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Alterations in the activities of hepatic plasma-membrane and microsomal enzymes during liver regeneration

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A marked increase in the activities of rat liver plasma-membrane ($Na^+ + K^+$)-stimulated ATPase and microsomal Ca^{2+}-stimulated ATPase was observed 18h after partial hepatectomy. Lipid analyses for both membrane preparations reveal that in partially hepatectomized rats the cholesterol and sphingomyelin content are decreased with a subsequent decrease in the cholesterol/phospholipid molar ratio compared with those of sham-operated animals. Changes in the allosteric properties of plasma-membrane ($Na^+ + K^+$)-stimulated ATPase by F^- (as reflected by changes in the Hill coefficient) indicated a fluidization of the lipid bilayer of both membrane preparations in 18h-regenerating liver. The amphipathic dodecyl glucoside incorporated into the hepatic plasma membranes evoked a marked increase in the ($Na^+ + K^+$)-stimulated ATPase and 5′-nucleotidase activities. The lack of effect of the glucoside on the Lubrol-PX-solubilized 5′-nucleotidase indicates that changes in the activities of the membrane-bound enzymes caused by the glucoside are due to modulation of the membrane fluidity. Dodecyl glucoside appears to increase the membrane fluidity, evaluated through changes in the Hill coefficient for plasma-membrane ($Na^+ + K^+$)-stimulated ATPase. The biological significance of these data is discussed in terms of the differences and changes in the interaction of membrane-bound enzymes with membrane lipids during liver regeneration.

The regenerating rat liver has been chosen as an *'in-vivo'* model to study eukaryotic cell growth because a well-defined, and to some extent synchronous, cellular proliferation occurs within the first 24h after partial hepatectomy (Hays, 1974). Some enzyme activities of rat liver plasma membranes have been reported to change during liver regeneration, mediated through alterations in membrane fluidity (Wright, 1977; Bruscalupi *et al.*, 1980). The effect of liver regeneration on the fluidity of the hepatic plasma membrane and endoplasmic reticulum, between 15 and 24h after partial hepatectomy, appears to be particularly important, since during this time the cells that are in the cell cycle of division are in the S or DNA-synthesis phase (Bruscalupi *et al.*, 1980).

We have studied previously the effects *in vitro* of small amphipathic molecules (i.e. cholesterol, octanol, dodecanol or their glucosides) on the activity of several membrane-bound enzymes (Alivisatos *et al.*, 1981*b*). The introduction of the water-soluble glucosides led to the discovery of the synergistic nature of the incorporation of these compounds into biomembranes, with subsequent co-operative functional changes of integral proteins (Papaphilis & Deliconstantinos, 1980; Alivisatos *et al.*, 1981*a*; Alivisatos & Deliconstantinos, 1982).

In the present study we performed experiments on the evoked functional changes in hepatic membrane-bound enzymes after partial hepatectomy. The water-soluble dodecyl glucoside was used as a tool to provide insight into the influence of membrane fluidity on some membrane integral enzymes of hepatic plasma membrane and microsomes during liver regeneration.

Materials and methods

Male Wistar rats (150–180g) were maintained in a temperature-controlled room with a 12h light period ((07:00h to 19:00h). Partial hepatectomy was performed under diethyl ether anaesthesia by the method of Higgins & Anderson (1931). The left

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and median lobes, which constitute 68–70% of total liver mass, were resected. Sham-operated rats were handled in an identical manner with respect to the incision and handling of the liver, but the ligation and resection were not performed. Animals were killed by cervical dislocation 18h after surgical treatment. During this period they were fed normally. Experiments were timed so that the rats were killed between 08:00h and 11:00h.

Isolation of plasma membranes and microsomes

Liver plasma membranes were prepared from control, sham-operated and hepatectomized rats by discontinuous-sucrose-density-gradient centrifugation by the method of Neville (1960) with 1mM-$NaHCO_3$, but with the modification described by Emmelot *et al.* (1964), in which another sucrose gradient was employed (1.18g/ml) in addition to the original gradients of 1.22 and 1.16g/ml used by Neville (1960). The membrane layer, which rises to the interface between 1.18 and 1.16g/ml, was removed and resuspended in 5mM-Tris/HCl, pH 7.4, to a final concentration of 1–2mg of protein/ml.

Liver microsomes were isolated from the resulting supernatant as described previously (Deliconstantinos, 1981; Deliconstantinos & Ramantanis, 1982) by first centrifuging at 17000*g* for 20min in a Sorvall RC-2B refrigerated centrifuge. The pellet was discarded and the supernatant was centrifuged at 105000*g* for 60min in a Beckman L5-75 Ultracentrifuge. The resulting pellet was resuspended in 1.15% KCl/5mM-Tris/HCl (pH 7.0) at a concentration of 10mg of protein/ml. The protein content was determined by the Lowry method as described by Miller (1959) with bovine serum albumin (Sigma) as standard.

Extraction of lipids

Plasma membranes and microsomes were extracted overnight with 20vol. of chloroform/methanol (2:1, v/v) by the method of Folch *et al.* (1957). Phospholipids were separated from the chloroform extracts by t.l.c. on silica-gel G with appropriate standards and with, as solvent system, chloroform/methanol/acetic acid/water (25:15:4:2, by vol.).

The phospholipids were identified by exposure to I_2 vapour, removed and the content of lipid phosphorus was measured by the method of Bartlett (1959) in the spots after digestion with 70% $HClO_4$ at 180°C. More than 95% of the phospholipid phosphorus was chromatographed with the standard phospholipid. Cholesterol was determined by g.l.c. with 5α-cholestane as internal standard.

Enzyme assays

The quality and the degree of purification of the isolated plasma membranes and microsomes was assessed by measuring the activities of the following enzyme markers. 5'-Nucleotidase (EC 3.1.3.5), characteristic of plasma membranes, was assayed at pH 7.5 in the presence of 10mM-AMP and 5mM-$MgCl_2$ by the method of Bodansky & Schwartz (1963). Cytochrome *c* oxidase (EC 1.9.3.1), a mitochondrial marker, was assayed as described by Cooperstein & Lazarow (1951). Glucose 6-phosphatase (EC 3.1.3.9), a microsomal marker, was measured by the method of Swansen (1955).

ATPase assay

Liver plasma-membrane preparations were assayed for $(Na^+ + K^+)$-stimulated ATPase activity in 0.07–0.1mg of plasma-membrane protein. Incubations were carried out in the reaction medium (1ml) for 20min at 37°C. The reaction was started with the addition of 100μl of 50mM-disodium ATP (Sigma Chemical Co., St. Louis, MO, U.S.A.) in a mixture containing 80mM-Tris/HCl, pH 7.4, 5mM-$MgCl_2$ and 0.2mM-EDTA, in the presence or absence of 10mM-KCl and 100mM-NaCl. Ouabain (1mM) was used in parallel incubations to estimate $(Na^+ + K^+)$-stimulated ATPase activity as the difference between total ATPase and ouabain-insensitive ATPase (i.e. Mg^{2+}-stimulated ATPase). The results showed that the ouabain-inhibitible component of ATPase activity is equal to that activity which is lost when Na^+ or K^+ is omitted from the reaction mixture. The reaction was terminated by adding ice-cold trichloroacetic acid to a final concentration of 10% and placing the tubes on ice. After centrifugation, P_i was determined in the supernatant by the method of Fiske & SubbaRow (1925). $(Na^+ + K^+)$-stimulated activity was taken to be the difference between total ATPase (with KCl and NaCl) and Mg^{2+}-stimulated ATPase (without KCl and NaCl). For the assay of the inhibition by F^- of the $(Na^+ + K^+)$-stimulated ATPase the reaction mixture contained increasing amounts of NaF as indicated in the Figures.

Assay of Ca^{2+} uptake

Ca^{2+} uptake by microsomes was assayed at 37°C in a medium described by Moore (1982) by using the Millipore-filtration technique The medium consisted of 100mM-KCl, 30mM-imidazole/histidine buffer, pH 7.0, 5mM-ammonium oxalate, 5mM-$MgCl_2$ and 5mM-dipotassium ATP (Sigma) in a total volume of 1.5ml. The assay was started by the addition of 100μg of microsomal protein. After exactly 60s, $CaCl_2$ (20μM, containing 0.1μCi of $^{45}Ca^{2+}$) was added and the mixture was thoroughly agitated. Samples (200μl) taken from the mixtures at defined intervals were placed on pre-wetted HAWP Millipore filters (0.45μm pore diameter) and filtered under suction by using a Millipore 3025 sampling manifold. The filters were washed three times by adding 2ml of 0.25M-sucrose/5mM-Tris/HCl, pH 7.0;

and transferred into counting vials. They were then shaken in a mixture of 1 ml of 1% sodium dodecyl sulphate and 10 ml of dioxan-based scintillation fluid (Bray, 1960) and counted for radioactivity. A portion of the incubation medium was also spotted on a Millipore filter, dried and counted for radioactivity under identical conditions. Radioactivity (c.p.m.) was converted into nmol by using the known specific radioactivity. The amount of radioactivity that was constantly absorbed on to microsomes at zero time (instantaneous deposition of radioactivity) was subtracted in every case from all samples. ATP-dependent Ca^{2+} uptake was calculated by subtracting Ca^{2+} binding of microsomes in the absence of ATP in the medium from total Ca^{2+} uptake in its presence. Binding in the absence of ATP was complete by 2 min.

Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase activity of the microsomes was estimated under similar conditions to those used for estimating Ca^{2+} uptake. The reaction was started by the addition of 0.35 mg of microsomal protein and stopped at defined intervals with 0.2 ml of 50% trichloroacetic acid. The liberated P_i was measured by the method of Fiske & SubbaRow (1925). The Ca^{2+}-stimulated ATPase activity was defined as the difference between the P_i liberated during incubation in the presence and absence of Ca^{2+}.

Effect of dodecyl glucoside

Dodecyl glucoside was synthesized as previously described (Alivisatos *et al.*, 1981*a*). Pre-incubations of plasma membranes with different amounts of dodecyl glucoside were also carried out as described previously (Alivisatos *et al.*, 1981*a*) for 3 h at 37°C in an incubation mixture of 1.15% KCl/5 mM-Tris/HCl, pH 7.4, containing 0.5 mg of plasma-membrane protein in a final volume of 3 ml with continuous stirring with a magnetic bar. $(Na^+ + K^+)$-stimulated ATPase activity was estimated in portions of the membranous suspensions as described above. Control experiments indicated that dodecyl glucoside did not interfere with the assay procedure.

Results

The purity of the hepatic plasma-membrane and microsomal preparations used in these studies was assessed by measuring the specific activities of 5′-nucleotidase, a marker enzyme for plasma membranes, as well as glucose 6-phosphatase and cytochrome *c* oxidase, which are well-established markers for the endoplasmic reticulum and the mitochondrion respectively. The average yield of plasma membranes from sham-operated animals was 1.55 ± 0.21 mg/g wet wt. of liver (mean ± S.D. for five animals) and from partially hepatectomized

Table 1. *Activities and yields of marker enzymes*

Homogenates, plasma membranes and microsomes were prepared from livers of sham-operated and partially hepatectomized rats 18 h after surgery, and assays were carried out as described in the Materials and methods section. Specific activities of the phosphatases are expressed as μmol of P_i/h per mg of membrane protein. Cytochrome *c* oxidase activity is expressed as average ΔA units/min per mg of protein. Percentage recovery equals total preparation activity as a percentage of the total homogenate activity. Relative specific activity (RSA) equals the ratio of the preparation specific activity to homogenate specific activity. Results are given as means ± S.D. for five animals.

	Sham-operated rats						Partially hepatectomized rats					
	5′-Nucleotidase			Glucose 6-phosphatase		Cytochrome *c* oxidase	5′-Nucleotidase			Glucose 6-phosphatase		Cytochrome *c* oxidase
	Specific activity	Yield (%)	RSA	Specific activity	Yield (%)	Specific activity	Yield (%)	Specific activity	Yield (%)	RSA	Specific activity	Yield (%)	Specific activity	Yield (%)
Plasma membrane	40.68 ± 6.08	13.32	18.92	2.15 ± 0.21	0.31	0.05 ± 0.01	0.01	36.16 ± 4.79	11.06	15.92	1.78 ± 0.17	0.26	0.04 ± 0.01	0.009
Microsomes	5.18 ± 1.30	22.01	2.40	24.78 ± 3.34	47.38	2.75 ± 0.35	7.47	5.05 ± 1.40	22.61	2.22	17.28 ± 3.08	42.61	3.05 ± 0.40	10.00

Table 2. *Effect of partial hepatectomy on rat liver plasma-membrane enzyme activities and lipid composition*
Liver plasma membranes were prepared from sham-operated and partially hepatectomized rats 18h after operation, and were analysed for enzyme activities and lipid composition as described in the Materials and methods section. Values given are means ± S.D. for five animals. The cholesterol/phospholipid ratio is shown in parentheses.

	Sham operation	Partial hepatectomy
Enzymes tested (μmol of P_i/mg of protein per h)		
$(Na^+ + K^+)$-stimulated ATPase	13.79 ± 1.18	22.87 ± 2.16*
Mg^{2+}-stimulated ATPase	41.16 ± 4.87	43.15 ± 5.61
Lipids (nmol/mg of protein)		
Cholesterol	230 ± 24	166 ± 17*
Phospholipids	357 ± 45 (0.64)	361 ± 51 (0.45)
Phosphatidylcholine	117 ± 14	125 ± 15
Sphingomyelin	64 ± 8	37 ± 5*
Phosphatidylethanolamine	71 ± 10	64 ± 9

* Statistically significant, compared with control (sham-operated) rats ($P < 0.01$).

Table 3. *Effect of partial hepatectomy on rat liver microsomal enzyme activities and lipid composition*
Liver microsomes were prepared from sham-operated and partially hepatectomized rats 18h after operation, and were analysed for enzyme activities and lipid composition as described in the Materials and methods section. Values are means ± S.D. for five animals. Values in parentheses are cholesterol/phospholipid ratio.

	Sham operation	Partial hepatectomy
Enzymes tested (nmol of P_i/mg of protein per h)		
Ca^{2+}-stimulated ATPase	105.5 ± 13.34	175.7 ± 22.3*
Mg^{2+}-stimulated ATPase	652.7 ± 33.4	675.8 ± 35.7
Lipids (nmol/mg of protein)		
Cholesterol	75 ± 9	52 ± 7*
Phospholipids	351 ± 48 (0.21)	478 ± 65* (0.11)
Phosphatidylcholine	201 ± 20	291 ± 28*
Sphingomyelin	46 ± 4	12 ± 2*
Phosphatidylethanolamine	82 ± 12	94 ± 13

* Statistically significant compared with control (sham-operated rats) ($P < 0.05$).

was 1.25 ± 0.20 mg/g (five animals). The specific activity of 5′-nucleotidase was increased 18-fold in plasma membranes compared with homogenates, and the recovery of this enzyme in plasma membranes was 11–13% of total homogenate (Table 1). Although 5′-nucleotidase was 18-fold-enriched, whereas Wright (1977) obtained 38-fold enrichment, the relative purification of liver plasma-membrane preparations did not differ significantly between respective sham-operated and partially hepatectomized animals. Thus as shown in Table 1, mitochondrial and microsomal contamination, as indicated by the activities of the marker enzymes, was similar in the plasma membranes and microsomes prepared from sham-operated and partially hepatectomized animals.

Preliminary experiments showed that at 18h after sham operation, the hepatic plasma-membrane $(Na^+ + K^+)$-stimulated ATPase activity was significantly affected. This observation, in combination with already published results, that surgical operation or anaesthesia alone changed the lipid structure of liver plasma membranes (Bruscalupi *et al.*, 1980), led us to use as 'controls', in the present study, membranes isolated from sham-operated rats. Thus, all the comparisons made here were between sham-operated and partially hepatectomized rats.

Table 2 shows that the activity of the hepatic plasma-membrane $(Na^+ + K^+)$-stimulated ATPase was increased approx. 65% at 18h after partial hepatectomy, whereas the activity of the 5′-nucleotidase exhibited a slight but not statistically significant decrease ($P > 0.05$). Results presented in Table 3 show a marked increase in the Ca^{2+}-stimulated ATPase activity (approx. 70%) and a slight but not statistically significant decrease ($P > 0.05$) in the glucose 6-phosphatase activity in microsomal membranes from 18h-regenerating liver. Mg^{2+}-stimulated ATPase activity did not show any change in both membrane preparations.

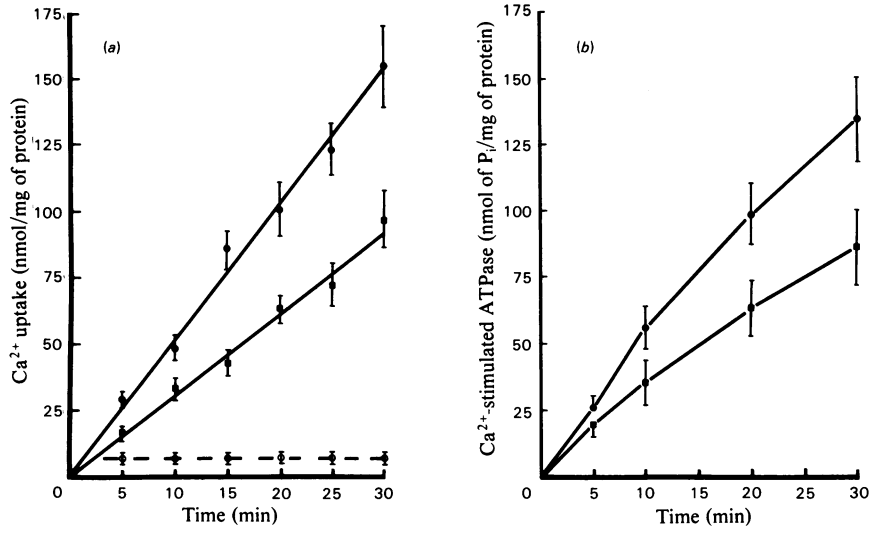

Fig. 1. *Time course of Ca^{2+} uptake (a) and Ca^{2+}-stimulated ATPase activity (b) for hepatic microsomal membranes from sham-operated (■) and partially hepatectomized rats (●)*

(*a*) Ca^{2+} uptake was assessed in the standard assay as described in the Materials and methods section. ○, Ca^{2+} uptake by microsomes in the absence of ATP. The straight lines were fitted by the method of least squares. Each point represents the mean ± S.D. of duplicate determinations for both (*a*) and (*b*) from five separate microsomal preparations.

Data in Fig. 1(*a*) are from experiments in which the progress of Ca^{2+} uptake by liver microsomes isolated from sham-operated and partially hepatectomized rats was followed at relatively short time intervals. Ca^{2+} was accumulated by microsomes in the presence of ATP but not in its absence. In the standard medium used, the initial rate of Ca^{2+} uptake was maintained at least for 30min. Computer analysis for curve fitting of Ca^{2+} uptake was performed with a programmable Hewlett–Packard HP-97 calculator. The correlation coefficients (r^2) for linear fitting were greater than 0.95. The ATP-dependent uptake of Ca^{2+} was measured at relatively low (20 μM) Ca^{2+} concentrations, comparable with those prevailing intracellularly. Liver microsomes isolated from partially hepatectomized rats exhibit a higher Ca^{2+} uptake (approx. 60%) than those isolated from sham-operated animals. A close relationship between enhanced microsomal Ca^{2+} uptake and increased microsomal Ca^{2+}-stimulated ATPase activity appears to exist (Figs. 1*a* and 1*b*).

Results from quantitative analysis of lipids in plasma membranes and microsomes (Tables 2 and 3) disclosed some interesting findings on the characteristics of regenerating liver in comparison with sham-operated animals. (*a*) There was markedly lower cholesterol and sphingomyelin contents per mg of protein in both plasma membranes and microsomes isolated from partially hepatectomized rats. (*b*) There was an increase in the amount of total phospholipids per mg of protein in the microsomes isolated from partially hepatectomized rats. The cholesterol/total phospholipid molar ratio in plasma membranes of sham-operated animals (0.64) was much higher than that in microsomal membranes (0.21). The ratio was decreased to 0.45 for the plasma membranes and to 0.11 for the microsomes at 18h after partial hepatectomy.

Fig. 2(*a*) shows that dodecyl glucoside incubated for 3h at 37°C with liver plasma membranes isolated from sham-operated animals markedly increases the activity of the $(Na^+ + K^+)$-stimulated ATPase, with a maximal effect of approx. 85% greater than the original value being achieved at glucoside concentrations around 0.1–0.2mM. Further increases in the glucoside concentration above this value led to an equally marked inhibition of the activity up to 35% lower than the original value, at a concentration of 1mM. Liver plasma membrane of partially hepatectomized rats treated in the same way with the glucoside showed a small but significant increase (approx. 20%) at concentrations of 0.1–0.2mM, whereas use of higher concentrations led to a progressive inhibition.

The effect of dodecyl glycoside on the activity of 5′-nucleotidase of liver plasma membranes from sham-operated and partially hepatectomized rats is

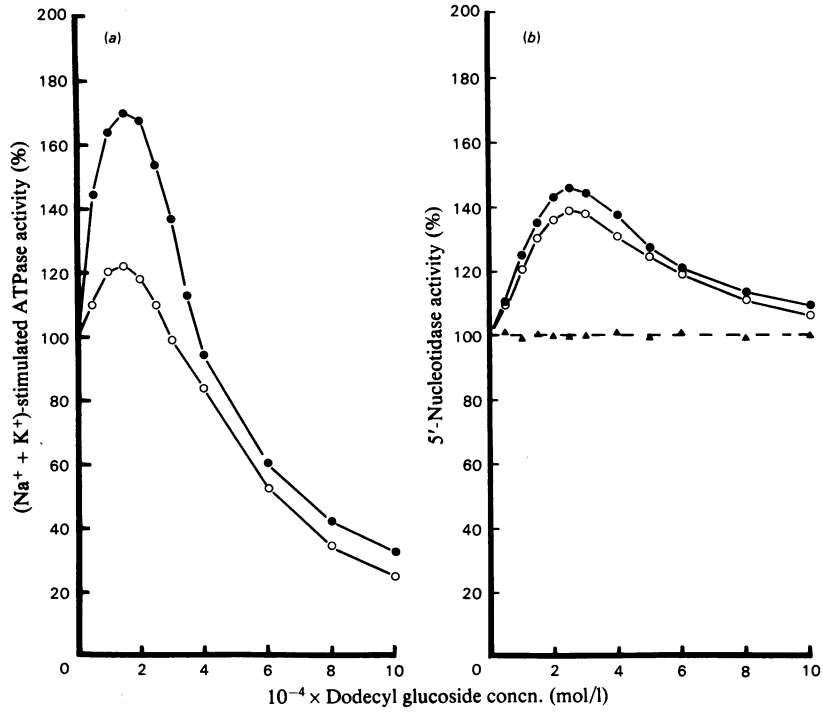

Fig. 2. *Effect of dodecyl glucoside on membrane-bound* $(Na^+ + K^+)$*-stimulated ATPase activity* (*a*) *and on the 5'-nucleotidase activity* (*b*) *of liver plasma membranes from sham-operated* (●) *and partially hepatectomized rats* (○) (*b*) shows the effect of dodecyl glucoside on 5'-nucleotidase activity in Lubrol PX-solubilized membranes (▲). Points in the drawn curves are mean values of duplicate determinations for both (*a*) and (*b*) from a typical experiment, which was repeated three times.

shown in Fig. 2(*b*). 5'-Nucleotidase activity exhibited a significant increase (approx. 45%) in both plasma membranes at around 0.2mM-dodecyl glucoside, whereas use of higher concentrations led to some inhibition of the activity compared with the maximal percentage of stimulation. Solubilization of the 5'-nucleotidase by using the non-ionic detergent Lubrol-PX by the method of Swislocki *et al.* (1975) resulted in no change in the enzyme activity at any dodecyl glucoside concentration used. The results obtained for the effect of the glucoside on the activity of the membrane-bound enzymes and on the solubilized enzyme indicate that it exerts its action not directly on the protein molecules but by changing the membrane fluidity.

The possible influence of the lipid environment and its fluidity on the membrane enzyme activity was studied from the inhibition of integral membrane enzymes by F^- the method of Farias (1980). Fig. 3 shows the curves obtained when the relative rates of the enzymic activities were plotted against F^- concentrations for $(Na^+ + K^+)$-stimulated ATPase of liver plasma membranes isolated from sham-operated and partially hepatectomized rats and liver plasma membranes from sham-operated rats pre-incubated with 0.2mM-dodecyl glucoside for 3h at 37°C. The Hill coefficient (slopes), *h*, for the F^- inhibition of $(Na^+ + K^+)$-stimulated ATPase activity for the sham-operated animals was 2.03, indicating the presence of co-operativity, which was decreased in membranes treated with dodecyl glycoside ($h = 1.35$) and abolished in partially hepatectomized rats ($h = 1.01$). These results suggest that partial hepatectomy and/or dodecyl glucoside increase the membrane bilayer fluidity and subsequently inhibit the allosteric behaviour of the membrane-bound enzymes.

Discussion

Rat liver regeneration provides an '*in-vivo*' model in which changes in the activities of plasma-membrane and endoplasmic-reticulum enzymes may be related to the regulation of the orderly cell proliferation (Kaplan, 1978). Changes in liver plasma-membrane fluidity have been observed

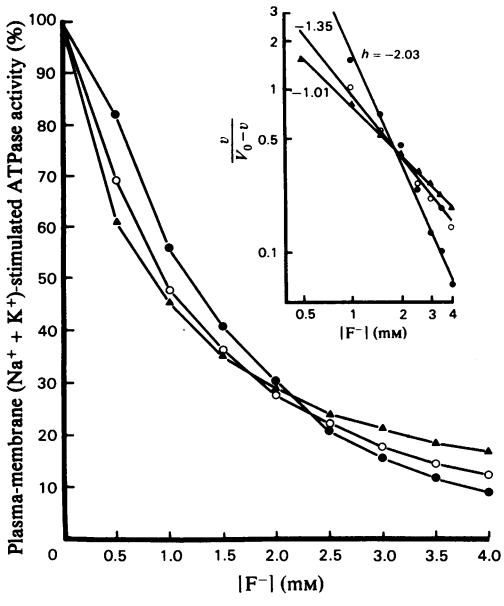

Fig. 3. *Effect of F^- on the reaction rate of the hepatic plasma-membrane-bound ($Na^+ + K^+$)-stimulated ATPase from sham-operated (●) and partially hepatectomized rats (▲) and from sham-operated rats treated in vitro with dodecyl glucoside* (○)

Experimental details are given in the text. The insert show Hill plots of the same data. Corresponding Hill coefficients (*h*) are as indicated. The correlation coefficient (r^2) for the straight lines in the insert are >0.95. *v* is the reaction velocity, V_0 is the rate of the reaction in the absence of F^-; the slope (*h* value) of each line is indicated in the Figure. Points in the drawn curves are mean values of duplicate determinations from a typical experiment, which was repeated three times.

between 15 and 24h after partial hepatectomy (S or DNA-synthesis phase) associated with alterations in the lipid composition and functional changes in liver plasma-membrane-bound enzymes (Van Hoeven *et al.*, 1979; Bruscalupi *et al.*, 1980).

In the present study, ($Na^+ + K^+$)-stimulated ATPase activity showed an approx. 65% increase in hepatic plasma membranes of partially hepatectomized rats in comparison with that of sham-operated rats. This finding is consistent with that reported by Wright (1977) of an increase ($Na^+ + K^+$)-stimulated ATPase activity in plasma membranes for 24h-regenerating liver, but it does not agree with the results obtained by Bruscalupi *et al.* (1980), who demonstrated that the enzyme activity was decreased at 15h after partial hepatectomy. These contrasting results may be due to differences in sex and strain of rats, time of surgery or methods of membrane isolation and also in the fact that, in various physiological conditions, the division and the specific tissue functions of the liver during regeneration present a mutually exclusive relationship and a specific diurnal rhythm (Desser-Wiest, 1975). Demel *et al.* (1977) showed that cholesterol has a preferential affinity for neutral phospholipids in the following order: sphingomyelin > phosphatidylcholine > phosphatidylethanolamine. This preferential interaction of cholesterol with sphingomyelin is noteworthy in connection with the present observation that partial hepatectomy lowers the contents of both sphingomyelin and cholesterol in liver plasma membranes and microsomes, effects that tend to increase the fluidity of the membrane lipid bilayer (Tables 2 and 3). It has been proposed by Farias (1980) that the co-operative behaviour of several membrane-bound enzymes could be used as a tool to detect modifications at the cell-membrane level. In their system the Hill coefficient for the inactivation of the membrane enzyme ($Na^+ + K^+$)-stimulated ATPase by F^- varied considerably when the membrane fluidity was changed. Their observations were confirmed in the present studies for the plasma-membrane ($Na^+ + K^+$)-stimulated ATPase of rat liver. As shown in Fig. 3 the value of the Hill coefficient, *h*, was decreased after partial hepatectomy, indicating a loss of co-operativity consistent with a general increase in lipid fluidity.

The water-soluble dodecyl glucoside exhibits profound effects on the activities of the liver plasma-membrane-bound enzymes ($Na^+ + K^+$)-stimulated ATPase and 5′-nucleotidase (Figs. 2*a* and 2*b*). The lack of effect of the glucoside upon the Lubrol-PX-solubilized 5′-nucleotidase reveals that it does not affect the enzyme directly, but the evoked functional effects in the membrane-bound enzyme are mediated through changes in membrane fluidity. The modulation of the membrane fluidity by the influence of the dodecyl glucoside was detected by determination of the Hill coefficient for the inhibition of plasma-membrane ($Na^+ + K^+$)-stimulated ATPase by F^- (Fig. 3). The decrease in the Hill coefficient observed in glucoside-treated membrane compared with sham-operated rats (controls) suggests a decrease of co-operativity in the enzymes tested, which is undoubtedly due to an increase in the fluidity of the membrane.

There is considerable evidence that Ca^{2+} plays a significant role in the regulation of a variety of functions associated with cell membranes, including the transduction of hormonal signals, neurotransmitter release and action, muscle contraction and relaxation, release of exocrine and endocrine secretory products etc. (Foreman *et al.*, 1973). We reported previously (Deliconstantinos *et al.*, 1982) the role of the Ca^{2+}-stimulated ATPase in the hepatic endoplasmic reticulum as a Ca^{2+}-pump and the alterations of calcium homoeostasis occurring in

liver cells owing to disturbances in the function of this pump. The results also suggest that certain of the functional effects of Ca^{2+} may be mediated through changes in membrane lipid metabolism and fluidity.

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