preparations were re-centrifuged in the small capacity VTi 65 (8×5.3 ml) vertical rotor. Ethidium bromide and caesium chloride were removed from the plasmid, the latter characterized by horizontal gel electrophoresis and electron microscopy[6], and the DNA concentration measured[7].

Results. The efficiency of the Ti 70 fixed angle and VTi 50 vertical rotors for Ti plasmid preparation can be compared, since both have a similar maximum radius and hold tubes of similar dimensions and capacities. The smaller VTi 65 is useful for estimating the purity of plasmid prepared in the larger volume Ti 70 and VTi 50 rotors. At DNA concentrations higher than 77.9 µg/ml, a plasmid band was not always visible in the Ti 70 rotor after centrifugation. However, in the vertical rotors, with the same and higher concentrations of DNA, separation of plasmid from chromosomal DNA was always achieved, and it was possible to use up to 155.8 µg/ml of total DNA without overloading the gradient (table 1). When a DNA concentration was used at which the separation of plasmid from chromosomal DNA could be achieved repeatedly in both rotors (51.9 µg of total DNA/ml of gradient), it was found that in the vertical rotors equilibrium was reached with distinct plasmid and chromosomal bands present after 12–16 h of centrifugation. However, in the fixed angle rotor, equilibrium was not achieved until 36–48 h (table 2). Resolution in the vertical rotors was superior to that in the fixed angle rotor (table 2; fig. A and B). Plasmid prepared in the fixed angle Ti 70 rotor usually contained 20–30% contaminating chromosomal DNA (fig. C), which increased when the gradient carried more than 50 µg/ml of total DNA. In contrast, plasmid prepared in the VTi 50 rotor contained little or no chromosomal DNA after re-centrifugation (fig. D), and was at least 70% supercoiled as judged by gel electrophoresis (fig. E) and electron microscopy (fig. F). Plasmid yield was routinely 50 to 80 µg/l of bacterial culture.

Discussion. The aim of this investigation was to speed up the large scale isolation of plasmid DNA from *A. tumefaciens* whilst maintaining a low level of contaminating chromosomal DNA and other macromolecules. Using the procedure described, the total DNA from 1.0 g wet wt of bacteria could be processed in a single large capacity vertical rotor tube, and plasmid bands collected after 12 h. If an adequate plasmid concentration step[8] is included in the isolation procedure, then the plasmid DNA can be re-centrifuged several times in a small volume vertical tube to give an ultra-pure preparation in the same time that it would take to achieve a single isopycnic separation in a fixed angle rotor.

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Effect of free fatty acids and cholesterol in vitro on liver plasma membrane-bound enzymes

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Summary. The effect of cholesterol and fatty acid treatment in vitro was tested on rat liver plasma membrane-bound enzymes and lipid fluidity. The observed alterations of membrane fluidity affect both (Na^+-K^+)-ATPase and Mg^{2+}-ATPase activities but not 5′-nucleotidase; basal adenylate cyclase as well as its hormonal sensitivity were differentially affected by changes of membrane microenvironment.

Recent evidence points to the role of lipids in the modulation of plasma membrane-bound enzymatic activities and hormonal responses[2–5]. Alterations in phospholipid content and fatty acid composition as well as changes in cholesterol levels, observed in vivo and in vitro, affect the function of membrane enzymes[6] which in turn are mainly dependent on the fluidity of the membrane microenvironment[5,7].

Few reports have been published so far as to the dependence of liver plasma membrane-bound enzymatic activities on the in vivo or in vitro alteration of membrane lipid composition[6,8]. In this experimental framework, the observations to be reported herein deal with the in vitro modulation of rat liver plasma membrane fluidity as related to the activity of plasma membrane-bound (Na^+-K^+) and Mg^{2+}-ATPase (EC 3.6.1.4 and 3.6.1.3 respectively), 5′-nucleotidase (EC 3.1.3.5) and adenylate cyclase (EC 4.6.1.1).

Materials and methods. Liver plasma membranes were isolated from male Sprague-Dawley rats (150 g, av. b.wt), fed ad libitum, according to Ray[9] as previously reported[10]. Fatty acids dissolved in absolute ethanol were added to plasma membrane suspended in 0.05 M Tris HCl (pH 7.5) at a final concentration of 1 µmole/3 mg membrane protein in a volume of 1 ml, the final ethanol concentration being 1%. Cholesterol was dissolved as above and added to the membrane suspension at a final concentration of 100 µg/3 mg membrane protein. Fatty acid and cholesterol treatment of isolated liver membranes was carried out for 10 min at 20 °C[11] whereas controls were treated with ethanol at a final concentration of 1% for the same time; this gave a 15% decrease in basal activity detectable only in the case of the cyclase assay[5].

Fatty acid- or cholesterol-treated membranes were washed twice with the Tris buffer and resuspended to the initial protein concentration in the same buffer. The measured cholesterol enrichment, estimated as already reported[12], was about 25% ($p < 0.001$) being the cholesterol content 70 ± 3.6 and 93 ± 5.3 (mean ± SD, $n = 4$) µg/mg membrane protein in control and treated membranes respectively. The

extent of the in vitro incorporation in liver membranes of both oleic and palmitic acid was tested using tracer amounts of the [^{14}C]-labelled analogs and proved to be 78 ± 4% (average ± SD, n = 4). This test was not carried out for cis-vaccenic acid owing to the unavailability of the labelled compound. After the in vitro treatment, plasma membranes were assayed as to ATPase activities and 5′-nucleotidase as already reported[13]; adenylate cyclase activity was also assayed as reported by us with the addition of 10 mM GTP when glucagon stimulation was tested[14]. All enzymatic assays were carried out at 37 °C.

Fluorescence labelling of control and treated plasma membranes and fluorescence polarization measurements were carried out as follows: $2 \cdot 10^{-3}$ M 1,6-diphenyl-1,3,5-hexatriene (Fluka A G, Buchs, Switzerland) in tetrahydrofuran was diluted 1:1000 immediately prior to use with Tris buffered saline (0.05 M Tris HCl, pH 7.5; 0.1 M NaCl) and then mixed in a 1:1 ratio with membrane suspension in the same medium to a final protein concentration of about 50 µg/ml; this mixture was then incubated 30 min at 37 °C. Fluorescence polarization was measured as recently reported[15] employing an Aminco-Bowman spectrophotofluorometer equipped with 2 Glan prism polarizers; the excitation was set at 366 nm and the emission recorded at 430 nm. The degree of fluorescence polarization, P, was calculated by the equation:

$$P = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp} = \frac{(I_\parallel / I_\perp) - 1}{(I_\parallel / I_\perp) + 1}$$

$I_\parallel$ and $I_\perp$ being the fluorescence intensities observed with the analyzing polarizer oriented, respectively, parallel and perpendicular to the direction of the polarized excitation beam[16]; correction for light scattering from the sample as well as for a grating correction factor[17] were introduced; the temperature of the sample was 37 ± 0.1 °C.

Proteins were estimated by the method of Lowry et al.[17] using bovine serum albumin as a standard.

Glucagon, 99% chromatographically pure cholesterol, and fatty acids of the highest purity available were from Sigma (St. Louis, MO, USA); ATP, AMP, GTP and cyclic AMP were from Boehringer, Mannheim (FRG). (8-^{3}H)-Adenosine 3′,5′-monophosphoric acid, ammonium salt, [1 − ^{14}C]palmitic and oleic acid were obtained from the Radiochemical Centre, Amersham, Bucks, UK.

Results and discussion. The effects of the fatty acid and cholesterol treatment on plasma membrane microenvironment have been studied by the fluorescence polarization technique, which gives an estimate of the average fluidity of the lipid bilayer[16], taking into account the possible microheterogeneity of the fluorophore solvation in the membrane interior[19]. Results reported in table 1 suggest that both oleic acid and cis-vaccenic acid treatment significantly increase liver plasma membrane fluidity as indicated by the decrease of fluorescence polarization values, whereas the cholesterol treatment markedly increases this parameter. 5′-nucleotidase appears to be unaffected under any condition tested (table 1); as far as ATPase is concerned, both (Na^+-K^+) and Mg^{2+}-dependent ATPase activities are significantly decreased after oleic acid and cis-vaccenic acid treatment (table 1), a result which agrees well with the in vivo observation made by Brivio-Haugland and coworkers[6].

Adenylate cyclase activity of cholesterol-treated plasma membranes was significantly increased, in basal conditions, with respect to controls (table 2), whereas its glucagon or fluoride sensitivity appears to be unaltered; on the other hand, cis-vaccenic acid abolished glucagon sensitivity of treated membranes, leaving a decreased fluoride stimulation. Oleic acid treatment acted differently from cis-vaccenic, leaving fluoride sensitivity nearly unaltered and glucagon response slightly decreased (table 2). This differential sensitivity of hormone- and fluoride-stimulated cyclase activity to the plasma membrane microenvironment has recently been reported in turkey erythrocytes[20].

Table 1. Effect of in vitro cholesterol and fatty acid treatment of liver plasma membrane on ATPase and 5′-nucleotidase activities and fluorescence polarization values

Treatment	Mg^{2+}-ATPase	(Na^+-K^+)-ATPase	5′-nucleotidase	p-value
Control	5.13 ± 0.80 (7)	1.28 ± 0.46 (7)	29.3 ± 2.5 (6)	0.268 ± 0.05 (9)
Palmitic acid	5.43 ± 1.09 (7)	1.05 ± 0.41 (7)	30.6 ± 2.0 (6)	0.250 ± 0.04 (9)
Oleic acid	4.00 ± 0.62* (7)	0.79 ± 0.38* (7)	31.6 ± 1.5 (6)	0.206 ± 0.06* (9)
Cis-vaccenic acid	3.74 ± 1.15* (7)	0.66 ± 0.28* (7)	29.4 ± 1.5 (6)	0.209 ± 0.05* (9)
Cholesterol	5.02 ± 0.59 (7)	1.03 ± 0.30 (7)	27.7 ± 2.2 (6)	0.322 ± 0.09* (9)

ATPase activities are reported as µmoles P_i hydrolyzed ± SD per mg protein per 5 min; 5′-nucleotidase activity is reported as µmoles P_i hydrolyzed ± SD per mg protein per h. Fluorescence polarization (P at 37 °C) values are reported as means ± SD. The number of separate membrane preparations tested is reported between brackets; * $p < 0.05$ at least, as estimated by paired data analysis with respect to untreated controls; all other differences being statistically not significant. Fatty acid and cholesterol treatment in vitro of isolated liver plasma membranes is reported in the text.

Table 2. Effect of in vitro cholesterol and fatty acid treatment of liver plasma membranes on adenylate cyclase activity

Treatment		Basal	Adenylate cyclase activity	
			Glucagon-stimulated	Fluoride-stimulated
Control	(9)	11.0 ± 4.2	55.5 ± 15.3	108.2 ± 15.4
Palmitic acid	(5)	9.1 ± 3.1	48.4 ± 10.5	100.3 ± 17.5
Oleic acid	(5)	15.3 ± 3.1	50.8 ± 14.7	133.0 ± 20.2*
Cis-vaccenic acid	(7)	14.5 ± 4.8	15.2 ± 3.2*	93.9 ± 7.5*
Cholesterol	(9)	21.1 ± 7.2*	94.6 ± 20.8	186.4 ± 30.2*

Enzymatic activity is reported as pmoles cyclic AMP ± SD per mg protein per 10 min; the number of different plasma membrane preparations tested is reported between brackets. * $p < 0.05$ at least, as estimated by Student's t-test with respect to untreated controls; all other differences being not statistically significant. Glucagon concentration was $1 \cdot 10^{-7}$ M; NaF was 10 mM.

The ineffectiveness of oleic acid treatment on liver plasma membrane basal adenylate cyclase activity is in good agreement with very recent results obtained in strictly comparable experimental conditions[8]; on the other hand, the stiffening of plasma membrane due to cholesterol incorporation exerts a stimulatory effect; which could be comparable to similar effects observed in tissue-culture cells by Sinensky and coworkers[21].

There results show that although, on the one hand, the effect of cis-vaccenic acid on the cyclase activity is at variance with previous observations on a different membrane system[5], on the other hand the hormonal sensitivity appears to be strictly dependent on the lipid environment whereas this is not the case for the less specific fluoride stimulation.

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Failure of cilia of reprogram following segmental ampullary reversal of the rabbit oviduct[1]

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Summary. Cilia exhibited unidirectional and coordinated movement within microsurgically reversed segments of rabbit ampulla when examined up to 13 months after surgery. The direction of ciliary beating was opposite that of the remainder of the oviduct.

Ciliated cells constitute over 50% of the cells lining the ampullary portion of the mammalian oviduct[3]. Ampullary cilia beat in the direction of the uterus and are thought to be a major determinant in effecting ovum transport through this portion of the female reproductive tract[4]. The technique of surgically reversing a segment of oviduct in order to assess the role of ciliary activity in ovum transport through the rabbit oviduct was first described over 50 years ago[5], but was not successfully achieved until the advent of tubal microsurgery[6]. Cilia within microsurgically reversed segments continue to beat normally, but in the ovarian direction, counter to that of cilia in the rest of the oviduct. Microsurgical segmental reversal performed at the level of the sparsely ciliated isthmus is consistent with normal fertility[6]. In contrast, reversal performed within the more densely ciliated ampulla effectively prevents pregnancy[7,8]. These data suggest that cilia are critical to ovum transport through the rabbit ampulla, but not through the isthmus. It has been suggested that cilia may reprogram several months following segmental reversal[9,10]. The present experiment was undertaken in order to determine if cilia within a reversed segment of rabbit ampulla are programmed to beat in a given direction indefinitely or if they become influenced by cilia in adjacent segments to beat in line with the entire oviduct following a protracted interval.

Materials and methods. 4 adult female New Zealand white rabbits, 2–3 kg, were selected at random and anesthetized with pentobarbital sodium (30 mg/kg). In 2 animals, a single oviduct underwent microsurgical double transection and reversal of a 1-cm segment of mid ampulla as previously described[6]. Briefly, this technique entails mobilizing a segment of oviduct connected to an intact vascularized pedicle of mesosalpinx. The segment is rotated 180° on its pedicle and a double tubal anastomosis is performed, using 6–8 interrupted sutures of 10-0 nylon passed through the serosa and myosalpinx, but avoiding the endosalpinx. The

Ratio of implantations to number of ovulations following unilateral segmental ampullary reversal and double transection without reversal

Animal No.	Operated side	Control side
	Segmental reversal	
1	0/3	2/4
2	0/5	2/3
	Double transection	
3	6/8	4/6
4	5/6	4/4