dolichol monophosphatase activities were assayed as earlier described [26]. In the case of the glycosylation studies using GDP-[^{14}C]mannose, UDP-[^{14}C]-*N*-acetylglucosamine and UDP-[^{14}C]glucose the procedure followed the methods described in earlier publications [14].

RESULTS AND DISCUSSION

This article summarizes certain differences in lipid metabolism in a human material consisting of healthy livers and hepatocellular carcinomas. Analyses was made on samples from fresh livers collected at surgery and from a group of autopsy cases. The lipid composition was analysed prior to and after freezing and was found to be similar in both groups. The data indicated that certain measurements on autopsy material reflect the *in vivo* level of the measured lipids, which was in agreement with earlier studies [21].

The radioactive incorporation studies, the enzyme measurements and phospholipid analysis were however made almost immediately on fresh surgical biopsies from healthy livers and from highly differentiated hepatocellular carcinomas. The microsomal fractions deriving only from hepatomas of a high degree of differentiation showed (judged by electron microscopy) a similar morphological appearance in comparison with healthy liver microsomes which was also in line with previous investigations [28]. When marker enzyme activities were compared the values did not differ between the two groups. Earlier studies [1] especially on **animal** models have to a large extent been dealing with fatty acids, phospholipids and cholesterol, however only a few studies have as mentioned been reported on a **human** material where the analyses are focused to the mevalonate pathway. Earlier experiments using a rat model with drug induced preneoplastic nodules showed an increase in the dolichol level and a disturbance in the dolichol mediated glycosylation [14]. These experiments showed a disturbance in the polyisoprenol metabolism and a correlation between the level of dolichyl

Table 1

Comparison of lipid levels in homogenates and in microsomes prepared from healthy human liver (control) and from human hepatocellular carcinomas (hepatomas) with a high degree of differentation[a]

Tissue	Lipid	Relative lipid values (Hepatomas:control)
Homogenate[b]	Cholesterol	2.60
	Total phospholipid	0.96
	Ubiquinone-10	0.45
	Total polyisoprenols (α-saturated + α-unsaturated polyprenols)	0.13
	Dolichyl ester	0.48
	Dolichyl phosphate	0.96
Microsome[c]	Cholesterol	1.53
	Dolichol	0.65
	Dolichyl phosphate	2.1
	Total phospholipid	0.68

[a]Data was taken from [26, 34, 35]; [b]values were initially expressed in μg/g wet weight; [c]values were initially expressed in μg/mg of protein.

phosphate and the degree of glycosylation which was in line with earlier reports [10]. The results from the analysis on lipid composition in hepatomas (Table 1) indicated also a disturbance in the metabolism of polyisoprenoid and cholesterol compounds which was confirmed by the studies using radioactive lipid precursors (Table 2). The results from the metabolic labelling studies (Table 2) could partly explain the level of certain lipids (that is the low dolichol, reduced ubiquinone-10 and high cholesterol levels, see Table 1). However, the low mevalonate labelling into dolichyl phosphate in tumours did not explain the dolichyl phosphate levels which showed a relatively unaltered concentration in the total hepatoma homogenates and an increased level in the microsomal hepatoma fractions. However, the increased dolichol kinase and a relatively unaltered dolichol monophosphatase activity in hepatoma microsomes (Table 3) helped to explain the higher dolichyl-P levels in microsomes. The results was thus in favor of the idea of an alternative regulatory system for the dolichyl phosphate level. These changes in the enzyme activities in hepatomas (Table 3) could theoretically explain that under pathological conditions, other factors than alterations in *de novo* synthesis regulate the concentration of dolichyl phosphate.

Table 2

Comparison of incorporation of [^{3}H]mevalonic acid into lipids in human liver homogenates and in hepatocellular carcinomas (hepatomas) of a high degree of differentiation[a]

Lipid	Relative lipid values[b] (Hepatomas:control)	
	A	B
Polyisoprenol fraction: (α-saturated and α-unsaturated polyisoprenol)	0.07	0.6
Dolichyl phosphate	0.05	0.02
Ubiquinone-10	0.53	1.1
Cholesterol	1.9	0.95

[a]Data was taken from [26, 27]; [b]values were in A — initially expressed as c.p.m./g tissue, and in B — c.p.m./μg phospholipid.

Table 3

Comparison of activities of various enzymes and of glycosylation activity in microsomal fractions from human healthy liver (control) and hepatocellular carcinomas (hepatomas) of a high degree of differentiation[a]

Enzyme	Incubation with labelled nucleotides as substrates	Relative enzyme activity[b] value	Relative dolichyl-P-monosaccharide incorporation value[b]	Relative total protein incorporation value[c]
		Hepatoma:control	Hepatoma:control	Hepatoma:control
HMG-CoA reductase	—	1.9	—	—
Dolichol kinase	—	1.5	—	—
Dolichol monophosphatase	—	1.05	—	—
—	GDP-mannose	—	1.03	1.25
—	UDP-*N*-acetyl-glycosamine	—	1.05	1.05
—	UDP-glucose	—	1.38	1.17

[a]Data was taken from [26, 27]; [b]values were initially expressed in c.p.m./mg of protein per 10 min; [c]values were initially expressed in c.p.m./mg of protein.

The increase of the dolichyl phosphate level in hepatoma microsomes (Table 1) was paralleled by a minor increase in the incorporation into dolichyl phosphate monosaccharides (Table 3) and an increased incorporation into the proteins (Table 3) which was in line with earlier investigations [14].

The increase in the human hepatoma microsomal HMG-CoA reductase activity (Table 3) and the following increase in cholesterol synthesis (Table 2) was also in line with earlier investigations on animal models [16].

When analysing the composition of the different polyisoprenoid length (free, esterified and phosphorylated compounds), the lipids exhibited minor changes with a minor shift towards shorter dolichols in hepatomas (Table 4). However, this was only in the case when controls and highly differentiated hepatomas were compared. In hepatomas of a low degree of differentiation the distribution of polyisoprenols with different length showed a clear shift towards shorter dolichols (Table 4). A shift in polyprenol length could experimentally be explained by a decrease in the size of the mevalonate pool which was earlier suggested by [29].

A difference in the mevalonate pool size thus cannot be excluded between tumours and controls but this seems unlikely when controls and hepatomas with a high degree of differentiation are compared. Since there are no reliable methods for measurement of the pool size of mevalonate the data from the mevalonic labelling studies (see Table 2) should be carefully evaluated. However, since the mevalonate molecule is a common precursor to the polyisoprenoid and cholesterol compounds, the specific incorporation pattern (i.e., 50% and 95% reduction in ubiquinone and polyisoprenols, respectively, and an almost 200% increase in cholesterol in hepatomas (HC)), thus cannot be explained only by an alteration in the mevalonate pool (Table 2).

Several studies have also concluded that the first enzymes in dolichol and ubiquinone biosynthesis have a much higher affinity for farnesyl pyrophosphate (FPP) than squalene synthetase which means that even if the mevalonate pool is small in tumours with a following decrease in the FPP concentration, saturation of the other FPP-utilizing enzymes in the dolichol

Table 4

Comparison of distribution of individual polyisoprenoids in homogenates in the free dolichol fraction in human homogenates from healthy livers (control), and hepatocellular carcinomas (hepatomas) of a high and low degree of diffrentiation[a]

	Relative values of dolichol length (Hepatomas:control)					
Group	D17[b]	D18	D19	D20	D21	D22
Hepatoma (high) : control	1.0	1.3	1.2	0.9	0.7	0.6
Hepatoma (low) : control	4.7	1.5	1.2	0.7	0.5	0.4

[a]Data was taken from [36]; [b]values were initially expressed as dolichol composition, percentage of total.

Table 5

Comparison of relative amounts of α-unsaturated polyprenols in polyisoprenoid compound containing 19 and 20 isoprene residues (saturated and unsaturated compounds) in homogenates prepared from control liver and from hepatocellular carcinomas (hepatomas) of a high and low degree of differentiation[a]

	Relative values of α-unsaturated compounds (Hepatomas:control)	
Group	Polyisoprenol-19 (α-unsaturated)[b]	Polyisoprenol-20 (α-unsaturated)[b]
Hepatomas (high) : control	1.30	1.26
Hepatomas (low) : control	7.0	5.7

[a]Data was taken from [34]; [b]the figures were initially expressed as percentage of the total polyisoprenoid alcohol (saturated plus unsaturated).

and ubiquinone biosynthetic pathways are still probably sufficient.

Considering the differences in both the recurrent pattern of incorporation and the clearly altered pattern of lipid levels it thus seems unlikely that all these differences are caused simply by changes in the mevalonate or farnesyl-pyrophosphate concentration, and/or an increased flow of metabolites into the cholesterol pathway resulting in the depletion of precursor for the ubiquinone or polyisoprenol pathways.

It seems thus more likely that the alterations in lipid metabolism is not only restricted to the regulatory enzyme HMG-CoA reductase but most probably also to other enzymes subsequent to farnesyl pyrophosphate, which regulate the flow of metabolites beyond this branch point to the three major polyisoprenoid entities, i.e. sterols, dolichols and ubiquinones. The results thus indicate an independent regulation of these three lipids, where the individual pathways in hepatomas are likely to differ from those in healthy livers. In order to explain the mechanism behind the recurrent pattern of the different lipid levels in hepatomas a battery of enzyme disturbances have to exist which hypothetically could be explained by an interference with the regulatory system controlling the mevalonate pathway somewhere down the line from the genome or more likely, in or close to the genome. When the total polyisoprenol 19 and 20 fractions were analyzed in healthy livers and in hepatomas with a high degree of differentiation only a few percent was found in the α-unsaturated form (not shown). A minor increase in the α-unsaturated form was found in the hepatoma fraction when compared to control (Table 5). On the other hand, in a material consisting of carcinomas of a low degree of differentiation the percentage of α-unsaturated polyisoprenols, both of the 19 and 20 length were found to be clearly increased in comparison with both controls and hepatomas of a high degree of differentiation (Table 5).

When comparing individual phospholipids in hepatomas of a high degree of differentiation with control, only minor changes in fatty acid composition were observed in the major microsomal phospholipid fractions, which consisted of a decrease in the relative amount of long chain polyunsaturated derivatives and an increase in the percentage of 18:0 fatty acids in hepatomas (not shown). The exact explanation to these disturbances in the fatty acid composition and constituents of α-unsaturated and α-saturated polyprenols are not fully understood. Recent investigations of model membranes suggest as already mentioned that changes in the amount of dolichol influences properties such as fluidity and stability [30, 7].

Other lipids like ubiquinone was earlier also reported to influence membrane fluidity [31]. Compared to normal hepatocytes, hepatoma cells were reported to demonstrate a lower fluidity of the plasma membrane [3, 32], which properties could partly be explained by the low dolichol, low ubiquinone and high cholesterol levels. It is thus tempting to speculate that the physical alterations in rat hepatoma membranes, which was described earlier, is dependent not only on changes in the levels of cholesterol and phospholipids and on the phospholipid composition of membranes, on the degree of unsaturation of bound fatty acids and on the amount of membrane protein, which are known natural modulators of lipid fluidity, but also in the case of highly differentiated humans hepatomas on the levels of ubiquinone and on the type and amount of polyisoprenoid compounds present. When summerizing the lipid metabolism in hepatomas of a high degree of differentiation the major disturbance at least for the bulk of lipids, thus seems to be localized to the mevalonate pathway and especially in the polyisoprenol metabolism. It is thus tempting to speculate that during the carcinogenesis some trigger mechanism in or outside the cell could start the earlier described isoprenylation of proteins with an activation of *ras*-oncogenic products [33]. This product could influence the function of the genome with a following disturbance in the mevalonate pathway.

A disturbance in the mevalonate metabolism and especially in the polyisoprenol metabolism could thus cause or contribute to the mentioned changes of membrane properties with the following altered behavior of the cancer cell.

REFERENCES

1. Bergelson, L.D. (1972) Tumor lipids; in *The chemistry of fats and other lipids;* vol. 13 (Part 2), pp. 1–56, Oxford, Pergamon Press.

2. Alm, R. & Eriksson, S. (1985) Biosynthesis of abnormally glycosylated hepatoma secretory proteins in cell cultures. *FEBS Lett.* **190**, 157–160.
3. Shinitzky, M. (1984) Membrane fluidity in malignancy adversative and recuperative. *Biochim. Biophys. Acta* **738**, 251–261.
4. Spector, A.A. & Yorek, M.A. (1985). Membrane lipid composition and cellular function. *J. Lipid Res.* **26**, 1015–1035.
5. van Hoeven, R.P. & Emmelot, P. (1972) Studies on plasma membranes. Lipid class composition of plasma membranes isolated from rat and mouse liver and hepatomas. *J. Membrane Biol.* **9**, 105–126.
6. Lai, C.-S. & Schutzbach, J.S. (1984) Dolichol induces membrane leakage of liposomes composed of phosphatidylethanolamine and phosphatidylcholine. *FEBS Lett.* **169**, 279–282.
7. Valtersson, C., van Duijn, G., Verkleij, A.J., Chojnacki, T., de Kruijff, B. & Dallner, G. (1985) The influence of dolichol, dolichol esters, and dolichyl phosphate on phospholipid polymorphism in model membranes. *J. Biol. Chem.* **260**, 2742–2751.
8. Struck, D.K. & Lennarz, W.J. (1980) in *The biochemistry of glycoproteins and proteoglycans* (Lennarz, W.J., ed.) pp. 35–83, New York, Plenum Press.
9. Hemming, F.W. (1983) in *Biosynthesis of isoprenoid compounds* (Porter, J.W. & Spurgeon, S.L., eds.) vol. 2, pp. 305–354, New York, Wiley.
10. Dallner, G. & Hemming, F.W. (1981) Lipid carriers in microsomal membrane; in *Mitochondria and microsomes* (Lee, C.P., Schatz, G. & Dallner, G., eds.) pp. 655–681, Reading, Addison-Wesley.
11. Mills, J.T. & Adamany, A.M. (1978) Impairment of dolichyl saccharide synthesis and dolichol-mediated glycoprotein assembly in the aortic smooth muscle cell in culture by inhibitors of cholesterol biosynthesis. *J. Biol. Chem.* **253**, 5270–5273.
12. Carson, D.D., Earles, B.J. & Lennarz, W.J. (1981) Enhancement of protein glycosylation in tissue slices by dolichyl phosphate. *J. Biol. Chem.* **265**, 11552–11557.
13. Potter, J.E.R., James, M.J. & Kandutsch, A.A. (1981) Sequential cycles of cholesterol and dolichol synthesis in mouse spleens during phenylhydrazine-induced erythropoesis. *J. Biol. Chem.* **256**, 2371–2376.
14. Eggens, I., Eriksson, L.C., Chojnacki, T. & Dallner, G. (1984) Role of dolichyl phosphate in regulation of protein glycosylation in 2-acetylaminofluorene-induced carcinogenesis in rat liver. *Cancer Res.* **44**, 799–805.
15. Hirayama, C., Yamanishi, Y. & Irisa, T. (1979) Serum cholesterol and squalene in hepatocellular carcinoma. *Clin. Chim. Acta* **91**, 53–57.
16. Siperstein, M.D. & Fagan, V.W. (1964) Deletion of the cholesterol negative feedback system in liver tumors. *Cancer Res.* **24**, 1108–1115.
17. Brown, M.S. & Goldstein, J.L. (1980) Multivalent feed-back regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21**, 505–507.
18. Faust, J.R., Goldstein, J.L. & Brown, M.S. (1979) Synthesis of ubiquinone and cholesterol in human fibroblasts: regulation of a branched pathway. *Arch Biochem. Biophys.* 192, 86–99.
19. Eggens, I., Elmberger, P.G. & Dallner, G. (1989) Conditions for quantitation of dolichyl phosphate, dolichol and cholesterol by high-performance liquid chromatography. *Biomed. Chromatography* **3**, 20.
20 Rupar, C.A. & Carroll, K.K. (1978) Occurrence of dolichol in human tissues. *Lipids* **13**, 291–293.
21. Tollbom, Ö. & Dallner, G. (1986) Dolichol and dolichyl phosphate in human tissues. *Br. J. Exp. Path.* **67**, 757–764.
22. Eriksson, L.C. (1973) Studies on the biogenesis of endoplasmic reticulum in the liver cell. *Acta Pathol. Microbiol. Scand. (Suppl.)* **239**, 1–72.
23. Beaufay, H., Amar-Costesec, A., Feytmans, E., Thines-Sempoux, D., Wibo, M., Robbi, M. & Berthet, J. (1974) Analytical study of microsomes and isolated subcellular membranes from rat liver. *J. Cell Biol.* **61**, 188–200.
24. Elmberger, P.G., Kalén, A., Appelkvist, E.L. & Dallner, G. (1987) *In vitro* and *in vivo* synthesis of dolichol and other main mevalonate products in various organs of the rat. *Eur. J. Biochem.* **168**, 1–11.
25. Gornall, A.G., Bardwill, C.J. & David, M.M. (1949) Determination of serum proteins by means of the Biuret reaction. *J. Biol. Chem.* **177**, 751–766.
26. Eggens, I., Ericsson, K. & Tollbom, Ö. (1988) Cytidine 5′-triphosphate-dependent dolichol kinase and dolichol phosphatase activities and levels of dolichyl phosphate in microsomal fractions from highly differentiated human hepatomas. *Cancer Res.* **48**, 3418–3424.
27. Eggens, I., Ekström, T.J. & Åberg, F. (1990) Studies on the biosynthesis of polyisoprenols, cholesterol and ubiquinone in highly diffe-

rentiated human hepatomas. *J. Exp. Path.* **71**, 219–232.

28. Ma, M.H. & Blackburn, C.R.B. (1973) Fine structure of primary liver tumors and tumor-bearing livers in man. *Cancer Res.* **33**, 1766–1774.
29. Ekström, T.J., Chojnacki, T. & Dallner, G. (1987) The α-saturation and terminal events in dolichol biosynthesis. *J. Biol. Chem.* **262**, 4090–4097.
30. van Duijn, G., Valtersson, C., Chojnacki, T., Verkleij, A.J., Dallner, G. & de Kruijff, B. (1986) Dolichyl phosphate induces non-bilayer structures, vesicles fusion and transbilayer movement of lipids. A model membrane study. *Biochim. Biophys. Acta* **861**, 211–223.
31. Lenaz, G. & Degli Espositi, M. (1985) Physical properties of ubiquinones in model system and membranes; in *Coenzyme Q, biochemistry, bioenergetics and clinical application of ubiquinone* (Lenaz, G., ed.) pp. 83–103, John Wiley and Sons.
32. van Hoeven, R.P., van Blitterswijk, W.J. & Emmelot, P. (1979) Fluorescence polarization measurements on normal and tumor cells and their corresponding plasma membranes. *Biochim. Biophys. Acta* **551**, 44–54.
33. Schmidt, R.A., Schneider, C.J. & Glomset, J.A. (1984) Evidence for posttranslational incorporation of a product of mevalonic acid into Swiss 3T3 cell proteins. *J. Biol. Chem.* **259**, 10175–10180.
34. Eggens, I., Elmberger, P.G. & Löw, P. (1989) Polyisoprenoid, cholesterol and ubiquinone levels in human hepatocellular carcinomas. *Br. J. Exp. Path.* **70**, 83–92.
35. Eggens, I., Bäckman, L., Jakobsson, Å. & Valtersson, C. (1988) The lipid composition of highly differentiated human hepatomas with special reference to fatty acids. *Br. J. Exp. Pathol.* **69**, 671–683.
36. Eggens, I. & Elmberger, G. (1990) Studies on the polyisoprenoid composition of hepatomas and its correlations to their differentiation. *APMIS* **98**, 535–542.