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Studies on the biosynthesis of polyisoprenols, cholesterol and ubiquinone in highly differentiated human hepatomas

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Summary. Surgical samples of human hepatic tissue were analysed morphologically and biochemically and highly differentiated hepatomas were compared with two control groups: morphologically normal liver tissue surrounding the tumour, and tissue from normal livers. In tumour homogenates cholesterol levels were more than twice, ubiquinone levels about half and the concentration of free dolichol about 10% of the control value. The levels of dolichyl phosphate were basically similar, whereas the phospholipid level was slightly lower in the tumours. In microsomes isolated from hepatomas, the level of cholesterol was about 30% higher than the control value. HMG-CoA reductase activity in microsomes isolated from hepatomas was elevated almost 100% in comparison to control. In hepatomas, no major alterations in the compositions of dolichol or dolichyl phosphate could be observed. The relative amounts of α-saturated and α-unsaturated polyprenols were also basically unaltered in hepatomas. Liver samples were incubated with ^{3}H-mevalonic acid and radioactivity was monitored in polyprenols. With control tissue, incorporation was considerably higher in α-unsaturated polyprenols than in their α-saturated counterparts. In the tumours the rates of incorporation into both polyprenol fractions were much lower, although still higher in the α-unsaturated fraction. Labelling of polyisoprenols containing 19 isoprene residues was higher than that of 20 residues. The pattern of labelling in the polyisoprenyl-P fraction was similar. In hepatomas the incorporation into cholesterol and ubiquinone-10 was about 100% higher and 50% lower respectively compared with control tissue. The results in this study of hepatomas indicate that the levels of various lipids may be influenced not only by the regulatory enzyme HMG-CoA reductase, but also by other enzymes catalysing reactions subsequent to this regulatory point. It is also suggested that levels of cholesterol, ubiquinone and dolichol may be regulated independently subsequent to the branch point at farnesylpyrophosphate.

Keywords: regulation of cholesterol, ubiquinone and dolichol, human hepatoma

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Alterations in lipid levels have been demonstrated in several neoplastic tissues (Bergelson 1972) and liver cell carcinoma has been reported to be associated with hyperlipidaemia (Chen *et al.* 1978). Defective control of lipid biosynthesis in cancerous and precancerous livers has been described in hepatoma-bearing animals (Siperstein & Fagan 1964; Sabine 1975). Since membranes of tumour tissue exhibit changes in their structure and fluidity (Shinitzky 1984), much interest has been focused on cholesterol and phospholipids in tumours, since it has been suggested that the ratio between these lipids influences membrane fluidity (Shinitzky 1984; Van Hoeven & Emmelot 1972). Other membrane lipids have also been thought to have an influence on properties of membranes. In model membranes, for instance, it has been shown that the fluidity of the fatty acids of phospholipids, membrane stability, and membrane permeability are all influenced considerably by the type and amount of dolichols present (Van Duijn *et al.* 1986). Moreover, studies in model systems have shown that ubiquinones also affect the fluidity of lipid bilayers (Lenaz & Degli Esposito 1985). Thus it has been suggested that alterations in lipid composition are responsible at least in part for some of the altered properties of hepatoma membranes (Shinitzky 1984; Spector & Yorek 1985).

Recent investigations have shown that all animal tissues and almost all membranes in eukaryotic cells contain α-saturated polyisoprenoid compounds (dolichols) (Struck & Lennarz 1980; Hemming 1983; Dallner & Hemming 1981) and that the dolichol content in human tissues is particularly high (Rupar & Carroll 1978). In the normal rat liver the α-unsaturated derivatives constitute only a few per cent of the total polyisoprenols (Ekström *et al.* 1984). The dolichols are a family of polyisoprenoid alcohols differing in chain length (Struck & Lennarz 1980; Hemming 1983; Dallner & Hemming 1981). The dolichol pattern varies slightly between different species but is relatively similar in different organs of the same species (Hemming 1983; Dallner & Hemming 1981). The polyisoprenoid alcohols are present either in the free form, esterified with a fatty acid or as the phosphorylated derivative (Struck & Lennarz 1980; Hemming 1983; Dallner & Hemming 1981; Parodi & Leloir 1979). Dolichyl phosphate serves as an obligatory intermediate in the synthesis of *N*-linked glycoproteins, where its level is suggested to be rate-limited under certain conditions (Mills & Adamany 1978; Carson *et al.* 1981; Potter *et al.* 1981; Eggens *et al.* 1984).

Preliminary studies on human hepatomas revealed an increased cholesterol level, a low concentration of dolichol and a relative enrichment in the shorter dolichols in certain tumours (Eggens *et al.* 1983; Eggens 1987). Cholesterol and squalene levels in human serum are also reported to be high in hepatoma-bearing individuals (Hirayama *et al.* 1979). The ubiquinone content in rat hepatomas was found to be lowered (Eggens 1987; Ostergberg & Wattenberg 1961; Sugimura *et al.* 1962).

The alterations in sterol metabolism observed in hepatomas may be partially due to an altered effect of dietary cholesterol on the mevalonate pathway (Siperstein & Fagan 1964). The rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, mainly localized in the endoplasmic reticulum, is regulated via a receptor-mediated uptake of LDL-cholesterol (Brown & Goldstein 1980) and this normal dietary 'feedback' inhibition of cholesterol synthesis has been proposed to be abolished in hepatoma-bearing animals (Siperstein & Fagan 1964). It has also been suggested that inhibition of squalene synthetase by LDL-cholesterol occurs under normal conditions and may prevent hepatocytes from synthesizing cholesterol while allowing continued formation of dolichol and ubiquinone (Faust *et al.* 1979).

In the present investigation we have studied the synthesis of cholesterol, ubiquinone and polyisoprenols in a number of highly

differentiated human hepatocellular carcinomas. A preliminary report of some of these data has appeared (Eggens 1987).

Materials and methods

Five normal human livers and five highly differentiated human hepatocellular carcinomas (hepatomas) were analysed directly after surgery. A portion of the material was stored at −70°C. The hepatic material obtained from the hepatoma patients was divided into material from the tumour itself and morphologically normal hepatic tissue outside the tumour. The patients were 45–60 years of age and all samples were subjected to histological examination before analysis. With tumours care was taken to exclude all fibrotic, necrotic or haemorrhagic areas. Samples were extracted as described below. Homogenates and microsomal fractions were prepared as described earlier (Tollbom & Dallner 1986; Autuori *et al.* 1975). Measurements are expressed on a gram wet weight and mg protein basis. Marker enzyme activities were determined using previously described procedures (Eriksson 1973; Beaufay *et al.* 1974). The values present in the tables are the means of two different experiments each involving five different hepatic samples handled independently. Each sample consisted of three or four pieces of tissue picked randomly from the area of interest. The samples were all homogenized with an Ultra Turrax blender. As internal standards, dolichol-23, dolichyl-23-phosphate, ubiquinone-9 and ^{14}C-cholesterol were added. When measuring ubiquinones the samples were extracted and purified as earlier described (Elmberger *et al.* 1989).

When total cholesterol, α-unsaturated polyprenols, dolichols and dolichyl phosphates were to be isolated, the samples were homogenized in chloroform–methanol–water (1:1:0.3) and HCl was then added to a final concentration of 0.1 M. Acid hydrolysis was performed first for 60 min at room temperature, then for 45 min at 55°C and, finally, for 10 min at 100°C. The mixture was subsequently neutralized with NaOH and evaporated to dryness. Alkaline hydrolysis was then performed in methanol–water–KOH (60%) (1:1:0.5) for 45 min at 90°C. The pH was then adjusted to 7.0 and chloroform added to give a chloroform–methanol–water ratio of 3:2:1. The upper phase was removed and lower phase was washed twice with theoretical upper phase (Folch *et al.* 1957).

After evaporation, the polyisoprenol mixture was dissolved in 200 μl chloroform–methanol (CM) (2:1), after which 10 ml methanol–water (MW) (98:2) containing 20 mM H_3PO_4 was added. This mixture was placed onto a C18 Sep-Pak column (which had been equilibrated with the same solution) and washed first with 10 ml MW (98:2) containing 20 mM H_3PO_4 and subsequently with 10 ml MW (98:2). These pooled washes contained cholesterol. The mixture of free and phosphorylated polyisoprenols were eluted from the C18 Sep-Pak column with CM (2:1). This eluate was supplemented with ammonia at a final concentration of 1% and then placed onto a silica Sep-Pak column. The free polyisoprenoid compounds were eluted with CM (2:1) containing 1% ammonia and the phosphorylated polyisoprenoid compounds were eluted with CMW (1:1:0.3). The CMW mixture was evaporated to dryness and redissolved in CM (2:1) containing 40 mM HCl.

When analysing the acid-labile α-unsaturated polyprenyl phosphates, the procedure was started with the alkaline hydrolysis (that is, the acid hydrolysis was eliminated), followed by the same sequence of columns as described above.

For analysis of only the free polyisoprenols (α-unsaturated polyprenols and dolichols) and cholesterol an initial alkaline hydrolysis was employed in order to ensure as complete recovery as possible. This hydrolysis was followed by separation of lipids on a C18 Sep-Pak column as described above, after which the samples were analysed by HPLC.

Phospholipids were extracted, purified and separated by thin layer chromatography

(Elmberger *et al.* 1989; Valtersson & Dallner 1982) as previously described. The lipid compounds dolichol, dolichyl-P, ubiquinone and cholesterol were analysed by HPLC using a Hewlett-Packard Hypersil ODS 3 μm reversed-phase column as described previously (Elmberger *et al.* 1989).

When both α-saturated and α-unsaturated polyprenols were to be measured, the individual peaks (containing both unsaturated and saturated compounds) were first collected from the reversed-phase HPLC column above and then reinjected (after evaporation) onto a SiO_2 column (1 × 50 cm) (Waters) as described earlier (Ekström *et al.* 1987).

When both the phosphorylated α-saturated and α-unsaturated polyisoprenyls were to be analysed, the individual polyisoprenyl phosphates peaks were collected from the reversed-phase column as described above. After dephosphorylation with wheat-germ acid phosphatase (Sigma) (Carson & Lennarz 1981) the resulting saturated and unsaturated free alcohols were separated on a SiO_2 column as described above.

For assay of HMG-CoA reductase activity, microsomes (1 mg protein in 0.25 M sucrose) were preincubated in a mixture containing 25 mM KH_2PO_4, pH 7.4, for 10 min at 37°C. The assay mixture with a final volume of 5 ml contained the preincubated microsomal suspension, 25 mM KH_2PO_4, pH 7.4, 30 mM KCl, 2 units of glucose-6-phosphate dehydrogenase (Sigma), 1 mM $NADP^+$, 7 mM glucose-6-phosphate, 20 mM NaCl, 30 mM EDTA and 3 mM dithioerythritol, with or without Triton X-100 at a final concentration of 0.05%. The reaction was started by the addition of 0.15 μmol of DL-3-^{14}C-HMG-CoA (about 230 000 d.p.m.).

The reaction was stopped by the addition of 0.5 ml 10 M NaOH containing trace amounts of 5-^{3}H-mevalonolactone (about 15 000 d.p.m.). After 15 min at room temperature, 1 ml concentrated HCl was added. The ^{14}C-labelled mevalonolactone product and the internal standard were then extracted into diethyl ether as described earlier (Erickson & Heller 1983) and the extracts taken to dryness under N_2. The residue was dissolved in a small amount of acetone and applied to silica gel plates, which were then developed with benzene–acetone (1:1). After chromatography, mevalonolactone was visualized under u.v. light and by scanning for radioactivity. The area containing mevalonolactone was scraped off and transferred into vials and its radioactivity was measured.

Homogenates were prepared in 0.25 M sucrose. Protein (35 mg) was incubated in a total of 5 ml medium at 30°C for 30 min with constant shaking. The medium contained 5 mM ATP, 10 mM phosphoenolpyruvate, 100 units pyruvatekinase (Sigma), 5 mM $MgCl_2$, 100 mM KH_2PO_4, 5 mM NADH ^{3}H-mevalonic acid (125 μCi). The pH was 7.5. The incubation was stopped with chloroform–methanol (2:1), after which internal standards were added to correct for losses. Polyisoprenols, cholesterol and ubiquinone were isolated as described above and their radioactivities then measured.

Protein was determined with the biuret procedure (Gornall *et al.* 1949). Phospholipids were quantitated by phosphate determinations (Valtersson & Dallner 1982). The isolated lipids were dissolved in 10 ml Aqualuma Plus and radioactivity determined by scintillation counting.

Results

The liver material in this study was used to prepare slices, homogenates and microsomal fractions. The latter fractions were analysed by electron microscopy and contained mainly rough and smooth microsomes (not shown). No major differences in the appearance of the microsomal fractions from the control and hepatoma groups were observed.

Both microsomal and mitochondrial marker enzymes were assayed and no major differences in NADPH-cytochrome *c* reductase or cytochrome *c* oxidase activities between hepatomas and controls was seen (Table 1). The cytochrome *c* oxidase activity

Table 1. Marker enzyme activities in microsomal fractions prepared from normal human liver, from morphologically normal liver tissue outside the tumours and from hepatomas. Tissues were diagnosed histologically prior to analysis. Experimental details are given in Materials and methods

Sample	Enzyme activities in the microsomal fractions*	
	NADPH-cytochrome *c* reductase†	cytochrome *c* oxidase‡
Control liver	68±5	16±1.4
Morphologically normal liver tissue outside the tumours	70±6	17±1.5
Hepatomas	61±5	14±1.2

* Values represent the mean±s.e.m. (10 experiments).
† nmoles/min/mg protein.
‡ nmoles cytochrome *c* oxidized/min/mg protein.

was about 100 times higher, whereas the NADPH-cytochrome *c* reductase activity was about 23 times lower in the mitochondrial than in the microsomal fraction (not shown). In some experiments the plasma membranes and lysosomal contents of the microsomal fractions were analysed and no major differences between the control and the hepatoma groups could be seen.

In homogenates and microsomal fractions

Table 2. Lipid levels in homogenates and microsomes prepared from normal human liver, from morphologically normal tissue outside the tumours and from hepatomas. The samples were diagnosed histologically prior to analysis. Experimental details are given in Materials and methods

Tissue fraction	Lipid	Lipid levels*		
		Control liver	Morphologically normal tissue outside the tumours	Hepatomas
			μg/g wet weght	
Homogenates†	Cholesterol	1820±132	2230±175	3860±269
	Total phospholipid	11480±752	12600±825	10390±689
	Ubiquinone-10	49±4	45±5	22±1.8
	Dolichol	578±42	545±36	65±5
	Dolichyl phosphate	15.5±1.6	14.9±1.2	14.7±1.1
			(μg/mg protein)	
Microsomes	Cholesterol	38.2±3.6	40.1±3.9	56.5±4.9

* Values represent the mean±s.e.m. (10 experiments).
† Control liver homogenate, tissue outside the tumours and hepatoma tissue contained 185, 192 and 178 mg protein per gram wet weight, respectively.

Table 3. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in microsomal fractions prepared from control liver, from morphologically normal tissue outside the tumours and from hepatomas. Microsomes were incubated with 3-^{14}C-HMG-CoA. The reaction was stopped and 5-^{3}H-mevalonolactone added in trace amounts as an internal standard. The reaction product 3-^{14}C-mevalonolactone was extracted and isolated by thin-layer chromatography. All tissues were diagnosed histologically prior to analysis. Experimental details are given in Materials and methods

	Microsomal HMG-CoA reductase activity* (pmoles min^{-1} mg protein^{-1})	
Sample	Incubation with Triton	Incubation without Triton
Control liver	43±3.5	38±3.1
Morphologically normal liver tissue outside the tumours	46±3.6	41±3.3
Hepatomas	79±6.3	69±5.7

* Values represent the mean±s.e.m. (10 experiments).

from the region outside the tumours only minor changes in lipid amounts could be observed in comparison with healthy control tissue (Table 2). Cholesterol levels in hepatoma homogenates were more than twice as high, while the amount of total phospholipid was slightly less than the control values. The concentration of dolichol in the tumours was radically lower, that is, about 10% of the control value. The ubiquinone level in hepatomas was about half the control value, while the amount of dolichyl phosphate was basically the same in the different groups. In microsomal fractions from hepatomas the level of cholesterol was about 30% higher than control. The protein content per gram wet weight of the hepatoma homogenates was basically similar to that of the controls.

When HMG-CoA reductase was measured (Table 3), microsomal fractions were incubated with and without Triton X-100 in order to exclude possible differences in membrane permeability between the two control and carcinoma fractions. The HMG-CoA activity in microsomal fractions from morphologically normal tissue outside the tumour was similar to that of control livers, while the corresponding activity of microsomes prepared from hepatomas was twice as high. The same pattern was seen in the absence and presence of Triton.

The distribution of individual polyisoprenoid compounds in the free and phosphorylated dolichol fractions from control liver found here is in agreement with earlier observations (Eggens *et al.* 1983), and no major difference could be observed between normal livers, tissue outside the tumours or the tumours themselves (Table 4). The two major derivatives were in all cases those with 19 and 20 isoprene residues of which only 2–4% was α-unsaturated (Table 5).

With microsomes from control liver, the incorporation of labelled mevalonic acid into α-unsaturated free polyisoprenols was much higher than into the saturated compounds (Table 6). A preferential labelling of α-unsaturated polyprenols in the initial phase of incubation was also observed with isolated rat hepatocytes (Ekström *et al.* 1984), but to a lesser extent than in the case of the human hepatomas. Incorporation into the polyisoprenol with 19 isoprene residues was higher than into the compound with 20 residues in agreement with previous studies on rat hepatocytes (Ekström *et al.* 1984). In the case of the phosphorylated polyisoprenyls, ^{3}H-mevalonic acid was not incorporated into the

Table 4. Compositions of dolichol and dolichyl phosphate in homogenates prepared from control liver, from morphologically normal liver tissue outside the tumours and from hepatomas. Experimental details are given in Materials and methods

Sample	Lipid	Composition* (% of total)					
		D17†	D18	D19	D20	D21	D22
Control	Dolichol	4±0.8	14±1.9	38±2.9	33±2.4	9±1.1	2±0.2
	Dolichyl phosphate	1±0.1	6±0.9	34±2.3	40±3.0	16±1.3	3±0.2
Morphologically normal tissue outside the tumours	Dolichol	3±0.7	14±2.1	39±3.0	34±2.5	8±0.9	2±0.2
	Dolichyl phosphate	1±0.1	7±1.1	37±2.4	40±2.8	12±1.2	3±0.3
Hepatomas	Dolichol	2±0.2	10±1.3	43±3.1	37±2.6	7±1.0	1±0.1
	Dolichyl phosphate	1±0.1	6±1.0	41±2.7	36±2.5	13±1.3	3±0.3

* Values represent the mean±s.e.m. (10 experiments).
† Dolichol length.

Table 5. Relative amounts of α-unsaturated polyprenols in polyisoprenoid compounds containing 19 and 20 isoprene residues (saturated and unsaturated compounds) in homogenates prepared from control liver, from morphologically normal liver tissue outside the tumours and from hepatomas. The samples were diagnosed histologically before analysis. Experimental details are given in Materials and methods. Values represent the mean ± s.e.m.

Samples	Polyisoprenol-19 α-unsaturated* (%)	Polyisoprenol-20 α-unsaturated* (%)
Control liver	2.0±0.3	3.5±0.3
Morphologically normal tissue outside the tumours	2.4±0.3	3.2±0.4
Hepatomas	2.6±0.4	4.4±0.4

* The figures represent the percentage of the total polyisoprenoid alcohol (saturated plus unsaturated) (10 experiments).

α-saturated compounds by either control or hepatoma tissue.

When ^{3}H-mevalonic acid was used as a precursor for cholesterol, high incorporation was obtained, as expected, in comparison with the labelling of polyisoprenols (Table 7). No differences in the incorporation into cholesterol by the non-hepatoma control liver tissue or by the morphologically normal tissue outside the liver tumours could be observed. The labelling of cholesterol with hepatoma material was about twice higher than the control (calculated on a gram wet weight or mg protein basis). The specific activity of cholesterol was, however, the same in the different groups.

When the incorporation of mevalonic acid into ubiquinone-10 was studied (Table 8), the labelling per gram wet weight by control tissue was in the same range as for the polyprenols. In the hepatomas, incorporation into ubiquinone was however only about half the control value (calculated in a gram wet weight or mg protein basis). The specific radioactivity of this compound in hepatomas remained unaltered in comparison to the control.

When the ratios of labelling of cholesterol and free polyisoprenols or cholesterol and phosphorylated polyisoprenols were calculated, the hepatoma values were about 20 and 40 times higher, respectively, than the control values (Table 9). The cholesterol to ubiquinone ratio was more than 3 times higher in the tumour tissue whereas the ratio of total dolichol to ubiquinone-10 in tumours was 9 times lower than in control tissue.

Discussion

The present studies have been focused on the synthesis of cholesterol, ubiquinone and polyisoprenoid compounds in control livers and hepatomas, since these compounds share a common initial pathway and, consequently, are dependent on common precursors (Rudney & Sexton 1986). Since the levels of sterols and polyisoprenoid compounds in hepatomas have been reported to be different from control tissue (Chen *et al.* 1978; Eggens *et al.* 1983; Eggens 1987; Osterberg & Wattenberg 1961; Sugimura *et al.* 1962) it was of interest to examine possible alterations in their rates of synthesis, in order to explain the altered levels.

In addition to changes in de-novo synthesis, other possible explanations for the altered lipid levels in hepatomas include changes in degradation and dietary uptake and/or excretion. However, in the case of dolichol no degradation has yet been

Table 6. Incorporation of ^{3}H-mevalonic acid into α-unsaturated and α-saturated polyisoprenols in both free and phosphorylated form by homogenates prepared from control liver and from hepatomas. Tissues were diagnosed histologically prior to analysis. Experimental details are given in Materials and methods

	Polyisoprenol labelling* (c.p.m./g homogenate)							
	Polyisoprenol-19		Polyisoprenol-20		Polyisoprenyl-19P†		Polyisoprenyl-20P†	
Sample	α-unsaturated	α-saturated	α-unsaturated	α-saturated	α-unsaturated	α-saturated	α-unsaturated	α-saturated
Control livers	880±75	92±10	400±41	44±4	1030±86	<10	500±48	<10
Hepatomas	67±8	15±2	25±3	<10	35±4	<10	<10	<10

* Values represent the mean±s.e.m. (10 experiments).
† P indicates phosphate.

Table 7. Incorporation of ^{3}H-mevalonic acid into cholesterol by homogenates prepared from control liver, from morphologically normal tissue outside the tumours and from hepatomas. The results were basically similar when liver slices were used. Experimental details are given in Materials and methods

	Cholesterol labelling*		
Sample	c.p.m./g homogenate	c.p.m./μg cholesterol	c.p.m./mg protein
Control liver	23450±1310	12.9±1.4	127±9.8
Morphologically normal tissue outside the tumours	24500±1386	11.0±1.2	127±9.6
Hepatomas	42300±2630	11.8±1.2	242±18.2

* Values represent the mean±s.e.m. (10 experiments).

Table 8. Incorporation of ^{3}H-mevalonic acid into ubiquinone-10 by homogenates prepared from control liver, from morphologically normal tissue outside the tumours and from hepatomas. The results were basically similar when slices were used. Experimental details are given in Materials and methods

	Incorporation into ubiquinone-10		
Sample	c.p.m./g homogenate	c.p.m./μg ubiquinone	c.p.m./mg protein
Control liver	1250±121	25.5±2.2	6.8±0.5
Morphologically normal liver tissue outside the tumours	1190±96	26.4±2.3	6.2±0.4
Hepatomas	665±55	29.5±2.4	3.8±0.3

* Values represent the mean±s.e.m. (10 experiments).

Table 9. The ratio of incorporation of mevalonic acid into sterols and nonsterols and into different nonsterols in control livers and hepatomas

Experimental group	Cholesterol/ free dolichol	Cholesterol/ phosphorylated dolichol	Cholesterol/ ubiquinone	Total dolichol/ ubiquinone
Normal liver	16.6	15.1	18.8	2.4
Hepatomas	377.7	650.8	63.6	0.27

observed and dietary uptake and/or excretion of this compound is suggested to play a minor role in determining its level in the normal liver (Elmberger *et al.* 1987; Chojnacki & Dallner 1983). On the other hand, it is still an open question as to whether the dolichol level is influenced by an altered uptake and/ or excretion under pathological conditions.

In order to select suitable samples for preparation, all the material was routinely examined histologically. Control and hepatoma livers were also subfractionated and the microsomal fraction obtained consisted

mainly of rough and smooth microsomes. Since more and altered mitochondria have been reported to be present in hepatomas (Ma & Blackburn 1973) it was necessary to investigate the possibility of an increased contamination of the microsomal fraction by this organelle. The analysis demonstrated that the mitochondria and the endoplasmic reticulum in our highly differentiated hepatomas were well developed and that no major differences in enzyme activities occurred compared to controls, which is in agreement with earlier studies (Ma & Blackburn 1973).

Since the cholesterol level fluctuates somewhat during diet, diurnal rhythm, hormonal status, etc., morphologically normal tissue outside the tumours was also used as a control to correct for some of these influences. A further control was to compare slices, homogenates and isolated microsomes in order to exclude a technical problem such as an altered uptake of the labelled precursors into the different liver samples.

When homogenates and microsomal fractions from different hepatomas were analysed, the amount of cholesterol was higher in all cases compared to controls. In the case of polyisoprenols, the dolichol levels in non-hepatoma livers and in the regions outside the tumours were similar, while the dolichol amount in the hepatomas was markedly lower.

During the de-novo biosynthesis of dolichols, α-saturation is catalysed by a NADH-dependent enzyme recently suggested to be localized in the cytoplasm (Ekström *et al.* 1987). Since recent unpublished data on poorly differentiated hepatomas collected by autopsy revealed a low level of dolichol and an increased percentage of α-unsaturated polyprenols, it was of interest to measure these parameters in our fresh surgical samples as well. In our highly differentiated hepatomas no significant difference in the level of saturation compared to controls could be observed.

Earlier investigations on human hepatomas with poor differentiation collected from autopsies revealed a shift in the polyisoprenoid pattern towards an accumulation of shorter polyisoprenols (Eggens *et al.* 1983). In this study on fresh, surgical samples of highly differentiated tumours, no major difference was found in any individual free or phosphorylated polyisoprene compared to control tissue. The altered dolichol pattern in poorly differentiated tumours was suggested, on the basis of in-vitro experiments, to be due to a decrease in the size of the mevalonate pool (Ekström *et al.* 1987). Our present results may thus indicate that the mevalonate concentration in highly differentiated hepatomas is the same as in control hepatic tissue.

The different lipid levels in hepatomas, ubiquinone (about 50% below control), dolichol (about 90% lower) and dolichyl-P (the same) is interpreted to reflect differential regulation of the biosynthesis of these compounds. The measurements of phospholipid and protein contents indicated that the total membrane mass was similar.

When ^{3}H-mevalonic acid was incubated with hepatoma liver fractions, considerably lower de-novo incorporations into α-unsaturated polyisoprenyl phosphates and free α-unsaturated polyisoprenols were obtained when compared to controls. These findings explain, at least in part, the low level of dolichol in hepatomas. The relatively unchanged level of dolichyl phosphate in tumours indicates, however, that other mechanisms than an altered de-novo synthesis may determine the concentration, as was also recently suggested (Eggens 1988). When incorporation of mevalonic acid into cholesterol was analysed, hepatomas exhibited an almost twice as high incorporation in comparison to controls.

These data indicate a moderately higher rate of de-novo cholesterol synthesis and a radically lower rate of de-novo polyisoprenol synthesis in the hepatomas, which is seen even more clearly when the ratios of incorporation into cholesterol and free polyprenols are compared.

When incorporation of mevalonic acid into ubiquinone was monitored a 50% lower

value was observed in the hepatomas, and the ratio of incorporation into cholesterol and ubiquinone was about 3 times higher in the tumour material. On the other hand, the dolichol/ubiquinone ratio of incorporation was about 9 times lower in the tumours. These ratios and the levels of cholesterol, ubiquinone and dolichol were thus clearly different, and it is suggested they reflect independent regulation of these lipids. The mechanism(s) behind these differences in lipid incorporation are, however, not clear. It was suggested earlier that the intermediate farnesylpyrophosphate has a relatively high affinity for the dolichol and ubiquinone biosynthetic pathways in comparison with the cholesterol pathway (Gold & Olson 1966; Faust *et al.* 1979), but this mechanism is not likely to operate under the pathological conditions examined here. This conclusion is also in line with a recent study on normal rat hepatocytes, where both the cholesterol and polyisoprenol pathway were saturated at the same concentrations of mevalonolactone (Keller 1986).

The increase in HMG-CoA reductase activity and increase in cholesterol synthesis which was observed in our hepatoma microsomal fractions is in agreement with earlier reports (Siperstein & Fagan 1964; Brown & Goldstein 1980; Siperstein *et al.* 1971). In addition to HMG-CoA reductase, other enzymes such as squalene synthetase are also suggested to be regulated by LDL cholesterol (Brown & Goldstein 1980). In fibroblasts exposed to LDL-cholesterol inhibition of the enzyme squalene synthetase was suggested to lead to an accumulation of farnesylpyrophosphate, a decrease in cholesterol synthesis and a flow of metabolites towards ubiquinone (Faust *et al.* 1979a). An increase in the latter enzyme activity would be consistent with the relatively high rate of mevalonate incorporation into cholesterol in our hepatomas.

The report that HMG-CoA reductase is also localized in peroxisomes (Keller *et al.* 1985) may indicate the presence of different intracellular pools of precursor metabolites, which would further modify the picture. Alterations in the level of dolichol have recently been suggested to be caused by changes in HMG-CoA reductase activity (Kabakoff & Kandutsch 1987), but the low amounts of polyisoprenoid compounds and the lowered polyisoprenol synthesis in our hepatomas could theoretically also be explained by a lowered *cis*-prenyl transferase activity and/or by a decrease in the size of the farnesylpyrophosphate pool secondary to a flow of metabolites into the cholesterol pathway. This last effect might arise as a result of a hypothetical loss of the inhibition of squalene synthetase activity by LDL-cholesterol in hepatomas.

Since the incorporation of mevalonic acid into ubiquinone also was lower in hepatomas, but not as low as in the case of the polyisoprenols, a simple alteration in the size of the farnesylpyrophosphate pool is unlikely to explain these different labelling patterns for both ubiquinone and polyisoprenols. Other explanations for the altered incorporation into ubiquinone are, however, possible, since the ubiquinone molecule is suggested to be assembled from the products of several different metabolic pathways localized in both mitochondria and microsomes (Kalén *et al.* 1987).

Considering the differences in both the patterns of incorporation and lipid levels, it thus seems unlikely that all these differences are caused simply by changes in the mevalonate or farnesylpyrophosphate concentrations, and/or an increased flow of metabolites into the cholesterol pathway resulting in the depletion of precursor for the ubiquinone or polyisoprenol pathways. The results indicate an independent regulation of these three lipids, where the individual pathways in hepatomas are likely to differ from those in control tissue.

We thus suggest that the alteration in lipid metabolism in hepatomas is not restricted to the regulatory enzyme HMG-CoA reductase but probably also involves other enzymes subsequent to farnesylpyrophosphate, which regulate the flow of metabolites

beyond this branch point to the three major polyisoprenoid entities, i.e. sterols, dolichols and ubiquinones. Future studies, for example on the independent regulation of squalene synthetase and of *cis* and *trans*-prenyltransferases, enzymes in the ubiquinone pathway, and measurements of the sizes of the mevalonate and farnesylpyrophosphate pools in hepatomas are, however, necessary to clarify these questions.

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