Because of the extraction procedures used and the small quantity of lipid removed, we are unable to determine accurately the surface density of the lipid in the respective cuticle. Hydrocarbons, whose nonpolar properties make them well suited for barrier function[8], are a major constituent of the surface lipids of both cuticle types. Differences in the molecular composition of the hydrocarbon fractions are probably not sufficient to significantly affect permeability, although the greater percentage of long chain hydrocarbon molecules in sclerotized cuticle should theoretically enhance its diffusion resistance[8]. Any advantage conferred by this compositional difference may very well be countered by the increased thickness of the lipid-rich inner epicuticle in arthrodial membrane. A thicker inner epicuticle in arthrodial membrane may also help compensate for the absent exocuticle; however, the latter's role in contributing to barrier function in sclerotized cuticle remains speculative.

The response of the different cuticle types to chemical treatments and mechanical abrasion (shams) sheds additional light on their barrier properties. The lipid barrier on the surface of arthrodial membrane appears to be quite labile judging by the marked increase in permeability following hexane application or simply mild rubbing. In contrast, the barrier function provided by surface lipids associated with sclerotized cuticle was unaffected by the same treatments. A strong base in combination with chloroform:methanol was required to disrupt this barrier and even then the increase in permeability was less than observed for arthrodial membrane treated with solvent alone. These data suggest that in sclerotized cuticle the epicuticular lipids are more tightly bound to proteins and/or are covered by a substance or layer that is essentially lipid-insoluble. There is histochemical evidence for an appropriately located layer consisting of acid and neutral mucopolysaccharides in the cuticle of the scorpion *Heterometrus liurus*[9]. Such a coating would dissolve when the cuticle is treated with KOH, leading to the increased permeability observed. Functionally, the layer would also protect that portion of the scorpion integument that is most likely to experience damage due to soil abrasion.

Despite the much greater permeability of cockroach cuticle, epicuticular lipids still represent a major barrier component, as their removal produces much higher transcuticular water loss rates. The reduced barrier effectiveness of cockroach lipids can be explained in part by their unique composition. Cockroach surface waxes are composed mainly of hydrocarbons (85–95%), with the unsaturated molecule *cis, cis*-6,9-heptacosadiene accounting for 71% of the total fraction[10]. Together, these hydrocarbons produce a mobile, grease-like coating rather than the hard wax coating found in scorpion cuticle and in most other xeric-adapted arthropods. Not only is this grease less effective in retarding water loss, but its thermal stability is also lower. In fact, phase changes that probably lead to the restructuring of these superficially-deposited hydrocarbons and, hence, to increased permeability begin at temperatures as low as 30 °C. Furthermore, the coating over the surface lipids that protects and contributes to the waterproofing barrier in sclerotized scorpion cuticle is either absent or poorly developed in the cockroach. Without an effective barrier to water efflux, transpiration across scorpion arthrodial membrane would lead to rapid dehydration, especially in gravid females during hot, dry summer conditions. Nonetheless, these findings cannot be applied indiscriminately to all arthropods or, for that matter, even to other scorpion species. During our study we also measured the permeability of the pleural membrane of the scorpion *Pandinus imperator*, a large, tropical species that occurs in lowland rainforests. The mean value for pleural membrane in two gravid females was 11.95 $\mu g \cdot cm^{-2} \cdot h^{-1} \cdot mm\ Hg^{-1}$. This value, which is comparable to rates observed for the cockroach pronotum, is also about 2.5 times greater than the permeability of the sternite in the same two scorpions. Obviously, species differences and habitat conditions will have a major bearing on the respective cuticular permeabilities and the mechanisms that have evolved to minimize water loss.

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Effect of glucose administration on bilirubin excretion in the rabbit

M.E. Muñoz, J. González and A. Esteller

Department of Animal Physiology, Faculty of Pharmacy, University of Salamanca, E-37007 Salamanca (Spain), 12 March 1986

Summary. The effect of i.v. infusion of glucose on the hepatic handling of bilirubin was examined in rabbits. A significant increase in the excretion of conjugated bilirubin into the bile was observed, accompanied by a decrease in bilirubinemia. Hepatic bilirubin concentrations were lowered and the UDP-glucose concentrations and liver UDP-glucuronosyl and UDP-glucosyl transferase activities increased.

Key words. Bilirubin; glucose; glucuronosyl transferase; rabbit; bilirubinemia; conjugates, bilirubin.

The hepatic handling of bilirubin depends on a series of different processes: plasma transport and translocation across the sinusoidal membrane of the liver cell, storage in the hepatocyte, conjugation and transfer into the bile. Previous studies in different species have demonstrated that glucose administration can lead to increased bilirubin excretion in fasted animals, with or without decreases in bilirubinemia[1,2]. The possibilities of increased bilirubin conjugation or of alterations in bilirubin bind-

Table 1. Biliary bilirubin excretion in the control rabbits and after glucose administration

	Total		Unconjugated (nmol/kg/min)		Conjugated	
	a	b	a	b	a	b
Control	4.57 ± 0.46	4.49 ± 0.48	0.57 ± 0.15	0.63 ± 0.12	4.02 ± 0.27	3.86 ± 0.48
Glucose	6.77 ± 0.64*	6.84 ± 0.36*	0.79 ± 0.09	0.75 ± 0.07	5.96 ± 0.14*	6.08 ± 0.29*

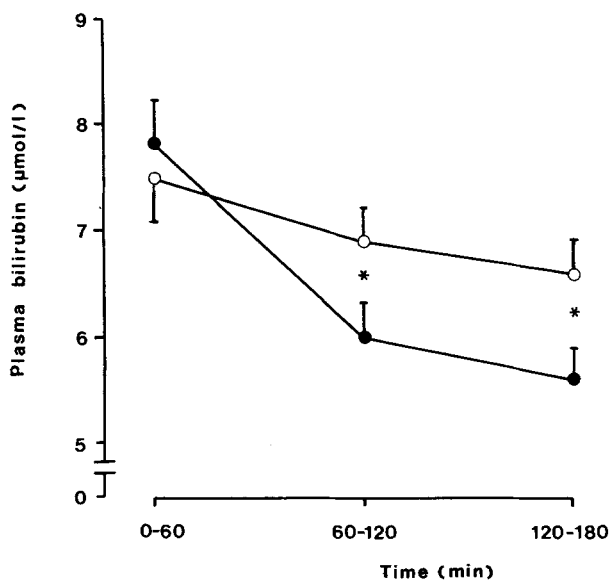
Each value represents the mean ± SEM from 8–10 rabbits; *$p < 0.05$ significantly different from the control. a) last 40 min of the glucose infusion period (min 80–120 of experiments) b) last 40 min of the postinfusion period (min 140–180 of experiments).

ing to serum albumin have been suggested[1,2], though no studies have been carried out regarding the modifications in the conjugation process, the excretion of bilirubin conjugates into bile or the hepatic concentrations of the pigment.

The present work was designed to study the effect of i.v. infusion of glucose on the hepatic handling of bilirubin in the rabbit, in an attempt to elucidate further the mechanisms of the stimulatory effect of glucose administration.

Materials and methods. Male New Zealand rabbits weighing 1.5–2.0 kg were used. They were housed in cages in a room kept at 22 °C with a 12-h dark/light cycle. Food but not water was withheld for 24 h prior to experiments. All procedures were initiated at approximately 09.00 h. Anesthesia was with pentobarbital (30 mg/kg i.v.). A tracheostomy was performed and the left femoral vein and artery were catheterized for infusions and blood sampling, respectively. The cystic duct was ligated and the common bile duct cannulated with polyethylene tubing (PE 50). A cannula was also inserted into the first part of the duodenum. Rectal temperature was monitored and maintained between 38.5 and 39 °C on a thermoregulated table.

After an equilibration period of 30 min, bile was collected for 180 min at 20-min intervals. Controls received an infusion of 0.154 M NaCl (10 ml/h) throughout. In a second group of animals, the normal saline infusion was replaced during the second hour of the experiments only by a solution of D-glucose (diluted in distilled water) at 83 µmol/kg/min. Samples were collected in darkness under melting ice and one part (< 10%) was kept for analysis; the rest was reinfused (after rewarming) through the duodenal cannula. At the midpoint of each bile collection, a 300-µl blood sample was obtained. Bile and plasma samples were stored in the dark at −20 °C for processing within 24 h. At the end of the experiments, animals were killed by exsanguination. The portal vein was perfused with ice-cold 0.154 M NaCl and the livers rapidly removed and stored at −20 °C.



Plasma bilirubin concentrations in the control rabbits (○) and after glucose administration (●). Each value is mean ± SEM from 5–6 rabbits; *$p < 0.05$ significantly different from the control.

Table 2. Determinations on liver in the control rabbits and after glucose administration

	Control	Glucose
UDP-glucose (µmol/g liver)	0.245 ± 0.048	0.888 ± 0.115*
UDP-glucuronosyl transferase (µmol/g liver/h)	0.504 ± 0.034	0.858 ± 0.100*
UDP-glucosyl transferase (µmol/g liver/h)	1.110 ± 0.029	1.349 ± 0.018*
Unconjugated bilirubin (µmol/g liver)	0.143 ± 0.021	0.085 ± 0.017*

Each value represents the mean ± SEM from 4–6 rabbits; *$p < 0.05$ significantly different from the control.

Total bilirubin levels in plasma and bile were determined after diazo cleavage with p-iodoaniline[3]. Unconjugated and conjugated bilirubin in bile were determined by alkaline methanolysis followed by thin layer chromatography[4]. UDP-glucuronosyl and UDP-glucosyl transferase activities were determined in digitonin-activated livers with bilirubin as acceptor substance at the standard concentration (164 µM)[5]. Unconjugated bilirubin in liver was assayed by the method of Piper and Hargreaves[6]. The hepatic concentration of UDP-glucose was estimated by the method of Kepler and Decker[7]. Glucose in plasma was assayed by the glucose-oxidase method[8]. Results were expressed as means ± SEM. The significance of the differences was calculated by Student's t-test. P-values of 0.05 or less were considered as statisitically significant.

Results. The mean plasma glucose concentration in the controls was 6.88 ± 0.07 mmol/l. Glucose administration gave a peak glucose concentration of 21.31 ± 0.13 mmol/l. A significant decrease in plasma bilirubin concentration was observed in glucose-treated rabbits as compared to the control values (fig.). Total bilirubin excretion into bile increased significantly after glucose infusion with respect to the controls, with maximal differences appearing during the third hour of the experiments (table 1); the conjugated form of the pigment showed values significantly higher than those found in the control rabbits (table 1). By the end of the assays, the hepatic concentration of UDP-glucose together with bilirubin UDP-glucuronosyl and UDP-glucosyl transferase activities were significantly increased in the glucose-infused group (table 2). A decreased concentration of unconjugated bilirubin in liver was observed in these animals (table 2).

Discussion. The results of this study clearly show that i.v. glucose administration lowers plasma bilirubin concentration and increases biliary bilirubin excretion in fasted rabbits, in a similar way to that reported earlier in other species such as the pony[1] or the monkey[2]. The phenomenon does not seem to be related to the effects on bile flow or bile acids, because both canalicular bile flow and bile acid secretion are decreased after glucose administration[9]. Although the underlying process is difficult to explain and the existence of additional mechanisms cannot be ruled out, it seems apparent that exogenous glucose modifies the process of hepatic conjugation of the pigment. The increase in bilirubin excretion is a progressive phenomenon with maintained stimulation during the postinfusion hour and is accompanied by

higher glucuronosyl and glucosyl transferase activities. Increases also occur in the hepatic concentration of UDP-glucose which, in the rabbit, constitute an important conjugating substrate for bilirubin[10].

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Intraluminal bile salt increases rate of firing in afferent fibers from the small intestine of the rat[1]

M.H. Tantisira, M. Jodal and O. Lundgren

Department of Physiology, University of Göteborg, S-400 33 Göteborg (Sweden), 20 February 1986

Summary. Perfusion of a rat intestinal segment with a solution containing sodium deoxycholate (8 mM) increases the rate of firing in periarterial afferent nerves from the gut. This observation indirectly supports our earlier proposal that bile salt evokes a net fluid secretion in the small intestine via an activation of the enteric nervous system.
Key words. Bile salt; intestinal afferent nerves.

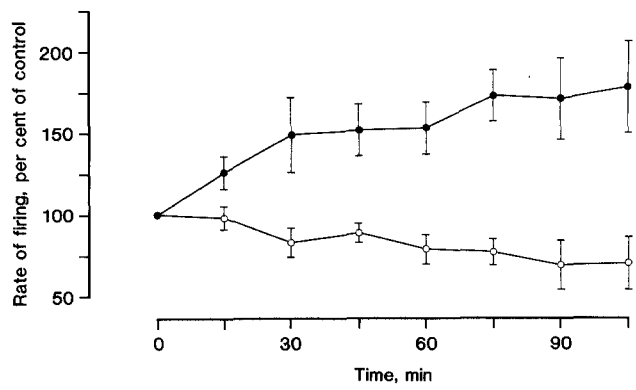
In an earlier series of experiments we provided evidence for the view that the intestinal net fluid secretion evoked by an increased concentration of a bile salt, sodium deoxycholate, in the intestinal lumen is at least in part caused by an activation of the enteric nervous system[2-5]. The present study was undertaken to obtain further evidence for the view that this bile salt can activate nerves, by recording afferent activity in periarterial nerves from the small intestine before and after exposing the intestinal lumen to a solution containing sodium deoxycholate.

Methods. The experiments were carried out on male Sprague-Dawley rats (Anticimex, Stockholm, Sweden), weighing 250–350 g. The animals were kept in the animal quarters under constant environmental conditions (22 °C, 50–60% relative air humidity, artificial lighting 06.00–18.00 h) for at least 7 days before the experiment. The animals were fasted 12 h before the experiment. Anesthesia was induced by i.p. injection of sodium methohexital (Bricanyl, Lilly Inc; 75 mg/kg b. wt) and maintained throughout the experiment by a continuous i.a. infusion of chloralose (1.25 mg/ml; 0.02 ml/min). The chloralose was administered in a glucose-bicarbonate solution via a catheter in the right femoral artery. Additional amounts of chloralose were administered through a catheter in the femoral vein if needed. The total dose of chloralose during an 8-h experiment did not exceed 150 mg/kg b.wt. Arterial pressure was measured in the femoral artery with a pressure transducer (Statham P23 DC).

After a tracheotomy, a midline abdominal incision was performed and a 15–20 cm long intestinal segment was selected and cannulated both proximally and distally. The proximal cannula was connected to a T-shaped tube which permitted the simultaneous perfusion of fluid and measurement of luminal pressure via a pressure transducer (Statham P23 AC). The perfusion was maintained at a constant rate of about 1 ml/min by means of a roller pump (Ismatec, Switzerland). The perfusate was heated to about 38 °C before entering the intestinal segment. After passing the segment, the perfusate was drained through the distal cannula into a beaker. The body temperature of the animal was kept at 38 °C by a lamp and a heating pad. The former was connected to a thermocouple thermometer in the mouth of the animal.

Bundles of periarterial nerve fibers around the superior mesenteric artery were dissected free from the surrounding adipose tissue distal to the coeliac ganglion. The proximal end of the bundle used for registration was ligated in order to eliminate the recording of efferent impulses. Afferent impulses were recorded via a bipolar platinum electrode that was kept in place with silicone rubber (Wacker Sil Gel 601). After amplification the signal was displayed on an oscilloscope (Tektronic Type 502). Two types of recordings were performed. In one the signal was rectified and the average response above base line (including background noise activity) was recorded. In another only signals greater than a certain preset magnitude were counted and recorded by means of a spike counter. The fact that the activity recorded was increased in response to a distension produced by clamping the drainage while introducing fluid into the segment was taken as evidence for the recording of afferent impulse traffic from the segment selected. All recordings were made on a Grass polygraph.

The modified Krebs-Henseleit solution used to perfuse the segment contained (mM): NaCl 122; KCl 4.7; KH_2PO_4 1.2; $NaHCO_3$ 25; mannitol 30. The pH of this solution was 7.7. In the



The effect of perfusing a rat intestinal segment with an isotonic solution containing sodium deoxycholate (8 mM) on the rate of firing in afferent fibers from the gut (n = 8). Bile salt (●——●) perfusion was started at time 0 which represents the control value. As a comparison is shown the corresponding results from control (○——○) experiments in which the intestinal perfusate contained no bile salt (n = 6). Bars indicate SE.