Zizyphus nummularia leaves (average dry matter 97%). Initially, the animals were allowed free access to water for a week. The volume of urine, voided by each animal and collected under liquid paraffin, was measured at 24-h intervals during this pre-experimental period. The different body fluid compartments (plasma volume, blood volume, extracellular fluid volume and total body water) of the animals, under normal and water deprived conditions, were measured (table). Plasma volume was obtained from the dilution of Evans blue (T1824) in plasma[5]. The total blood volume was calculated from the plasma volume and the packed cell volume. Extracellular fluid volume (ECF) (Thiocyanate space) was determined by Bowler's[6] method. The total body water (urea space) was calculated from the dilution of urea in the plasma[7]. The intracellular fluid volume was obtained by subtracting ECF from total body water. The interstitial fluid volume was obtained by difference from ECF and plasma volumes.

A 43% cell and gut water loss accounted for nearly 90% of the total loss in body water brought about by water restriction. Apparently, these animals tend to maintain the fluidity of the blood at the cost of intracellular and gut water when faced with acute water stress. This may be a part of the desert goat's strategy for adaptation which is akin to that in the camel[8], but which appears somewhat different from that apparently operating in the Merino[9] and the Marwari sheep[10]. There was only a minor reduction in plasma volume in the water-deprived goat while a 40–50% reduction in this parameter has been reported in sheep[9,10] of different breeds under more or less similar environmental conditions. Interestingly, the camel reportedly loses less than 10% of its normal plasma volume at a body weight loss of 20% due to dehydration[11]. Under approximately similar environmental and experimental conditions, plasma water loss in the Rajasthan desert sheep[10] has been reported to constitute about 8% of the total body water loss, i.e. 3 times more than in the desert goat, as shown in table. Thus, the desert goat's physiological ability to maintain normal haemodynamic conditions during acute water stress would give it a greater chance of survival than the sheep during prolonged drought spells. This finds corroboration in the almost 70% increase in the goat population of the Rajasthan desert during the drought-stricken decade 1961–71, when the sheep population registered a mere 18.5% increase[12].

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2 A. Shkolnik, A. Borut and J. Choshniak, Symp. Zool. Soc. Lond. *31*, 229 (1972).
3 G.M.O. Maloiy and C.R. Taylor, J. Agric. sci. *77*, 203 (1971).
4 M.S. Khan, T.O. Sasidharan and P.K. Ghosh, J. Arid Envir. *1*, 351 (1978).
5 P.S. Hawk, B.L. Oser and W.H. Summerson, in: Practical Physiological Chemistry. 14th ed. Blakiston, New York 1965.
6 R.G. Bowler, Biochem. J. *38*, 385 (1944).
7 S. Natelson, in: Microtechniques of Clinical Chemistry for the Routine Laboratory, p.381. C.C. Thomas, Springfield, Illinois 1957.
8 W.V. Macfarlane, R.J.H. Morris and B. Howard, Nature *197*, 271 (1963).
9 W.V. Macfarlane, R.J.H. Morris, B. Howard, J. McDonald and O.E. Budtz-Olsen, Aust. J. agric. Res. *12*, 889 (1961).
10 G.R. Purohit, P.K. Ghosh and G.C. Taneja, Aust. J. agric. Res. *23*, 685 (1972).
11 K. Schmidt-Nielsen, in: Desert animals: Physiological problems of heat and water. Clarendon, Oxford 1964.
12 Statistical Abstract, Rajasthan (1961, 1972). Directorate of Economics & Statistics, Rajasthan, Jaipur.

Inhibition of hepatic Na^+, K^+-adenosinetriphosphatase in taurolithocholate-induced cholestasis in the rat[1]

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Summary. Na^+, K^+-adenosinetriphosphatase (Na^+, K^+-ATPase) activity was decreased in liver plasma membranes from rats in which cholestasis had been induced by i.v. administration of sodium taurolithocholate (5 µmoles/100 g b. wt). Incubation of liver plasma membranes with taurolithocholate (10–1300 µM) caused significant and dose dependent reductions of Na^+, K^+-ATPase activity at taurolithocholate concentrations above 100 µM. These findings lend support to the hypothesis that cholestasis induced by monohydroxy bile acids is at least partially the result of an inhibition of hepatic Na^+, K^+-ATPase activity.

The potential role of monohydroxy bile acids in various cholestatic conditions has attracted increasing interest in recent years[3–7]. Taurolithocholate-induced cholestasis has been shown to be a useful experimental model for studies of the mechanism by which monohydroxy bile acids induce cholestasis[8–13]. Besides precipitation of taurolithocholate within the bile canaliculi[9], alteration of canalicular membrane function[11,13,14], and increased biliary membrane permeability to solutes[12], inhibition of bile acid-independent bile formation has been suggested as cause of taurolithocholate-induced cholestasis[10]. This component of bile flow appears to be related to the activity of Na^+, K^+-adenosinetriphosphatase (Na^+, K^+-ATPase) in liver plasma membranes[15–19]. The present study was conducted to investigate the in vivo and in vitro effects of taurolithocholate on the activity of this enzyme in rat liver plasma membranes.

Methods. Male Sprague-Dawley rats (Tierfarm Hartmut and Vos, Tuttlingen, FRG) weighing 278 ± 19 g were maintained on a standard rat diet (Altromin 300R, Altromin GmbH, Lage, FRG) and tap water ad libitum. In the in vivo experiments, the animals were anesthetized with i.p. sodium pentobarbital (5 mg/100 g b. wt) and the common bile duct was cannulated with PE 10 tubing. Body temperature was monitored with a rectal thermometer and maintained at 37.5 ± 0.5 °C. Groups of 5 animals each received 5 µmoles/100 g b. wt of sodium taurolithocholate (Calbiochem, San Diego, California, USA) or the solvent (0.15 M NaCl containing 10% w/v bovine serum albumin) into the inferior vena cava. This dose of taurolithocholate has been shown to produce cholestasis within 20 min after i.v. injection[3]. Bile was collected for a period of 20 min before and after the administration of the bile acid solution or the solvent. Immediately after completion of bile collection, heparin (250 IU/100 g b. wt) was injected into the inferior vena cava and the animal was sacrificed by severing the thoracic aorta and vena cava. The liver was perfused with

ice-cold physiologic saline via the portal vein to eliminate erythrocytes and it was then removed and weighed.

Liver plasma membranes rich in bile canaliculi were prepared according to a modification of the method of Song et al.[19] as described previously[20]. For the enzyme assays, the final pellets were resuspended by aspirating them 3 times through a 23-gauge hypodermic needle.

Na^+, K^+-ATPase activity was determined as previously described[20] using adenosine-5'-triphosphate disodium (Sigma Chemical Co., St. Louis, Mo.) as substrate. Total ATPase activity was assayed in an incubation medium containing 120 mM Na^+, 12.5 mM K^+, 5 mM Mg^{2+}, 1 mM EGTA and 5 mM ATP in 125 mM Tris-HCl buffer (pH 7.4). The non-Na^+, K^+-ATPase was determined under identical conditions in the presence of 1 mM ouabain octahydrate (Sigma Chemical Co.). The latter represented Mg^{2+}-ATPase. Na^+, K^+-ATPase was calculated as the difference between total and Mg^{2+}-ATPase activity.

The activities of the following marker enzymes were determined in the homogenate and in the membrane fractions: 5'-nucleotidase (E.C. 3.1.3.5.) as a marker for canalicular membranes[17,19] according to Bodansky and Schwartz[22], succinate dehydrogenase (E.C. 1.3.99.1.) as a marker for mitochondrial contamination by the method of Earl and Korn[23] and finally glucose-6-phosphatase (E.C. 3.1.3.9.) as a marker for microsomal contamination according to De Duve et al.[24]. All enzyme assays were linear with time and protein concentration under the experimental conditions used. The ATPase assays were performed in triplicate and the marker enzyme assays in duplicate.

In the in vitro experiments, liver plasma membranes were prepared from untreated rats and incubated with various concentrations (10–1300 μM) of taurolithocholate in Tris-HCl buffer pH 7.4 for 1 h at 4 °C. Thereafter, Na^+, K^+-ATPase activity was assayed as described above.

Protein was estimated by a modification of the Lowry method[25], using bovine serum albumin as standard. Total bile acids in bile were estimated enzymatically, using the 3α-hydroxy-steroid dehydrogenase method[26].

All results are expressed as mean ± SEM. Means of 2 samples were compared by Student's t-test after testing the equality of the variances by an F-test[27]. If the variances were unequal, the modification of the t-test described by Welch[28] was employed. $P < 0.05$ was regarded as statistically significant.

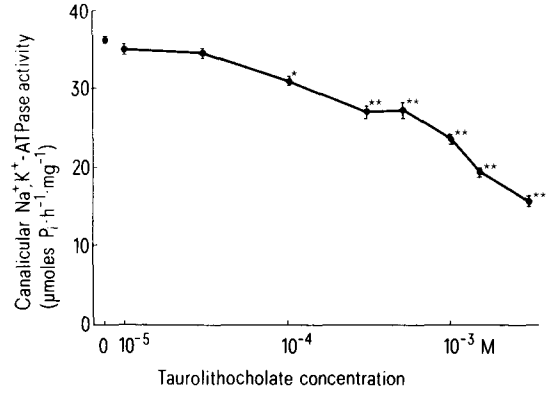
Results. In vivo administration of taurolithocholate decreased bile flow and bile acid excretion by 79 and 84%, respectively (table 1). Whereas the protein content of the liver homogenates did not differ significantly ($p < 0.40$), the protein recovery in the liver plasma membrane fractions was reduced by 38% ($p < 0.01$) after taurolithocholate administration (table 2). No difference was observed in the specific activities of the different marker enzymes. Microsomal and mitochondrial contaminations were small as judged from the corresponding marker enzymes, glucose-6-phosphatase and succinate dehydrogenase (table 2). While no significant difference was observed in the activity of Mg^{2+}-ATPase, Na^+, K^+-ATPase was reduced by 55% in the liver plasma membrane fractions of taurolithocholate treated animals (table 3).

In vitro incubation of the liver plasma membranes with taurolithocholate at concentrations ranging from 10 to 1300 μM showed significant and dose-dependent reductions of Na^+, K^+-ATPase activity at taurolithocholate concentrations above 100 μM. At a taurolithocholate concentration of 1300 μM Na^+, K^+-ATPase activity was reduced by 56% (figure).

Discussion. Several studies showing a relationship between Na^+, K^+-ATPase activity in liver plasma membranes and bile flow[17–29] support the hypothesis that sodium transport plays an important role in bile formation[15,16]. This view is supported by the demonstration of reduced Na^+, K^+-ATPase activity of liver plasma membranes in cholestasis induced by taurolithocholate. Since inhibition of Na^+, K^+-ATPase activity by taurolithocholate could also be demonstrated in vitro a direct effect of this bile acid on Na^+, K^+-ATPase appears likely. However, extrapolation of the in vitro studies to the in vivo situation must be made with caution, since the concentration of taurolithocholate to which the plasma membrane is exposed in vivo is unknown.

Although a membrane fraction rich in bile canaliculi was studied, activities of Na^+, K^+-ATPase measured in this preparation may be associated with parts of the lateral plasma membrane which cannot be completely separated from canalicular membranes. Recent evidence suggests that Na^+, K^+-ATPase is located in the lateral and sinusoidal (basolateral) rather than in the canalicular (apical) portion of the hepatocellular plasma membrane[30,31]. This location of the enzyme is compatible with the notion that the enzyme's primary role is to provide an electrochemical gradient for sodium across the cell membrane. This gradient appears to be essential for Na^+- coupled transports of anionic solutes, the main driving forces of hepatocellular bile flow.

The recent findings of Kakis and Yousef[14] that lithocholate but not taurolithocholate inhibits Na^+, K^+-ATPase activity in a liver plasma membrane fraction enriched in bile canaliculi is at variance with the results of our studies. This



Inhibition of Na^+, K^+-ATPase activity of liver plasma membranes incubated with various concentrations of taurolithocholate. Each point represents the mean ± SEM of 5 experiments.

Table 1. Effect of taurolithocholate on bile flow (μl · min^{-1} · g^{-1} liver) and bile acid excretion (nmoles · min^{-1} · g^{-1})[a]

Groups	Bile flow		Bile acid excretion	
	Before	After	Before	After
Control	2.72 ± 0.32	2.34 ± 0.06	84.6 ± 9.4	76.7 ± 1.9
Taurolithocholate	2.20 ± 0.29	0.46 ± 0.11[b]	95.3 ± 10.4	15.6 ± 2.7[c]

[a] Values are means ± SEM of 5 rats; [b] Significantly different ($p < 0.001$) from the respective value before treatment. [c] Significantly different ($p < 0.005$) from the respective value before treatment.

may be due to differences in methodology. Thus, taurocholate has been administered at a relatively low infusion rate (0.2 μmoles/min/100 g b. wt) by Kakis and Yousef[14] as compared to the single injection of 5 μmoles/min/100 g b. wt in the present study. Other differences are related to the method used for the isolation of liver plasma membranes.

The relative proportions of bile acid dependent and bile acid independent bile flow have not been determined in the present study. From the decrease in bile acid excretion it can be concluded that not only bile acid independent but also bile acid dependent bile formation was inhibited by taurolithocholate. Although one previous study[4] in the isolated hamster liver showed an inhibition of mainly bile acid-independent bile flow, other investigations in hamsters[9] and rats[9,12] have demonstrated that not only bile flow but also bile acid excretion is diminished in taurolithocholate-induced cholestasis.

The reductions in bile flow by 79% and in bile acid excretion by 84% were more pronounced than the decrease in Na^+, K^+-ATPase activity (55%). It must, however, be taken into consideration that the protein recovery in the membrane fraction was reduced in taurolithocholate-induced cholestasis. If canalicular Na^+, K^+-ATPase activity was calculated per g of liver its reduction by taurolithocholate treatment amounted to 72%, a value closer to the observed inhibition of bile flow. However, additional factors by which taurolithocholate may reduce bile flow, such as an increase in biliary membrane permeability[12], may contribute to the discrepancy between canalicular enzyme activity and bile flow.

Several possibilities could account for the inhibition of Na^+, K^+-ATPase by taurolithocholate. Na^+, K^+-ATPase has been shown to be a phospholipid dependent integral membrane enzyme[32]. Thus, solubilization of essential membrane phospholipids could readily explain the deleterious effect of taurolithocholate on Na^+, K^+-ATPase activity. Solubilization of membrane components may be reflected by the structural changes of the microvilli[8,11-14] as well as by the reduced recovery of canalicular membrane protein in the present study. Another explanation is suggested by the demonstration of an increased cholesterol incorporation into canalicular membranes of lithocholate treated animals[14,33]. Employing the model of ethinyl estradiol induced cholestasis Simon et al.[34] have shown that an increase in cholesterol content of liver plasma membranes is associated with decreases in membrane fluidity and Na^+, K^+-ATPase activity.

The results of the present investigation lend support to the hypothesis that cholestasis induced by monohydroxy bile acids is at least partially the result of an inhibition of hepatic Na^+, K^+-ATPase activity. Whether this inhibition is due to loss of enzyme, decrease of membrane fluidity or to masking of active sites by membrane alterations cannot be decided from the present results.

Table 2. Protein content ($mg \cdot g^{-1}$ liver) and activity of marker enzymes ($\mu moles \cdot h^{-1} \cdot mg^{-1}$ protein) in liver homogenate (H) and liver plasma membranes (LPM) in control and taurolithocholate treated animals[a]

	Control	Taurolithocholate
Protein		
H	153 ±3	161 ±9
LPM	2.39±0.16	1.48±0.20[b]
5-Nucleotidase		
H	3.4 ±0.1	3.1 ±0.5
LPM	74.2 ±2.0	74.0 ±3.8
Glucose-6-phosphatase		
H	14.1 ±0.8	13.8 ±0.9
LPM	0.4 ±0.2	0.3 ±0.1
Succinate dehydrogenase		
H	6.3 ±0.4	6.0 ±0.4
LPM	0.16±0.02	0.15±0.03

[a] Values are means±SEM of 5 rats. [b] Significantly different ($p<0.01$) from control.

Table 3. Activity of Mg^{2+}- and Na^+, K^+-ATPase ($\mu moles\ P_i \cdot h^{-1} \cdot mg^{-1}$ protein) in liver homogenate (H) and liver plasma membranes (LPM) in control and taurolithocholate-treated animals[a]

	Control	Taurolithocholate
Mg^{2+}-ATPase		
H	3.5±0.2	3.0±0.1
LPM	70.4±4.4	62.7±2.9
Na^+, K^+-ATPase		
H	1.5±0.2	1.1±0.2[b]
LPM	41.5±3.3	18.6±1.5[b]

[a] Values are means±SEM of 5 rats. [b] Significantly different ($p<0.001$) from control.

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2 The authors thank Mr H. Sägesser and Miss B. Schütz for technical assistance.
3 B.G. Priestly, M.G. Côté and G.L. Plaa, Can. J. Physiol. Pharmac. *49*, 1078 (1971).
4 J.E. King and L.J. Schoenfield, Proc. Mayo Clin. *47*, 725 (1972).
5 G.M. Murphy, F.H. Jansen and B.H. Billing, Biochem. J. *129*, 491 (1972).
6 P. Back, Klin. Wschr. *51*, 926 (1973).
7 R.E. Jenner and E.R. Howard, Lancet *2*, 1073 (1977).
8 F. Schaffner and N.B. Javitt, Lab. Invest. *15*, 1783 (1966).
9 N.B. Javitt and S. Emerman, J. clin. Invest. *47*, 1002 (1968).
10 J.E. King and L.J. Schoenfield, J. clin. Invest. *50*, 2305 (1971).
11 K. Miyai, V.M. Price and M.M. Fisher, Lab. Invest. *24*, 292 (1971).
12 T. Layden and J.L. Boyer, Gastroenterology *73*, 120 (1977).
13 K. Miyai, A.L. Richardson, W. Mayr and N.B. Javitt, Lab. Invest. *36*, 249 (1977).
14 G. Kakis and I.M. Yousef, Gastroenterology *75*, 595 (1978).
15 S. Erlinger, D. Dhumeaux, P. Berthelot and M. Dumont, Am. J. Physiol. *219*, 416 (1970).
16 J.L. Boyer, Am. J. Physiol. *221*, 1156 (1971).
17 T.J. Layden and J.L. Boyer, J. clin. Invest. *57*, 1009 (1976).
18 F.R. Simon, E. Sutherland and L. Accatino, J. clin. Invest. *59*, 849 (1977).
19 C.S. Song, W. Rubin, A.B. Rifkind and A. Kappas, J. Cell Biol. *41*, 124 (1969).
20 J. Reichen and G. Paumgartner, J. clin. Invest. *60*, 429 (1977).
21 O. Lindberg and L. Ernster, Biochem. Analysis *3*, 1 (1956).
22 O. Bodansky and M.K. Schwartz, J. biol. Chem. *238*, 3420 (1963).
23 D.C. Earl and A. Korn, Biochem. J. *94*, 721 (1965).
24 C. De Duve, B.C. Pressman, R. Gianetto, R. Wattiaux and F. Appelmans, Biochem. J. *60*, 604 (1955).
25 E.F. Hartree, Analyt. Biochem. *48*, 422 (1972).
26 G. Paumgartner, W. Horak, P. Probst and G. Grabner, Naunyn Schmiedeberg's Arch. Pharmac. *270*, 98 (1971).
27 K. Diem, Documenta Geigy scient. Tables, p. 145 (1962).
28 B.L. Welch, Biometrika *29*, 350 (1937).
29 G. Paumgartner, R. Herz, K. Sauter and H.P. Schwarz, Naunyn Schmiedeberg's Arch. Pharmac. *285*, 165 (1973).
30 B.L. Blitzer and J.L. Boyer, J. clin. Invest. *62*, 1104 (1978).
31 W.H. Evans, Biochem. J. *116*, 833 (1970).
32 J.L. Dahl and L.E. Hokin, A. Rev. Biochem. *43*, 327 (1974).
33 F. Bonvicini, A. Gautier and D. Gandiol. Lab. Invest. *38*, 487 (1978).
34 F.R. Simon, R. Sinensky, F. Kern and R.A. Davis, Clin. Res. *25*, 318 (1977).