Biochemical Pharmacology, Vol. 35, No. 3, pp. 479–486, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

DEMONSTRATION OF CELL SIDEDNESS IN HEPATIC TRANSFER OF MORPHINE AND MORPHINE GLUCURONIDE IN THE RAT*

ELIZABETH F. BERMAN† and JAMES M. FUJIMOTO‡

Toxicology Laboratory, Research Service, Veterans Administration Medical Center, Wood, WI 53193; and Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226, U.S.A.

(*Received* 2 *October* 1984; *accepted* 5 *July* 1985)

Abstract—The purpose of this study was to demonstrate hepatic cell sidedness for the transfer of [^{14}C]morphine and [^{14}C]morphine glucuronide. A tracer dose of [^{14}C]morphine was administered by segmented retrograde intrabiliary injection into the bile duct cannula of the urethane-anesthetized rat or the *in situ* isolated perfused liver preparation. The bile from the first, and the single pass perfusate from the second preparation, respectively, were analyzed for the radioactive components. Various doses of morphine were given intraportally in these preparations 5 min before the [^{14}C]morphine to influence the recovery of the radioactive components. As the dose of morphine increased, the recovery in bile of [^{14}C]morphine glucuronide decreased while [^{14}C]morphine remained unchanged. In the perfusate, the morphine loading increased the recovery of [^{14}C]morphine, but the [^{14}C]morphine glucuronide content was unchanged. These sets of results indicated that morphine loading inhibited the formation of morphine glucuronide by isotope dilution, which should have led to an increase in [^{14}C]morphine in the cell. Because of the presence of cell sidedness, the increased intracellular [^{14}C]morphine was directed toward the perfusate and not toward the bile. The decrease in [^{14}C]morphine glucuronide synthesis was manifested by a decrease in its excretion into bile, but not into perfusate. Further demonstration of cell sidedness was obtained by manipulation of these systems by chlordecone and *trans*-stilbene oxide pretreatment of the rats. The directionality of flow of [^{14}C]morphine and [^{14}C]morphine glucuronide out of the liver emanates from liver cell sidedness.

Biliary excretion of many drugs is facilitated by metabolism of the parent compound in the liver and conjugation of the metabolite to form highly polar compounds which are then transported into bile [1]. Induction of mixed-function oxidases and glucuronosyltransferases can increase the amount of polar metabolite formed. When such an increase in metabolism does not produce the expected increase in the amount of metabolite excreted into bile, then this exceptional situation emphasizes the paucity of our understanding of the mechanisms involved in the transfer process for biliary excretion. Induction of mixed-function oxidases and glucuronosyltransferases with phenobarbital and *trans*-stilbene oxide (TSO) increases morphine glucuronide formation, but decreases excretion of this metabolite into bile [2–5]. With TSO treatment, the markedly decreased biliary excretion of morphine glucuronide was ascribed to compartmentation within the hepatocyte and to differences between canalicular and sinusoidal mechanisms for transfer of morphine glucuronide [4]. The chlorinated hydrocarbon pesticide chlordecone decreases the biliary content of polar glucuronide metabolites of imipramine [6, 7] even though chlordecone is a microsomal enzyme inducer [8].

The purpose of the present study was to further consider cell sidedness in relation to the morphine/morphine glucuronide system. In the rat more than one-half of a tracer dose of [^{14}C-N-*methyl*]morphine will be conjugated to [^{14}C]morphine-3-glucuronide. In rats with a bile fistula, the majority of this glucuronide will be excreted in bile along with about 5% of the dose as free morphine. The present approach is to preload anesthetized, bile duct cannulated rats with large doses of non-radioactive morphine prior to the injection of a tracer dose of [^{14}C]morphine. This morphine preload should compete with [^{14}C]-morphine for the enzymatic and other binding sites; also the presence of large amounts of morphine glucuronide might affect the transfer of [^{14}C]morphine into bile. Similar experiments were performed on rats pretreated with chlordecone and TSO. Part of the experiments involved using the *in situ* isolated perfused rat liver to gain information into the corresponding changes on the blood side as measured by the changes in the radioactive components which occur in the perfusate. These various manipulations

* This investigation was supported by USPHS Grant GM 16503 and the Veterans Administration. A preliminary report was presented at the 1983 ASPET meetings in Philadelphia, PA.

† Postdoctoral Trainee, USPHS Grant 5T32 ES 07043.

‡ Research Career Scientist, Veterans Administration. Author to whom correspondence should be addressed at the Toxicology Laboratory, Research Service, VA Medical Center, 5000 West National Ave., Wood, WI 53193.

provide added insigit into the mechanisms involved in biliary excretion of morphine and morphine glucuronide.

MATERIALS AND METHODS

Male Sprague–Dawley rats (King Laboratories, Oregon, WI), weighing 270–350 g, were housed five to six per cage, on an alternating 12-hr light–dark cycle, with food (Purina Lab Chow) and water supplied *ad lib*. On the day of the experiment, rats were anesthetized with urethane (1200 mg/kg, i.p., with supplementation as necessary). A tracheotomy was performed if breathing difficulty occurred. After laparotomy, the bile duct was cannulated with a 10 cm length of PE20 polyethylene tubing which had a short piece of 27 gauge metal tubing attached at its distal end. The abdomen was covered with damp gauze, and core temperature was monitored by a rectal thermistor probe (Yellow Springs Instruments, Yellow Springs, OH) and maintained at 37 ± 1° by the use of an overhead incandescent lamp. Segmented retrograde intrabiliary injection (SRII) of [^{14}C]morphine followed the method of Olson and Fujimoto [9]. Briefly, a segment of PE20 tubing containing 40 μl (0.4 μCi) of radioactive morphine solution was connected to an infusion pump (Harvard Apparatus Co., Millis, MA), containing a 0.9% (w/v) sodium chloride solution. For the SRII, this apparatus was connected to the bile duct cannula and the pump was turned on to infuse the 40 μl of solution plus 110 μl of saline flush solution at a flow rate of 2.3 μl/sec. At the conclusion of the injection, the segment was disconnected quickly from the bile duct cannula, and bile was collected dropwise (drops 1–10 plus even numbered drops from 12 to 40) into polypropylene test tubes for later extraction and separation of [^{14}C]-morphine and [^{14}C]morphine glucuronide. In one study, bile was collected in the same manner immediately following intraportal injection of 70 μl (0.7 μCi) of [^{14}C]morphine. The [^{14}C]morphine glucuronide was separated from free [^{14}C]morphine by partitioning the two between 0.5 ml bicarbonate buffer (pH 8.8) and 1 ml water-saturated ethylacetate:*n*-butanol (7:3, v/v) by the procedure of Imamura and Fujimoto [10]. A standard of 10 μl of [^{14}C]morphine was run through the extraction procedure in duplicate for every experiment. Recoveries were calculated for each bile sample as the percentage of the total injected counts. Recovery of radioactivity in odd numbered bile drops was estimated by averaging the recoveries in the preceding and the succeeding bile drops. Total recovery of [^{14}C]morphine glucuronide and [^{14}C]morphine was determined by summing the recoveries from the individual bile drops.

Sinusoidal transfer of morphine and morphine glucuronide were measured in *in situ* liver perfusion experiments. Rats were prepared as for bile collection. In addition to cannulation of the bile duct, the portal vein and inferior vena cava were cannulated. The rat was placed in a perfusion cabinet maintained at 37°. The liver was perfused with oxygenated blood-free Krebs–Henseleit buffer (pH 7.4) containing 2.5% bovine serum albumin and 0.3% dextrose. The time for tying off the portal vein to the beginning of liver perfusion was kept to no more than 2 min. The liver was allowed to equilibrate with recirculating perfusate for 0.5 hr, and the flow rate was adjusted to be between 12 and 18 ml/min. To start the experiment, the perfusate outflow cannula from the liver was connected to a fraction collector (Gilson Instruments, Middleton, WI); [^{14}C]morphine was given by SRII into the bile duct cannula. Forty samples of perfusate were collected, one every 12 sec. At that point there was no recirculation of perfusate so the reservoir was refilled as needed with fresh perfusate. The volume of perfusate in each tube was determined indirectly by measuring the volume of an equivalent amount of water after which 1 ml of the perfusate was transferred to a new test tube. After adjusting the pH of the 1-ml sample to 9 with 16% (w/v) sodium carbonate solution, 2 ml of ethylacetate:*n*-butanol (7:3, v/v) was added, and the [^{14}C]morphine was separated from [^{14}C]morphine glucuronide. Aliquots of organic and aqueous phases of bile and perfusate extracts were counted by liquid scintillation spectrometry.

Using the two basic preparations described above, several additional experimental conditions were superimposed. In one set of experiments, rats (three to seven per dose) were injected intraportally with non-radioactive morphine sulfate (hereafter called morphine) 5 min prior to [^{14}C]morphine SRII. The dose of morphine ranged from 1.5 to 60 mg/rat and was injected in a volume of 0.3 ml. The injection for the rats given the 30 and 60 mg doses also included 0.3 mg of naloxone HCl to prevent respiratory failure from the large dose of morphine. In another set of experiments, the *in situ* isolated perfused livers were given an intraportal injection of morphine (3–60 mg) 5 min before the SRII of [^{14}C]morphine.

Further sets of experiments involved the same *in vivo* and *in situ* preparations in groups of rats pretreated with TSO and chlordecone. These pretreatments involved daily administration for 4 days of TSO (200 mg/kg/1.5 ml corn oil, i.p.) or 5 days of chlordecone (10 mg/kg/5 ml corn oil, i.p.). The animals were used 1 day after the last dose.

Urethane and TSO were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Chlordecone was obtained from Supelco, Inc. (Bellefont, PA). Morphine sulfate was purchased from Mallinckrodt Chemical Works (St. Louis, MO). The [^{14}C-N-*methyl*]morphine hydrochloride (sp. act. 56 mCi/mmole, radiochemical purity 98%) was from Amersham (Arlington Heights, IL). Significant differences ($P \leq 0.05$) between one treatment group and a number of treatment groups were determined by Dunnett's test, whereas a significant difference ($P \leq 0.05$) between two treatment groups was determined by Student's *t*-test.

RESULTS

Figure 1 gives an example of the recovery of radioactive components in bile after the administration of [^{14}C]morphine in the urethane-anesthetized rat. Expressed in terms of the amount administered, 62.3% of the dose of radioactivity was recovered as [^{14}C]morphine glucuronide. A small amount (3.1%) was recovered as [^{14}C]morphine. These data indicate that after the SRII administration of [^{14}C]morphine

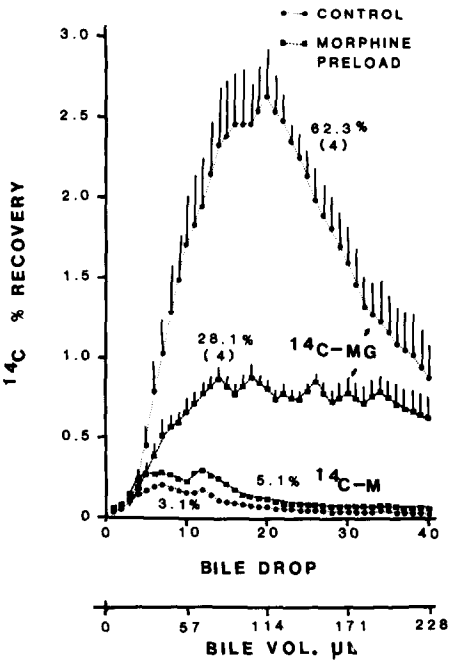

Fig. 1. Recovery of [^{14}C]morphine glucuronide ([^{14}C]MG) and [^{14}C]morphine ([^{14}C]M) for individual bile drops collected in urethane-anesthetized rats in the control group (● · · · ·) and the group injected i.v. with 15 mg morphine (■– – –■) 5 min before [^{14}C]morphine was given by SRII. Mean ± S.E.M. recovery values are expressed as the percent of the radioactive dose of [^{14}C]morphine injected. The numerical values and numbers in parentheses given beside each curve are the total (cumulated) recoveries and number of animals per group respectively.

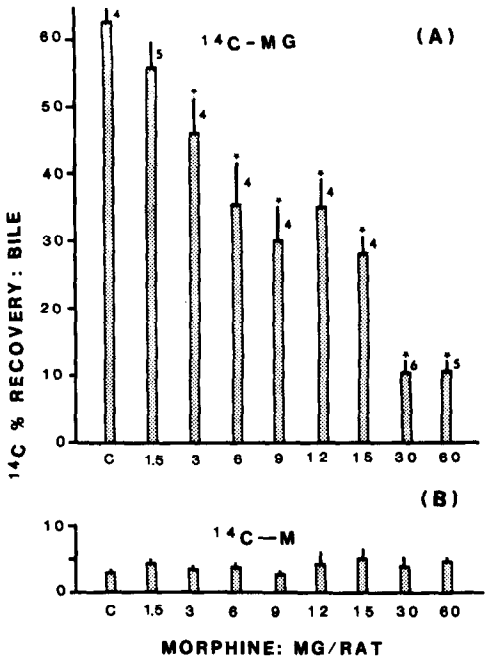

Fig. 2. Cumulative recovery of [^{14}C]morphine glucuronide and [^{14}C]morphine in bile (40 drops) in the control group (C) and the groups injected with morphine (dose given on abscissa) i.v. 5 min before [^{14}C]morphine was given by SRII. Asterisks indicate recoveries from morphine preload groups which were significantly different ($P \leq 0.05$) from the control group as assessed by Dunnett's test. The vertical bar above each mean value represents the S.E.M.; the number of number of animals in each group is given next to the S.E.M.

most of the morphine is absorbed from the biliary tree; the 150 µl SRII volume is more than three times the capacity of the biliary tree volume [9]. A large portion of the morphine was conjugated, most likely in the hepatocyte, to form [^{14}C]morphine glucuronide which was then excreted into bile. In the figure, it is seen that, when the rats were given 15 mg of morphine 5 min before the SRII of [^{14}C]morphine, the recovery of [^{14}C]morphine glucuronide was reduced to a value of 28.1%. On the other hand, this morphine preloading had no effect on the recovery of the [^{14}C]morphine (5.1%).

In Fig. 2, the cumulative recoveries of [^{14}C]morphine and [^{14}C]morphine glucuronide were derived in experiments as above and are shown in relation to various doses of morphine used to preload the animals. Figure 2 includes the data from Fig. 1. In Fig. 2A, a gradual decrease in the recovery of [^{14}C]-morphine glucuronide was seen as the dose of morphine increased. Half as much [^{14}C]morphine glucuronide was recovered when the dose of morphine was in the range of 6 to 15 mg. When the dose reached 30 mg, the recovery of [^{14}C]morphine glucuronide dropped to about 10%. Doubling this dose to 60 mg produced no further effect. In contrast to [^{14}C]morphine glucuronide, the amount of [^{14}C]-morphine in bile remained at about 5% and was not affected by even the largest doses of unlabeled morphine (Fig. 2B). Based on the results from panel A, there was no doubt that the unlabeled morphine had the effect of diluting the [^{14}C]morphine to account for the decrease in [^{14}C]morphine glucuronide in bile. This effect may arise from inhibition of glucuronidation or biliary excretion. Inhibition of glucuronidation would have been a clear choice had the [^{14}C]morphine in bile shown a large increase; excretion of [^{14}C]morphine was not affected by preloading with morphine.

To see what was happening to these components on the blood (sinusoidal) side in comparison to the bile (canalicular) side, the transfer of these components into the perfusate on a single pass perfusion was evaluated in the *in situ* isolated liver preparation. From the curves shown in Fig. 3 for the SRII of [^{14}C]morphine in the control situation, the recovery of [^{14}C]morphine glucuronide was 21.0% and [^{14}C]-morphine 6.19% for the 40 samples of single pass perfusate collected (12 sec/sample × 40 samples = 480 sec = 8 min). Note that the [^{14}C]morphine appeared as a peak in sample number 6. We assume from the work of Imamura and Fujimoto [10] that this [^{14}C]morphine represents both that which was never taken up by the cells (extracellular pathway) in passing from the bile to perfusate and also that which had passed through cells before entering the perfusate. Imamura and Fujimoto based their

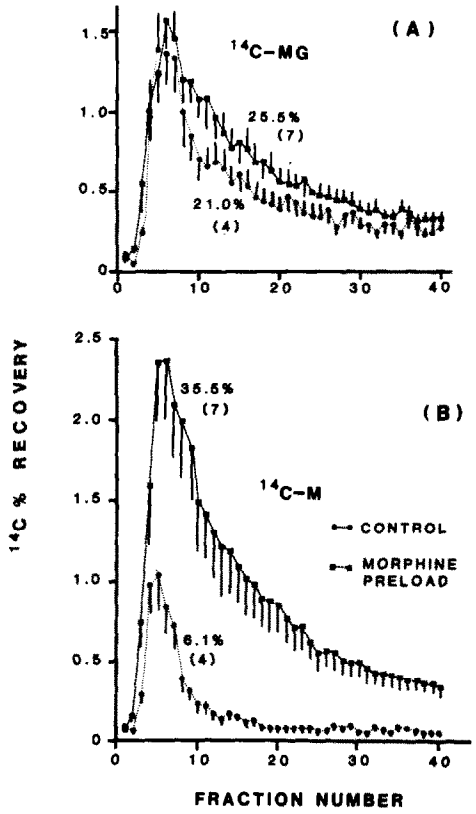

Fig. 3. Recovery of [^{14}C]morphine glucuronide and [^{14}C]-morphine in the single pass perfusate fractions collected after the administration of [^{14}C]morphine in the *in situ* isolated perfused rat liver. The data are presented as described in the legend of Fig. 1 for the control group (● · · · ●) and the group preloaded with 15 mg morphine (■– – –■) i.v. 5 min before [^{14}C]morphine was given by SRII.

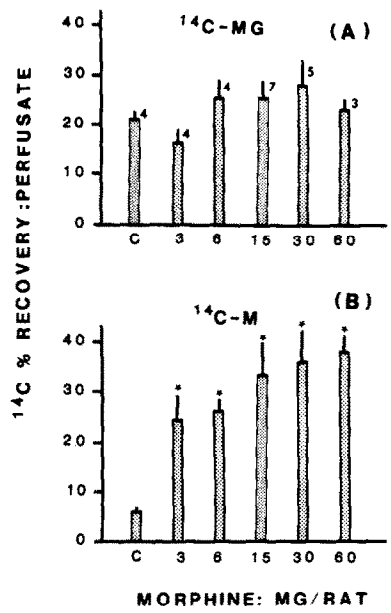

Fig. 4. Cumulative recovery of [^{14}C]morphine glucuronide and [^{14}C]morphine in the perfusate (40 samples) of the *in situ* isolated rat liver in the control group (C) and the groups preloaded with morphine (dose on abscissa) i.v. 5 min before [^{14}C]morphine was given by SRII. Asterisks indicate recoveries from morphine preload groups which were significantly different ($P \leq 0.05$) from the control group as assessed by Dunnett's test. Other designations are as in Fig. 2.

reasoning on the fact that the transit time for [^{14}C]-morphine was longer than that for dextran (which enters the perfusate through extracellular passage). For [^{14}C]morphine glucuronide, we assume that [^{14}C]morphine would have had to enter the hepatocyte to form the morphine glucuronide and then leave the hepatocyte in order to appear in the perfusate. Figure 3 also shows what happens when the preparation was preloaded with 15 mg of morphine given into the perfusate and recirculated for 5 min before the [^{14}C]morphine was given by SRII. The free [^{14}C]morphine curve is raised on the right side compared to the control. This rightward skewing is taken as evidence that the morphine preloading increased the egress of intracellular [^{14}C]morphine. If this were primarily an effect of [^{14}C]morphine coming into the perfusate by purely an extracellular route, we would expect the left side of the curve to be raised as much as the right side. The curve for [^{14}C]morphine glucuronide was unaffected by the 15 mg morphine preload.

Figure 4 shows the results for different preloading doses of morphine. It is seen that, even with the highest dose of morphine, the preloading had no effect on the recovery of [^{14}C]morphine glucuronide in the perfusate compared to the control situation (group C). On the other hand, even at a preloading dose of 3 mg of morphine, there was an increase in the amount of [^{14}C]morphine in the perfusate. Doubling the doses beyond the 15 mg of morphine did not increase [^{14}C]morphine much more. The conclusion derived from these experiments was that the morphine loading increased the amount of free [^{14}C]morphine, but not [^{14}C]morphine glucuronide, in the perfusate. It therefore appears that the morphine loading inhibits the formation of [^{14}C]morphine glucuronide to reduce the amount of [^{14}C]-morphine glucuronide that appears in bile (Fig. 2) and there is an increase in [^{14}C]morphine in the perfusate. The increase in [^{14}C]morphine however, does not fully reflect the decrease in [^{14}C]morphine glucuronide recovery.

Since in all of these experiments the sample collection period was limited, some insight into the total recovery of the ^{14}C originally administered could be obtained by adding the bile and perfusate recoveries together even though these values were derived from different experiments. At the morphine preload dose of 0, 3, 6, 15, 30, and 60 mg, the total ^{14}C accounted for in the analyzed samples was, respectively, 92, 90, 91, 94, 78 and 76%. This hysteresis in total recovery may occur because inhibition of [^{14}C]morphine glucuronide formation caused more [^{14}C]morphine to be present in the liver. It appears that [^{14}C]morphine has more difficulty getting out of the liver (longer transit time and larger volume of distribution) than does [^{14}C]morphine glucuronide (as determined by

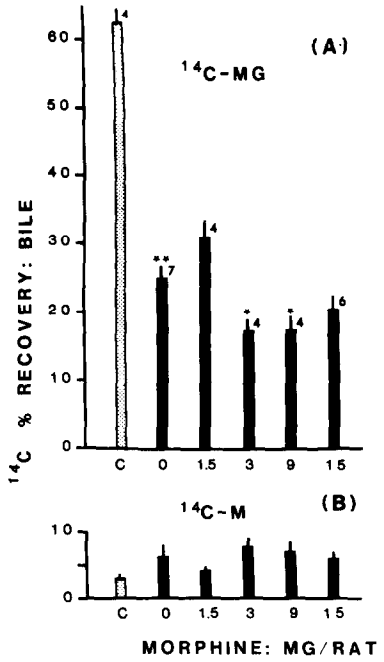

Fig. 5. Effect of chlordecone pretreatment of the rat on the cumulative recovery of [^{14}C]morphine glucuronide and [^{14}C]morphine in bile in the control group (C) and the group preloaded with morphine i.v. 5 min before [^{14}C]-morphine was given by SRII. The dotted bar represents the recovery for the control group (C) from Fig. 2. The cross-hatched bars represent the recoveries in the chlordecone pretreated groups. The single asterisk indicated a significant difference ($P \leq 0.05$) compared to the O morphine group as assessed by Dunnett's test. The double asterisk indicates a significant difference ($P \leq 0.05$) between the C and O groups as determined by Student's *t*-test.

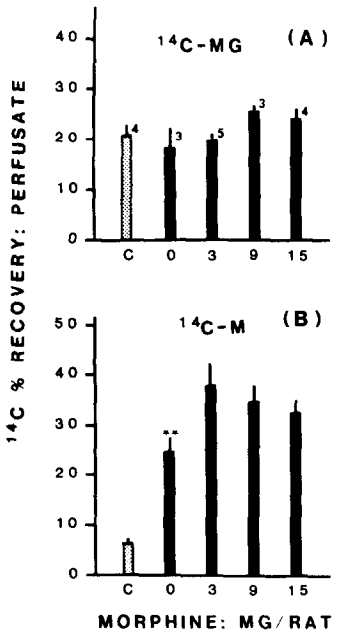

Fig. 6. Effect of chlordecone pretreatment of the rat on the cumulative recovery of [^{14}C]morphine glucuronide and [^{14}C]morphine in the single pass perfusate of the *in situ* isolated liver in the control group (C) and the groups preloaded with morphine i.v. 5 min before [^{14}C]morphine was given by SRII. The double asterisk indicates a significant difference ($P \leq 0.05$) between the C and O groups, as determined by Student's *t*-test. Other designations are as in Fig. 5.

giving [^{14}C]morphine glucuronide by SRII [10]). Carrying out the present sample collections to further times might have decreased the hysteresis in recovery. However, the presence of this hysteresis does not alter the major interpretation of the results.

Experiments similar to Fig. 2 and 4 for SRII were performed for the intravenous (portal vein) injection of [^{14}C]morphine. As in the SRII experiment, the preloading with morphine decreased [^{14}C]morphine glucuronide and had no effect on [^{14}C]morphine excretion in bile for [^{14}C]morphine given into the portal vein (data not given). In the *in situ* isolated perfused liver preparation, the single pass perfusate contained 65% of the [^{14}C]morphine given by intraportal administration. Since over half of the [^{14}C]-morphine was not extracted by the liver from the perfusate on single pass, we felt that this mode of administration of [^{14}C]morphine would not be as sensitive for observing changes produced by morphine loading as would be the SRII administration of [^{14}C]morphine. Thus, the intravenous route of administration of [^{14}C]morphine was not used any further.

We went on to modify the system with chlordecone and TSO pretreatment while the effect of morphine preloading was assessed. As seen in Fig. 5 for the SRII of [^{14}C]morphine, the excretion of [^{14}C]morphine glucuronide decreased from the control value (group C) of 62% to 25% for the chlordecone-treated (O morphine) group. Excretion of [^{14}C]morphine remained unchanged between the two groups. In this experiment and the subsequent one with TSO treatment, control groups treated with corn oil were included. Since the vehicle treatment did not have a significant effect compared to group C, the results for the vehicle controls were not included in the data presented. Next, the effect of morphine loading in the chlordecone-treated animals was examined (Fig. 5). The morphine loading appeared to have no consistent effect on the [^{14}C]morphine glucuronide excretion in that two doses of morphine slightly decreased [^{14}C]morphine glucuronide recovery while a higher dose did not.

In Fig. 6, it is seen that the chlordecone treatment (O morphine group) had no effect on the amount of [^{14}C]morphine glucuronide in the perfusate compared to control (group C). For [^{14}C]-morphine recovery, the chlordecone pretreatment (O morphine) increased the recovery compared to the control. In addition, the morphine loading further increased the [^{14}C]morphine recovery.

The results for the experiment with TSO are given in Figs. 7 and 8. First, note that in the TSO group (O morphine) the recovery of [^{14}C]morphine glucuronide in bile (20%) was reduced drastically from the control value of 62%. The biliary excretion of

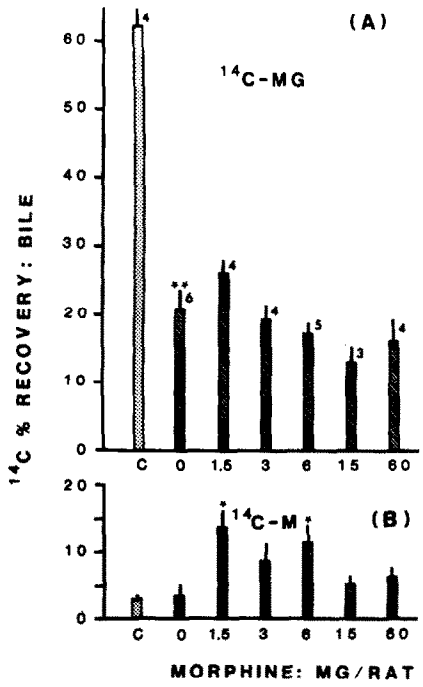

Fig. 7. Effect of TSO pretreatment of the rat on the cumulative recovery of [^{14}C]morphine glucuronide and [^{14}C]-morphine in bile in the control (C) group and the groups preloaded with morphine i.v. 5 min before [^{14}C]morphine was given by SRII. The dotted bar represents the recovery for the control group (C) from Fig. 2. The striped bars represent the recoveries in the TSO-pretreated groups. A single asterisk indicates a significant difference ($P \leq 0.05$) compared to the O morphine group as assessed by Dunnett's test. The double asterisk indicates a significant difference between the C and O groups as determined by Student's *t*-test.

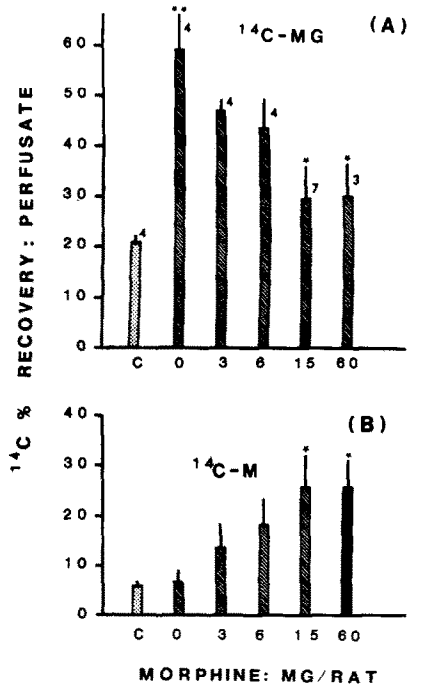

Fig. 8. Effect of TSO pretreatment of the rat on the cumulative recovery of [^{14}C] morphine glucuronide and [^{14}C]-morphine in the single pass perfusate of the *in situ* isolated liver in the control group (C) and the groups preloaded with morphine i.v. 5 min before [^{14}C]morphine was given by SRII. A single asterisk indicates a significant difference ($P \leq 0.05$) compared to the O morphine group as assessed by Dunnett's test. The double asterisk indicates a significant difference between the C and O groups as determined by Student's *t*-test. Other designations are as in Fig. 7.

[^{14}C]morphine was unaffected. For the perfusate, the TSO group had a large increase in [^{14}C]morphine glucuronide (59%) compared to the 21% value for the control. The [^{14}C]morphine remained the same. These sets of results indicate that TSO treatment decreased [^{14}C]morphine glucuronide excretion in bile, but increased [^{14}C]morphine glucuronide transfer to the perfusate as shown by Fuhrman-Lane and Fujimoto [4]. Under this condition, the loading with morphine was much less effective in further reducing the excretion of [^{14}C]morphine glucuronide in bile. Now, the morphine loading was more effective in reducing the amount of [^{14}C]morphine glucuronide in the perfusate. A rise in the [^{14}C]morphine in bile also was seen at the 1.5 and 6 mg doses of morphine. Since at the 3 and 15 mg doses of morphine the rise was not significant, it would appear that the interpretation of these rises in [^{14}C]morphine would not be particularly meaningful. The morphine loading did increase the amount of [^{14}C]morphine in the perfusate in a dose-dependent manner. The particularly important finding is that, by altering the amounts of [^{14}C]morphine which go into the bile as compared to perfusate, the TSO treatment changed the way in which the morphine–morphine glucuronide system responded to the morphine loading.

DISCUSSION

The present study is based on the premise that preloading the liver with various amounts of unlabeled morphine before the administration of [^{14}C]-morphine should modify the amounts of [^{14}C]morphine and its metabolites which appear in the bile and blood. In the present study, the major components measured were [^{14}C]morphine and [^{14}C]morphine glucuronide since results in earlier studies [10, 11] indicated that these accounted for most of the ^{14}C components present within the short period covered by these experiments. In the bile duct cannulated rat, when morphine-3-glucuronide is administered, it remains intact [11]. Morphine-3-ethereal sulfate is not formed in the rat [11, 12]. Changes in recovery of ^{14}C would thus indicate major alterations in amounts of [^{14}C]morphine and [^{14}C]morphine glucuronide by the liver.

The present study expands upon the one dose preload experiment conducted in the pentobarbital-anesthetized rat by Sweeney *et al.* [13]. They showed that preloading the rat with 45 mg of morphine sulfate intraportally 5 min before [^{14}C]morphine SRII decreases the biliary recovery of [^{14}C]morphine glucuronide and increased the amount of [^{14}C]morphine

in the perfusate. They concluded that the unlabeled morphine inhibited the formation of [^{14}C]morphine glucuronide. In the present experiment, a clear relationship between the dose of morphine and the decrease in biliary excretion of [^{14}C]morphine glucuronide was seen (Fig. 2). In the single pass perfused liver preparation (Fig. 4), there was a corresponding increase in the perfusate content of [^{14}C]morphine. Even though the recovery values in the latter experiments did not show a one-to-one relationship between the decrease in [^{14}C]morphine glucuronide and the increase in [^{14}C]morphine, it is likely that the hysteresis in the [^{14}C]morphine recovery mentioned previously can be attributed to the longer transit time and larger volume of distribution of [^{14}C]morphine than [^{14}C]morphine glucuronide in the liver. Thus, the present results support the view that the loading with unlabeled morphine inhibited the formation of [^{14}C]morphine glucuronide from [^{14}C]-morphine.

The results demonstrate the existence of another important factor, cell sidedness. Inhibition of [^{14}C]-morphine glucuronide formation by the morphine load would be expected to decrease both the biliary and perfusate recoveries of [^{14}C]morphine glucuronide in relatively equal proportions. Compensatory increases in perfusate and bile concentrations of [^{14}C]morphine should also occur in relatively equal proportions. Examination of the pairs of results for the bile and perfusate for this decrease in [^{14}C]morphine glucuronide and increase in [^{14}C]morphine certainly does not support this expectation. Instead, the effects are unequally manifested. The biliary content of [^{14}C]morphine glucuronide decreased but the perfusate content stayed unchanged (Figs. 2 and 4). We interpret these results to mean that, even though the amount of [^{14}C]-morphine glucuronide formed in the liver decreased, the amount available for transfer to the perfusate remained the same. The differential change in biliary (canalicular), but not perfusate (sinusoidal), [^{14}C]-morphine glucuronide content points to the existence of different controlling factors, cell sidedness, in handling [^{14}C]morphine glucuronide.

The results also provide support for the existence of cell sidedness for transfer of [^{14}C]morphine. The results in Figs. 2 and 4 indicate that increasing preloading doses of morphine which decrease [^{14}C]morphine glucuronide in bile have no effect on biliary excretion of [^{14}C]morphine. Since morphine preloading increases [^{14}C]morphine in the perfusate, it is safe to conclude that this morphine loading must have increased intracellular concentrations of [^{14}C]-morphine. This increased [^{14}C]morphine should have been available for transfer into both perfusate and bile. The fact that it was not transferred to bile in increased amounts, but transferred only to perfusate, must indicate that the control mechanism for transferring [^{14}C]morphine at the canalicular membrane is different from that at the sinusoidal membrane.

Further results can be interpreted to support the cell-sidedness argument. Comparing the control (group C) to the TSO-treated group (O morphine load) given an SRII of [^{14}C]morphine (Figs. 7 and 8), the results confirm the work of Fuhrman-Lane and Fujimoto [4] that TSO treatment decreases the [^{14}C]morphine glucuronide excetion in bile and increases it in the perfusate. TSO concomitantly induces glucuronosyltransferase [4, 5]. Despite other alternatives, they conclude that cell sidedness exists for [^{14}C]morphine glucuronide since biliary [^{14}C]-morphine glucuronide decreases in spite of the increased formation of the glucuronide. As shown in Fig. 7, with increasing morphine doses, the biliary excretion of [^{14}C]morphine glucuronide decreased even further (from 21 to 13% at 15 mg morphine). The incremental decrease was not as great as in the comparable groups in Fig. 2, probably because the original starting amount of [^{14}C]morphine glucuronide excreted was lower in the TSO-treated group. When the [^{14}C]morphine glucuronide amounts in the perfusate were high for the TSO group, morphine loading decreased [^{14}C]morphine glucuronide recovery in the perfusate but had no effect on biliary [^{14}C]morphine glucuronide. It is likely that the morphine preload here again inhibited morphine conjugation as in the control situation, since the amount of [^{14}C]morphine increased in the perfusate. This increase in [^{14}C]morphine occurred along with a drop in [^{14}C]morphine glucuronide in perfusate but not in bile. Again, the effects of morphine preloading indicate that transfer of both [^{14}C]-morphine glucuronide and [^{14}C]morphine at canalicular and sinusoidal sites are controlled differently. The decreased formation of [^{14}C]morphine glucuronide produced by the morphine load is manifested by incremental increases in [^{14}C]morphine in the perfusate but not in the bile.

Chlordecone pretreatment decreased the amount of [^{14}C]morphine glucuronide in bile. An indication as to how this decrease occurs is found in the results seen in this group (Figs. 5 and 6) compared to those seen with morphine loading in the control group (Figs. 2 and 4). The chlordecone group (O morphine load) manifested a decrease in [^{14}C]morphine glucuronide in bile (Fig. 5) along with an increase in [^{14}C]morphine in perfusate as compared to group C (not treated with chlordecone), where the [^{14}C]-morphine in bile and the [^{14}C]morphine glucuronide in perfusate remained unchanged. In the quantitative sense, these sets of changes suggest that chlordecone is inhibiting the formation of [^{14}C]morphine glucuronide (rather than affecting [^{14}C]morphine glucuronide transport). This conclusion would place the effect of chlordecone at a site of action different than that suggested by Mehendale [6, 7] for its effect on the imipramine system. The effect was attributed to impaired transport of the metabolites from the cell to the bile based on the inability to excrete the metabolites even in the presence of adequate levels of these metabolites in the liver. In our situation, it appears that the decrease in biliary content of [^{14}C]-morphine glucuronide is reflected by the increase in perfusate content of [^{14}C]morphine; since the overall effect would be a decrease in the amount of [^{14}C]-morphine glucuronide formed, it would be difficult to support an argument for the primary effect being that of inhibition of transport of [^{14}C]morphine glucuronide into bile. The evidence for decreased transport of [^{14}C]morphine glucuronide into bile is more convincing with the TSO treatment than the chlor-

decone because in the case with TSO the decrease in biliary [^{14}C]morphine glucuronide occurred with an increase in perfusate [^{14}C]morphine glucuronide. The results of the experiments where loading with morphine was performed in the chlordecone-treated animals are consistent with inhibition of metabolism. Since the chlordecone treatment had already inhibited formation of [^{14}C]morphine glucuronide, administration of the various doses of morphine had little effect in further decreasing [^{14}C]morphine glucuronide in bile or in the perfusate. [^{14}C]Morphine in the perfusate was raised by the chlordecone treatment so that superimposing morphine doses had only a slight effect in raising the perfusate concentration of [^{14}C]morphine further. The alternative that chlordecone treatment inhibits [^{14}C]morphine glucuronide transport would not fit the observed change.

At this point the argument for cell sidedness can be summarized in more general terms. No matter what manipulation we used, the biliary content of [^{14}C]morphine was fully resistant to change. This characteristic is surprising since even the raising of the intracellular content of [^{14}C]morphine by morphine preloading had no effect. The fact that, in Fig. 7, for TSO pretreatment, [^{14}C]morphine recoveries were increased at two doses of morphine loading does indicate that, under certain conditions, [^{14}C]-morphine excretion may become sensitive to manipulation.

On the sinusoidal side, [^{14}C]morphine changed with morphine loading and, as with chlordecone treatment, was indicative of inhibition of metabolism of [^{14}C]morphine. The change in [^{14}C]morphine glucuronide recovery across the canalicular and sinusoidal sides was not always in the same direction. Inhibition of [^{14}C]morphine glucuronide formation (as by morphine loading or chlordecone treatment) was seen as a decrease in [^{14}C]morphine glucuronide in bile but not in perfusate, whereas it was possible to increase the [^{14}C]morphine glucuronide in the perfusate (TSO treatment) at the same time that [^{14}C]morphine glucuronide excretion in bile was decreased. These various sets of data indicate that there must be mechanisms within the cell which determine the directionality of flow of morphine and morphine glucuronide. We wish to emphasize the fact that such directional flow of drugs and their metabolites is difficult to study and recognize in such *in vitro* systems as isolated hepatocytes and subcellular preparations.

REFERENCES

1. G. J. Dutton, *Glucuronidation of Drugs and Other Compounds*, Chap. 1. CRC Press, Boca Raton, FL (1980).
2. R. E. Peterson and J. M. Fujimoto, *J. Pharmac. exp. Ther.* **184**, 409 (1973).
3. D. L. Roerig, A. T. Hasegawa, R. E. Peterson and R. I. H. Wang, *Biochem. Pharmac.* **23**, 1331 (1974).
4 C. Fuhrman-Lane and J. M. Fujimoto, *J. Pharmac. exp. Ther.* **222**, 526 (1982).
5. R. B. Franklin, C. Fuhrman-Lane, E. F. Sweeney and C. W. Christensen, *Fedn Proc.* **41**, 1732 (1982).
6. H. M. Mehendale, *Toxic. appl. Pharmac.* **40**, 247 (1977).
7. H. M. Mehendale, *Fedn Proc.* **38**, 2240 (1979).
8. H. M. Mehendale, A. Takanaka, D. Desaiah and I. K. Ho, *Life Sci.* **20**, 991 (1977).
9. J. R. Olson and J. M. Fujimoto, *Biochem. Pharmac.* **29**, 205 (1980).
10. T. Imamura and J. M. Fujimoto, *J. Pharmac. exp. Ther.* **215**, 122 (1980).
11. D. S. Smith, R. E. Peterson and J. M. Fujimoto, *Biochem. Pharmac.* **22**, 485 (1973).
12. S. Y. Yeh, H. A. Krebs and C. W. Gorodetzky, *J. pharm. Sci.* **68**, 13 (1979).
13. E. F. Sweeney, C. Fuhrman-Lane and J. M. Fujimoto, *J. Pharmac. exp. Ther.* **228**, 19 (1984).