Use of cell-free translation systems in such studies should be recognized as a fruitful approach to investigation of molecular effects of oxypyridines at the level of biomembranes.

In summary, intraperitoneal injection of different amounts of Epygid-(2-ethyl-6-methyl-3-oxypyridine) to 3- and 18-month-old rats led to significant reduction of translating activity *in vitro* of membrane-bound polysomes of rat brain cells, but not of free polysomes. This regularity is more marked in case of endoplasmic membranes of 18-month-old animals than those of 3-month-old. Separation of polysomes from membranes by Triton X-100 resulted in restoration of template activity of the former to the level of free polysomes. It has been proven that the observed phenomenon is associated with incorporation of Epygid into the composition of membranes of endoplasmic reticulum which contain a part of cell polymers on their surface.

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Effect of the cholangiographic agent, ioglycamide, on the β-glucuronidase activity in rat liver and bile: relevance with regard to bilirubin deconjugation

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A fraction of bilirubin conjugated in the liver subsequently undergoes deconjugation [1–3]. In the rat, this fraction amounts to approximately 6–7% of the administered load of bilirubin mono- and diglucuronides [2, 3]. The mechanism of this process is unknown. Involvement of β-glucuronidase (EC 3.2.1.31) is suggested by the observations that the enzyme is abundantly present in liver tissue [4] and that its activity is associated with bilirubin granules in cholestatic liver tissue [1].

In rats, biliary excretion of the iodinated cholangiographic agent ioglycamide (the meglucamine salt of ioglycamic acid, Biligram®, Schering A.G., Berlin, F.R.G.) induced a marked choleresis together with a decrease in the biliary output of bilirubin conjugates [5]. In addition, ioglycamide treatment also led to a significant increase in the diconjugated to monoconjugated bilirubin ratio in both bile and serum [5]. The question thus arose as to whether deconjugation of bilirubin diglucuronide could be decreased by ioglycamide. Indeed, iodinated contrast agents inhibit the activity of purified β-glucuronidase presumably by non-specific binding to the enzyme [6]. We therefore investigated whether infusion of ioglycamide into rats decreases the activity of β-glucuronidase in bile and/or liver tissue, and whether this could in turn lead to decreased hydrolysis of conjugated bilirubins.

Male Wistar rats (330–345 g body wt) provided with a biliary cannula were infused via a jugular vein catheter with glucose 5% (w/v) in saline. After 18–20 hr, two 20-min basal bile samples were collected and rats were then infused for 70 min with ioglycamide (5.5 μmol/min/kg body wt), a load saturating its hepatic transport capacity (Tm) [7]. After a 50-min equilibration period, a 20-min bile sample was collected in the dark, into a pre-weighed plastic tube cooled on ice. Control rats received saline alone.

At the end of the 70 min infusion period, animals were killed and their liver rapidly removed and homogenized in ice-cold 0.25 M sucrose, pH 7.4 containing 1 mM disodium EDTA. Homogenates were centrifuged to separate nuclei, unbroken cells, and tissue debris (pellet or N-fraction) from other organelles and cell sap (supernatant or E-fraction) [8]. Studies were performed on aliquots of bile and N- and E-fractions. In some experiments, the E-fraction was further separated into four additional fractions by ultracentrifugation [8], in order to investigate the subcellular distribution of the β-glucuronidase activity.

Bile volume was determined gravimetrically without correction for specific gravity. The concentration of bilirubin conjugates in bile was measured after diazo-coupling with ethyl anthranilate [9]. β-glucuronidase activity (EC 3.2.1.31) was assayed according to Gianetto and De Duve [10]. Other enzyme activities (their putative subcellular localisation is given in brackets) were determined by established methods: lactate dehydrogenase (cytosol) EC 1.1.1.27 [11], 5′-nucleotidase (plasma membrane) EC 3.1.3.5 [12]. Activities are expressed as μmol substrate hydrolysed per hour. Values per g total liver weight were

calculated as the sum of the activities found in the E- and N-fractions [8]. Protein was estimated by a modification of the Lowry method [13] designed to remove interfering, non-protein material.

As shown for other species [14, 15] excretion of ioglycamide into rat bile induced a marked choleresis. Each μmol of the contrast agent excreted, was accompanied by an additional 22 μl of bile, resulting approximately in a three-fold increase in bile flow. This choleresis led to an equal decrease in the biliary activity of the enzymes assayed (Table 1). However, biliary enzyme output remained unchanged.

According to the Michaelis–Menten theory, the initial velocity of an enzymatic reaction is equal to the rate of breakdown of the enzyme–substrate complex. Thus, the 3-fold decrease in biliary β-glucuronidase activity together with the 8-fold decrease in the concentration of bilirubin conjugates (Table 1) would result in a decreased rate of the possible β-glucuronidase-catalyzed hydrolysis of bilirubin conjugates in bile. Such a decrease could in turn lead to preservation of bilirubin diconjugates and possibly to an increased di- to monoconjugated bilirubin ratio, assuming that the enzyme is far from saturation in the bile samples considered. Nevertheless, mammalian β-glucuronidase preparations saturated with substrate display an acid pH optimum [8], the enzyme activity at the pH of rat bile in both control (8.2 ± 0.2, $N = 8$) and treated animals (8.1 ± 0.2, $N = 4$) being minimal or even negligible. This suggests that the biliary β-glucuronidase activity probably does not play a major role in hydrolysis of conjugated bilirubins.

Under the present experimental conditions, ioglycamide does not alter bile salt output [7]. This probably explains why excretion of 5′-nucleotidase was unaffected by infusion with this contrast agent. In fact, plasma-membrane enzymes are thought to be released from the biliary canalicular membrane as a result of bile salt attack [16]. Moreover, ioglycamide did not seem to cause significant hepatic damage, as evidenced by the normal release of the cytosolic enzyme lactate dehydrogenase.

The concentration of total protein in bile decreased only by 45% under ioglycamide treatment. The output of total protein thus increased from 20.8 ± 2.0 to 35.4 ± 3.5 mg/hr/kg body wt ($N = 8$, $P < 0.01$). In the rat, most of biliary proteins derive from serum, and enter bile by leakage across tight junctions [17, 18] and/or direct transfer across the hepatocyte [17, 19]. The osmotic choleresis elicited by ioglycamide [14] could increase transfer of serum protein into bile by both processes. Indeed, osmotic choleresis induced, i.e. by taurocholate, has been shown to increase the biliary excretion of horseradish peroxidase, presumably by enhancing the formation of endocytic vesicles at the hepatocyte sinusoidal surface [19]. On the other hand, an increased permeability of junctional complexes between the hepatocytes occurs during osmotic choleresis induced by dehydrocholate and iodipamide [20, 21]. The latter compound is a cholangiographic agent, structurally related to ioglycamide.

The total hepatic β-glucuronidase activity averaged 85 μmol/hr/g of protein; approximately 55 and 26% of which activity was recovered in the mitochondrial-lysosomal and microsomal fractions, respectively. Protein content equalled 206 mg/g of liver. No significant differences were observed in the activity and subcellular localisation of hepatic β-glucuronidase, or the protein content in the liver, when ioglycamide-treated rats ($N = 8$) were compared to saline-treated controls ($N = 6$). Addition of ioglycamide in concentrations up to 30 mM (ninetyfold the serum concentration reached) to liver homogenates of saline-treated controls did not affect the β-glucuronidase activity. In contrast, a dose-related inhibition was observed for purified β-glucuronidase (Fig. 1a). This different behaviour is probably explained by the low protein content of the purified preparation, since addition of bovine albumin or liver cytosol reversed the inhibition (Fig. 1b).

Concentrations of ioglycamide attained in the hepatocyte, *in vivo*, are unknown. However, since this contrast agent does not seem to undergo hepatic storage [22], and is thought to be excreted in bile by a carrier-mediated transport system [14], one might assume those concentrations to be low. We therefore suggest that ioglycamide does not inhibit the hepatic β-glucuronidase activity *in vivo*.

In summary, infusion of rats with ioglycamide did not affect the biliary output of β-glucuronidase, lactate dehydrogenase and 5′-nucleotidase. A three-fold decrease in the activity being compensated for by an equal increase in bile flow. Due to only a slight fall in the biliary protein concentration, excretion of total protein increased by 60%. Pretreatment of rats with ioglycamide or addition *in vitro* of the contrast agent to liver homogenates, did not alter the hepatic β-glucuronidase activity. It is suggested that, *in vivo*, ioglycamide does not inhibit the β-glucuronidase activity either in bile or in liver tissue, in contrast to observations with purified β-glucuronidase preparations. Thus, the increased diconjugated to monoconjugated bilirubin ratio which occurs during ioglycamide-induced bilirubinostasis does not appear to result from an altered β-glucuronidase activity. Whether or not β-glucuronidase plays a role in long term mechanical bile duct obstruction [1] cannot be answered from the present studies.

Table 1. Effect of ioglycamide on some biliary constituents

	Basal N = 8	Ioglycamide N = 8
Bile flow (ml/hr/kg body wt)	2.6 ± 0.2	8.0 ± 0.5
Concentration of bilirubin conjugates (μmol/l)	135.5 ± 10.0	16.2 ± 1.5
Enzyme activities (μmol/hr/ml of bile)		
β-glucuronidase	2.0 ± 0.3	0.7 ± 0.1
lactate dehydrogenase	0.6 ± 0.1	0.2 ± 0.1
5′-nucleotidase	5.8 ± 0.5	1.9 ± 0.2
Total protein content (mg/ml)	8.0 ± 0.6	4.4 ± 0.4

Bile samples were collected in basal conditions and after 1 hr of ioglycamide infusion at 5.5 μmol/min/kg body wt. Means ±1 S.D. are given. Values obtained under ioglycamide infusion were statistically different ($P < 0.05$) from basal values, by the Wilcoxon signed rank test.

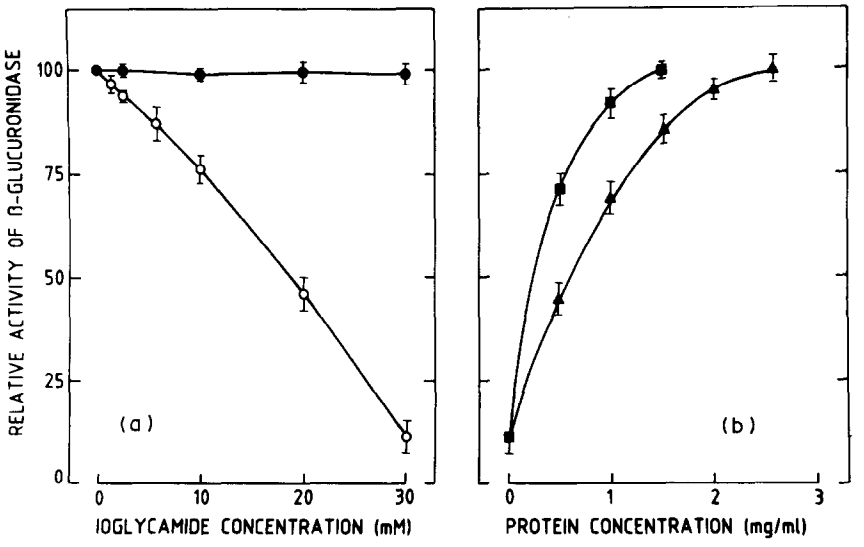

Fig. 1. (a) Effect of ioglycamide on the β-glucuronidase activity, (closed circles) in liver homogenates (final protein concentration of 4 mg/ml), or (open circles) in a purified enzyme preparation (final protein concentration of approximately 5 μg/ml). Mean values ±1 S.D. (N = 6) are given. (b) Effect of bovine serum albumin (closed squares) or liver cytosol (closed triangles) on the activity of a purified β-glucuronidase preparation, in the presence of ioglycamide (30 mM) as the inhibitory substance. Mean values ±1 S.D. are given.

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Purine effects on (^{3}H)-clonidine binding to rat brain

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A potentiation of the effects of noradrenaline by adenosine has been shown in electrophysiological experiments [1]. In the vas deferens Holck and Marks [2] demonstrated interactions of adenosine on noradrenaline evoked responses as well as the rate of resensitisation, findings which have recently been extended by Long and Stone [3] with the recognition that adenosine can potentiate the effects of noradrenaline at α_1 receptors while modifying the rate of resensitisation of noradrenaline receptors by an action at α_2 receptors.