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EFFECT OF INTRABILIARY ADMINISTRATION OF TRITON X-100 ON BILIARY EXCRETORY FUNCTION IN THE RAT*

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Abstract—Intrabiliary administration of Triton X-100 is of interest in producing effects on biliary tree permeability and canalicular biliary excretory function. Treatment with 0.4% Triton (40 μl) was shown to increase the biliary excretion of intraportably administered [^{3}H]sucrose. It also decreased recovery of [^{3}H]sucrose given into the biliary tree. Thus, we concluded that Triton treatment increased biliary tree permeability. Using a different set of marker compounds, canalicular transport of bromphenol blue, [^{14}C]morphine glucuronide and [^{3}H]ouabain was found to be decreased. The fact that [^{3}H] taurocholate excretion into bile was not affected whereas that of [^{3}H]ouabain was lends support to the concept that taurocholate and ouabain are not transported by a common pathway.

Triton X-100 has been used to solubilize microsomal membrane-bound mixed-function oxidases and to activate microsomal glucuronyltransferases *in vitro* [1]. Since a variety of agents induce and influence glucuronyltransferases [2], the matter of activation is of interest in considering glucuronyltransferase activities in the intact cells [3]. Activation has not been demonstrated by *in vivo* administration of Triton X-100 [3–5]. We found that intrabiliary injection of Triton X-100 in the rat inhibited the canalicular transport of morphine-3-glucuronide and perhaps decreased slightly rather than increased the conjugation of morphine to form the glucuronide metabolite. Previously, investigators [6, 7] had shown that such *in vivo* treatment with Triton inhibited canalicular membrane transport without an effect on plasma membrane sinusoidal transport function. They further demonstrated that Triton treatment released a canalicular membrane protein into bile which would bind bromphenol blue and was presumably a carrier involved in bromphenol blue transport [6]. Intrabiliary administration thus appears to provide a way of obtaining localized effects on canalicular membrane function which would not be feasible in intact cell preparations *in vitro*. For these various reasons, we felt it would be interest to study further the effect of disruption of canalicular membrane function by Triton X-100. Our observation that ouabain transport into bile was inhibited while taurocholate transport was not affected is of interest in view of the question as to whether certain bile salts share a common transport pathway with ouabain [8, 9]. In addition, we show that intrabiliary administration of Triton X-100 increased biliary tree permeability.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (ARS, Sprague–Dawley, Madison, WI) (300–375 g) were allowed food (Purina Rodent Chow) and water *ad lib.* and were maintained on a 12-hr light/dark cycle. The animals were anesthetized with intraperitoneal pentobarbital sodium (45 mg/kg). Following a tracheotomy and laparotomy, the right femoral vein of the animal was cannulated with PE-50 polyethylene tubing. The common bile duct was cannulated with a 10 cm segment of PE-20 tubing just distal to the first bifurcation of the biliary tree. The rectal temperature of the animal was maintained at 37.5 ± 0.5° by use of an incandescent lamp. The temperature was monitored with a Tele-Thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Bile flow rate was recorded by timing the rate of drop formation and assuming a volume of 5.7 μl/drop [10].

Administration of radioactive solutions (0.2 to 0.5 μCi) by segmented retrograde intrabiliary injection (SRII) consisted of injection into the bile duct cannula of an initial 40-μl "segment" of solution containing the marker compound followed by 110 μl of 0.9% saline [10]. The doses of the radioactive compounds given by SRII were, respectively: [^{3}H] sucrose, [^{3}H]ouabain and [^{3}H]taurocholate, 0.067, 0.053 and 0.306 nmoles/kg; [^{14}C]morphine and [^{14}C] mannitol, 24.1 and 17.8 μmoles/kg. The injection rate was 2.3 μl/sec (Harvard infusion pump, Harvard Apparatus Co., Dover, MA). Immediately upon

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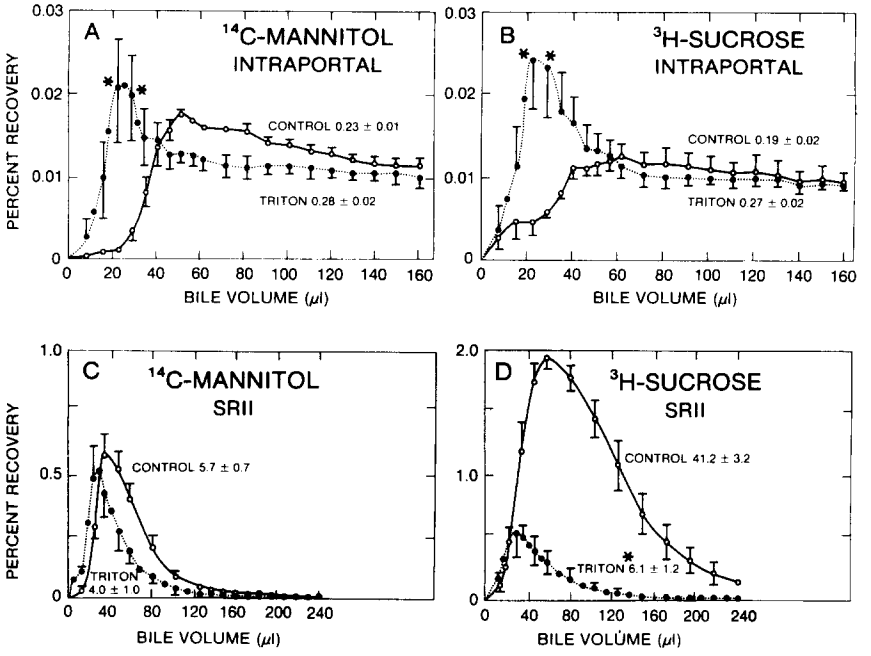

Fig. 1. Effects of 0.4% Triton X-100 treatment on the recoveries in bile of [^{14}C]mannitol and [^{3}H]sucrose given intraportally (A and B) and by SRII (C and D). The rats were renal-ligated only in the experiment in which the mannitol and sucrose were given intraportally to prevent loss of these compounds into urine. The Triton was administered as a 40-μl segment into the cannulated bile duct of the rat, followed by 31 μl of saline, at a rate of 2.3 μl/sec. Controls received 71 μl of saline. Five minutes after this treatment, the radiolabeled marker compounds were administered. The points on each curve represent the percent recovery (mean ± S.E.) of the administered dose in each bile drop collected following injection of the marker in six control and six Triton-treated animals. Student's *t*-test was used to compare the control to the Triton-treated group at $P < 0.05$ (designated with *). The cumulative recovery values ± S.E are given for each group but comparisons between these values were not made for values in panels A and B.

completion of the SRII, bile flow was re-established by disconnecting the bile duct cannula from the infusion pump. Bile drops number 1–10 and the even numbered drops 12–40 were serially collected. The radioactive content was determined by liquid scintillation spectrometry with an internal standard for quench correction. Bromphenol blue (0.4 mg sodium salt) was also administered by SRII as above. Serially collected bile drops were then assayed by adding 3.5 ml of alkaline buffer, pH 10.6 to 10.7, to the individual bile drops. Optical densities were read at 600 nm on a Gilford spectrophotometer.

The following radiolabeled compounds were injected into the hepatic portal vein (in renal-ligated animals in designated cases) in 70 μl of 0.9% saline followed by a 0.2 ml saline flush: [^{3}H]ouabain, [^{3}H] sucrose, [^{14}C]mannitol, [^{3}H]taurocholate, and [^{14}C] morphine (0.4 to 0.7 μCi). The doses were, respectively: [^{3}H]sucrose, [^{3}H]ouabain and [^{3}H] taurocholate, 0.11, 0.08 and 0.48 nmole/kg; [^{14}C] morphine and [^{14}C]mannitol, 28 and 38 μmoles/kg. Bromphenol blue (3 mg sodium salt) was given as a solution in 0.2 ml and flushed in with 0.1 ml of saline. In these experiments bile was collected for 30-min intervals for 180 (morphine) or 90 (all others) min in preweighed vials; the volume was calculated assuming a specific gravity of one. Samples of 10 or 20 μl were taken for analysis.

For the treatment with Triton X-100, 40 μl of buffered Tris–HCl–Triton (0.4%, w/v) (pH 7.4) plus 31 μl of 0.9% NaCl were injected into the biliary tree at a rate of 2.3 μl/sec. At the end of the injection, the infusion pump was detached from the bile duct cannula and bile flow resumed immediately. Control animals received 71 μl of buffered 0.9% NaCl. Five minutes were allowed to elapse, bile flow was recorded, and then marker compounds were administered i.v. or by SRII as described above.

Statistical analysis. Results were evaluated by Student's unpaired *t*-test. The procedure of Dunnett [11] was used to compare the effect of Triton treatments to the control on the biliary recoveries of [^{3}H]sucrose and [^{14}C]morphine in the dose–response and time–course studies. $P \leq 0.05$ was taken as indicating a significant difference in both tests.

Drugs and chemicals. Sources of drugs and chemicals were as follows: Triton X-100 (alkyphenoxy-ethoxy-ethanol, purity grade B) was from Calbiochem, San Diego, CA; pentobarbital sodium was obtained from Henry Schein, Inc., Port Washington, NY; bromphenol blue was obtained from the Sigma Chemical Co., St. Louis, MO. [N-$^{14}CH_3$]Morphine hydrochloride (58 μCi/mmole), [6,6′(*N*)-^{3}H]-sucrose (15.5 Ci/mmole), and [G-^{3}H]ouabain (19.5 Ci/mmole) were obtained from Amersham Searle, Arlington Heights, IL. D-[1-^{14}C]Mannitol (43

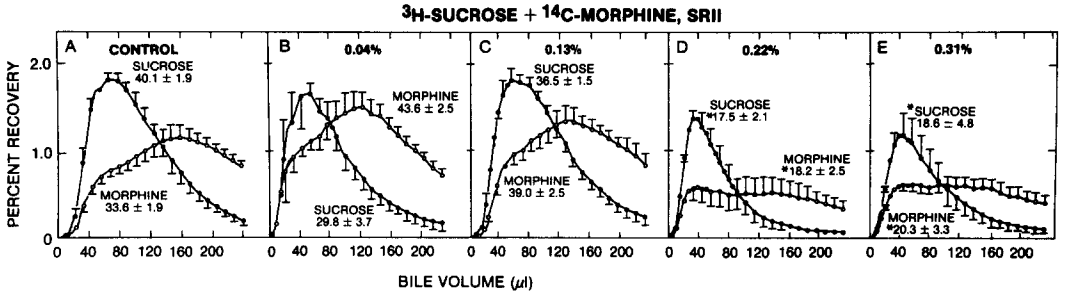

Fig. 2. Effects of various concentrations of Triton on the biliary recoveries of radioactivity after SRII of [^{14}C]morphine and [^{3}H]sucrose together. The data are plotted as in Fig. 1. N = 4. Key: *differs significantly from control, $P < 0.05$.

mCi/mmole) and [G-^{3}H]taurocholic acid (3.39 Ci/mmole) were obtained from the New England Nuclear Corp., Boston, MA. All compounds were administered in 0.9% sodium chloride. All other chemicals were of reagent grade.

RESULTS

Figure 1 illustrates the biliary excretion of [^{14}C] mannitol (panel A) and [^{3}H]sucrose (panel B) when intraportally administered in renal-ligated animals. These compounds enter bile by diffusion and convection down their concentration gradients from blood into bile [12]. However, the amount excreted in bile is limited by the relatively large volumes of plasma and tissue space in the liver which contain these solutes compared to the small volume of bile into which diffusion and convection must take place. Following intraportal administration in control animals, 0.19% of the labeled sucrose and 0.23% of the mannitol were excreted into bile over the collection period. Treatment with 0.4% Triton X-100 administered 5 min before the [^{14}C]mannitol (panel A) and [^{3}H]sucrose (panel B) caused peak concentrations to shift in recovery of these radioactive compounds to earlier bile volumes.

In panels C and D, the experiments illustrate the intrabiliary administration and recovery of [^{14}C]mannitol and [^{3}H]sucrose given by SRII in control and Triton-treated groups. When given by SRII, mannitol and sucrose are filtered and part of the solute is retained by the biliary tree epithelium. The amount of retained solute depends on its molecular size and is recollected in the bile as new bile is produced into the canalicular lumen. The control recovery in bile of [^{14}C]mannitol (5.7%) was less than that of [^{3}H] sucrose (41%) as expected from their molecular weight. The Triton treatment had little effect on the [^{14}C]mannitol recovery. [^{3}H]Sucrose recovery was affected much more; its value decreased from 41 to 6.1%. Based on the results in Fig. 1, we concluded that Triton X-100 treatment increased the permeability of the biliary tree as measured by the use of sucrose.

The results given in Figs. 2 and 3 show the effect on biliary recovery of [^{3}H]sucrose given by SRII and, respectively, the various doses of Triton X-100 used and its duration of action. In addition, the experiments include results for the SRII of [^{14}C]morphine given together with the [^{3}H]sucrose. [^{14}C]Morphine was included here because previously we had shown that treatment with 0.4% Triton had little effect on the conjugation of [^{14}C]morphine to [^{14}C]morphine-3-glucuronide but decreased biliary excretion of [^{14}C] morphine glucuronide, presumably by inhibiting canalicular transport of the glucuronide [5]. In Fig. 2, increasing the concentration of Triton up to 0.13% (panel A vs B and C) had no consistent effect. At

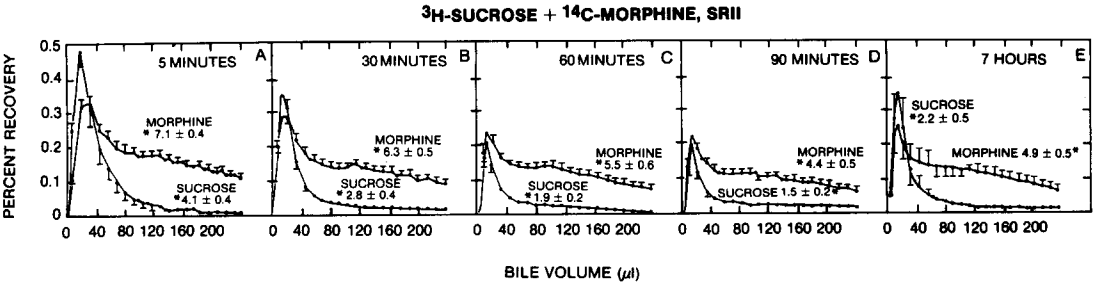

Fig. 3. Time-course of the effect of 0.4% Triton treatment on the biliary recoveries of [^{14}C]morphine and [^{3}H]sucrose following simultaneous SRII administration of the radioactive compounds were from 5 min to 7 hr, panels A–E. The data are plotted as in Fig. 1. N = 6. Key: *differs significantly from control, $P < 0.05$.

Table 1. Effect of Triton X-100 treatment to decrease the biliary excretion of bromphenol blue and radioactivity after intraportal or SRII administration of bromphenol blue, [^{14}C] morphine or [^{3}H]ouabain and its lack of effect on [^{3}H]taurocholate excretion in the rat*

Marker given	Intraportal		SRII	
	Control	Triton	Control	Triton
[^{14}C]Morphine	44.4 ± 6.4	9.8 ± 1.8†	37.6 ± 2.6	6.2 ± 0.3†
Bromphenol blue	55.6 ± 3.5	28.4 ± 4.2†	50.8 ± 4.4	13.1 ± 0.8†
[^{3}H]Ouabain	35.3 ± 5.5	10.9 ± 4.5†	49.2 ± 6.3	2.3 ± 3.8†
[^{3}H]Taurocholate	48.1 ± 13.6	48.7 ± 6.8	30.7 ± 0.6	33.5 ± 4.8

* Values in the table are mean ± S.E. of percent recoveries in bile. Triton-X-100, 0.4%, was given as described in Materials and Methods 5 min before the administration (intraportal or SRII) of the marker compound. Each group consisted of six animals except in the [^{14}C]morphine experiment where the groups were four animals each.

† $P < 0.05$ (Student's t-test).

0.22 and 0.31% (panels D and E), decreases in the radioactivity derived from both [^{14}C]morphine and [^{3}H]sucrose in bile were observed. The effect of the highest concentration, 0.4%, is shown in Fig. 3, panel A, where a further decrease in [^{14}C] and [^{3}H] recovery in bile was observed.

The result in Fig. 3, panel A, was the continuation point for the next experiment and served to demonstrate the response to the 0.4% concentration of Triton X-100, given 5 min before the SRII of the radioactive compounds. Subsequent panels (B through E) illustrate the results as the time between the administration of Triton and the SRII with [^{14}C] morphine and [^{3}H]sucrose was increased further to 30, 60, 90 min and 7 hr. It is seen that the effect of Triton treatment to decrease both ^{14}C and ^{3}H recovery was steadily maintained from 5 min to the 7-hr time period. It appears that even though the effect may increase slightly by 90 min (panel D) the response was similar at 7 hr.

In the next series of experiments, the 0.4% Triton was given as before by intrabiliary injection. Then, a number of marker compounds were examined for their biliary excretion after either intraportal or SRII administration. In Table 1, the results for [^{14}C]morphine given by SRII agree with those in Figs. 2 and 3. In addition, the results in Table 1 show that the Triton treatment decreased the biliary excretion of ^{14}C after intraportal administration of [^{14}C]morphine (control, 44 vs Triton, 9.8%; collection period 180 min).

The results given in Table 1 confirm the finding of Takada *et al.* [6] that Triton X-100 treatment causes a depression of biliary excretion of bromphenol blue given intravenously (BPB). In the control group, 56% of the administered dose of this non-metabolized organic anion was recovered in the 90-min collection of bile. Following treatment with 0.4% Triton X-100, the biliary recovery of BPB decreased to 28%. Since this anion is concentrated in bile compared to blood, the reduction in biliary excretion following Triton would be consistent with a disruption of the active transport of this compound [6]. The biliary recovery of BPB given by SRII in control animals was approximately 51%. Following Triton treatment, it decreased to 13%.

The next set of data shows that the Triton treatment also inhibited the biliary excretion of [^{3}H] ouabain whether the [^{3}H]ouabain was given by intraportal or SRII routes.

The striking finding was that for [^{3}H]taurocholate the Triton treatment had no effect on the biliary excretion of [^{3}H]taurocholate which was given by the intraportal or SRII routes.

DISCUSSION

Several details of our protocol for administration of Triton were different from that of previous workers [6, 7]. They gave intrabiliary injections of 50 μl of 4% Triton which was held in place for 10–15 min. We administered 40 μl of 0.4% Triton followed with 31 μl of saline. Bile flow was allowed to resume immediately. The location of the peak for the [^{14}C]mannitol by SRII in the control group (Fig. 1C) indicated a volume of about 42 μl for the distended biliary tree capacity as based on the work of Olson and Fujimoto [10]. Our rationale was that 40 μl of Triton plus 31 μl of saline would apparently put the peak concentration of Triton at about the canalicular membrane. Immediate resumption of bile flow would minimize further exchange between the bile and liver which would otherwise take place during 10–15 min of bile duct occlusion [12].

Retrograde intrability treatment with 0.4% Triton X-100 increased the permeability of the biliary tree. First, Triton treatment caused the biliary excretion peak of both [^{3}H]sucrose and [^{14}C]mannitol, given intraportally, to appear earlier in bile. Sucrose and other larger molecular weight water-soluble nonionic compounds, given i.v. appear to pass from sinusoidal blood through the tight junction by the paracellular pathway into the canalicular lumen [13, 14]. After attainment of the steady state, their bile to plasma concentration ratio is less than 1 [15]. A smaller molecule such as mannitol or erythritol may pass through not only the tight junction but through the cell as well and reach a bile to plasma ratio of 1 [15]. These latter compounds have a large volume of distribution in the liver and are indicators of canalicular bile flow [15]. Thus, the present results appear to indicate that, measured in the orthograde direction, tight junction permeability is increased by the Triton treatment.

Second, Triton treatment decreased the recovery in bile of [^{3}H]sucrose given by SRII. The same class

of compounds as above with larger molecular weights (sucrose, dextran, etc.), when given by SRII, have small volumes of distribution, appear rapidly in that blood (short transit times), and are not stored in hepatic cells [16]. These compounds probably pass from canalicular lumen to enter blood through the tight junctions. The small compounds (mannitol, erythritol) on SRII have larger volumes of distribution and longer transit times from canalicular lumen to blood [16]. Also, there is a gradation in their passage such that the smaller compounds pass in larger quantity from bile to blood [10, 16]. These characteristics make it likely that the latter compounds pass through both the paracellular and transcellular pathways. Since the Triton treatment reduced the recovery in bile of [^{3}H]sucrose given by SRII much more than [^{14}C]mannitol, we conclude that the Triton treatment increased the permeability of the tight junction. For comparative purposes, it is of interest that certain other drug treatments [17, 18] that increase biliary tree permeability did not differentially affect paracellular and transcellular permeabilities using the SRII technique.

At this point, a distinction should be made between the biliary tree permeability changes measured by the marker compounds above and the inhibition of biliary excretion of morphine, bromophenol blue, and ouabain produced by Triton X-100 apparently acting on the canalicular membrane. In a previous study [5], we had shown that 0.4% Triton treatment inhibited the biliary excretion of morphine-3-glucuronide formed intracellularly from [^{14}C]morphine. Biliary excretion of [^{14}C]morphine after SRII of [^{14}C]morphine was unchanged by the Triton treatment and was never more than 6.4% of the administered ^{14}C. Thus, in the present study, the decrease in the ^{14}C excretion on SRII of [^{14}C] morphine produced by the 0.4% (Fig. 3 and Table 1) or lower concentrations of Triton (Fig. 2) must be due to a decrease in [^{14}C]morphine glucuronide. Such a decrease largely results from inhibition of canalicular transport of [^{14}C]morphine glucuronide rather than an effect on its formation intracellularly [5]. It would appear from the Triton dose response (Fig. 2) and duration of action (Fig. 3) studies that concentrations of Triton X-100 lower than 0.4% affect canalicular transport and increase biliary tree permeability.

The results in Table 1 support the work of Takada *et al.* [6] that Triton treatment decreases bromophenol excretion into bile. Nakae *et al.* [7] showed that acetyl procaineamide ethobromide excretion into bile was also inhibited but to a lesser degree. Our demonstration in the results in Table 1 that, even though [^{3}H]ouabain excretion into bile was inhibited by Triton, excretion of [^{3}H]taurocholate was not, requires further comment. Meijer *et al.* [8] demonstrated in the rat that ouabain and dehydrocholate share a common pathway for biliary excretion but taurocholate does not. The fact that newborn rats have the capacity to excrete taurocholate much before the system develops for handling ouabain also is strong evidence for separate systems [9]. Our results with Triton X-100 clearly show that it is possible to decrease transport of ouabain without any effect on the biliary excretion of [^{3}H]taurocholate. The one advantage our present method may have over that used by Meijer *et al.* is that we might have separated the canalicular membrane carriers. That is, the work of Takada *et al.* [6] indicated that Triton X-100 removes the bromphenol blue carrier from the canalicular membrane. It seems likely that the present treatment, in addition, either removed or inactivated the canalicular carrier for dehydrocholate and ouabain. The taurocholate carrier, if indeed there be one, would thus remain intact in its original state in the canalicular membrane in spite of the treatment with Triton X-100.

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