Facilitated method for measurement of biliary secretion rates in healthy humans

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Abstract We have developed a facilitated method for determining secretion of constituents into bile. The ratio of constituent/bilirubin was measured in gallbladder bile and multiplied by bilirubin secretion rate, estimated by measuring endogenous production of carbon monoxide (V_{CO}) by breath sampling. Accuracy of this method was assessed by measuring secretion rate of ^{99m}Technetium-labeled disofenin during steady-state constant intravenous infusion. In nine subjects, mean (± SEM) secretion of disofenin by the CO method was 104.2 ± 7.2% of expected and by standard marker perfusion was 97.8 ± 13.1% of expected. In ten subjects, secretion rate of cholesterol by the CO method averaged 103 μmol/h by the CO method compared to 113 by marker perfusion (NS). Compared to marker perfusion (which is believed to reflect 24-h secretion rate), the CO method significantly underestimated secretion rate of bile acid (1110 vs. 1332 μmol/h, $P = 0.076$) and lecithin (295 vs. 413 μmol/h, $P = 0.01$), probably because gallbladder bile contained a disproportionate amount of fasting versus postprandial bile. Thus, this new method provides an accurate secretion rate for biliary constituents secreted at a relatively constant rate, including cholesterol, with less variability than marker perfusion. However, it can be used to estimate secretion of bile acid and lecithin only when a 20–30% underestimation of 24-h secretion is acceptable.—**Duane, W. C., M. D. Levitt, and M. K. Elson.** Facilitated method for measurement of biliary secretion rates in healthy humans. *J. Lipid Res.* 1993. **34:** 859–863.

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It is often useful to know the biliary secretion rate of either natural constituents of bile or exogenously administered compounds. In the past these rates have been measured by marker perfusion methods in which a nonabsorbable marker is perfused into the duodenum and samples are obtained from one or more distal ports, a technique most frequently applied to measurement of lipid secretion rates (1–7). Because of the presence of a gallbladder, this approach requires either prolonged (12–14 h) intraduodenal infusion of formula to maintain gallbladder contraction (1) or even longer studies with more physiologic feeding to account for movement in and out of the gallbladder (3, 8). Relatively few potential study subjects will consent to such arduous procedures and even fewer will consent to repeated procedures for comparative studies. We therefore attempted to develop an alternative method that would not require either prolonged intubation or formula infusion.

It is known that bilirubin is secreted into bile at a constant rate (9). If that rate is known, one can calculate secretion rate of a given biliary constituent from the ratio of constituent/bilirubin in bile (9). It is also known that CO is released exclusively and stoichiometrically during the conversion of heme to bilirubin, and that the production rate of CO in the body (V_{CO}) equals bilirubin production (10, 11). Because there is virtually no enterohepatic circulation of bilirubin (12), and in health nearly all bilirubin excretion occurs via the bile, bilirubin production should be equivalent to bilirubin secretion into bile. We therefore measured V_{CO} on the breath to estimate bilirubin secretion rate and multiplied by the ratio of constituent/bilirubin in bile to calculate secretion rate of that constituent. The method was assessed first by comparing breath V_{CO} measurements to direct measurements of bilirubin secretion; second, by using the CO method to measure a known secretion rate, that of ^{99m}Technetium-labeled disofenin during constant intravenous infusion; and third, by comparison of lipid secretion measured by the CO method versus a standard marker perfusion method.

METHODS

Twelve male volunteers ranging in age from 44 to 75 years were studied. All were without significant medical problems as judged by previously published criteria (13). Following a detailed explanation of study procedures, each gave his written consent to participate. All study protocols were approved by committees overseeing use of human subjects in research at both the Minneapolis VA

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Medical Center and the University of Minnesota. Three of the subjects did not undergo measurements involving administration of radioactivity.

^{99m}Technetium-labeled disofenin (hereafter referred to as disofenin) was prepared in the Nuclear Medicine Department of the Minneapolis VA Medical Center using Hepatolite kits (E. I. duPont de Nemours & Co., Billerica, MA). Shelf-life of this material is usually taken to be about 6 h. However, we found that when exposure to oxygen was minimized, the material was stable for at least 24 h as judged by thin-layer chromatography. An amount of isotope calculated to provide an initial infusion rate of about 8 μCi/h was sterilely transferred to an H-TRON V100 pump usually used for insulin infusion (Disetronic Medical Systems, Plymouth, MN). In addition to providing very precise delivery rates over long periods of time, this pump protected the isotope from degradation by atmospheric oxygen. Intravenous infusion was then accomplished by "piggybacking" the pump effluent into a standard intravenous infusion of normal saline set to run at 40 ml/h by an IVAC volumetric pump (IVAC Corp., San Diego, CA). Infusion was begun 12–18 h before secretion rates were to be measured to provide adequate time to reach a steady state. Actual infusion rate of isotope was measured by collecting timed samples of this infusate before and after completion of the study. In addition, because a small amount (10–15%) of isotope was excreted by the kidneys, urine was quantitatively collected during all infusions. All samples of infusate, bile, and urine were assayed for radioactivity simultaneously using a Packard Cobra auto-gamma counter. Expected biliary excretion was calculated by subtracting the urinary excretion rate from the intravenous infusion rate.

We determined V_{CO} by measuring the elimination of CO on the breath. This was accomplished by having the subject sit with his head in a Plexiglass hood containing CO_2 absorber as described in a previous publication (14). Air was drawn through this hood at a constant rate. The atmosphere within the hood was sampled periodically for measurement of CO concentration by gas chromatography as previously described (14). The difference between CO concentration in the hood and that in room air was multiplied by flow rate through the hood to calculate total CO excretion rate. However, some CO excretion is a result of previously assimilated atmospheric CO. To determine the fraction of breath CO that was exogenous, each subject wore an "equilibrator" for 24 h prior to sampling. This apparatus, which has been described previously (14), is specifically designed to equilibrate with atmospheric CO at the same rate as the subject. The partial pressure of CO (P_{CO}) in the equilibrator could be divided by P_{CO} in the subject's end-alveolar air to determine the fraction of the subject's CO that was of exogenous origin. This exogenous fraction was then subtracted from the total CO excreted by the subject to calculate V_{CO}.

Measurement of lipid or disofenin secretion using the CO method was calculated by multiplying V_{CO} by the ratio of lipid or disofenin/bilirubin in gallbladder bile obtained in the morning before breakfast. We chose to use gallbladder bile obtained before breakfast for these measurements because it should represent bile secreted partially in the postprandial and partially in the fasting state. Samples of bile were obtained using a peroral duodenal tube and intravenous cholecystokinin octapeptide (Kinevac, Squibb & Sons, Inc., Princeton, NJ) as previously described (7, 13). Samples were analyzed for bile salt, phospholipid, and cholesterol also as previously described (7, 13). Analysis for bilirubin was performed by standard diazo reaction (15).

Marker perfusion measurements of secretion rates for lipids, bilirubin, and disofenin were performed as previously outlined (7) using the method originally described by Grundy and Metzger (1). When bilirubin secretion was to be measured, all tubing and collection flasks were protected from light. When marker perfusion measurements were to be compared to secretion measured by the CO method, both were carried out within 2 weeks of each other with the subject on the same diet. With the exception of studies requiring administration of radioactivity, measurements were made at least twice to reduce random variability.

Statistical testing was performed using SAS software (SAS Institute, Cary, NC) on a Northgate 486 personal computer. Comparisons were done by paired *t*-test.

RESULTS

Although other studies have suggested that bilirubin output into bile can be equated with V_{CO} (10–12), we tested that premise in our subjects as well. As shown in **Fig. 1,** V_{CO} measured by breath sampling provided a reasonable estimate of bilirubin secretion measured by standard marker dilution.

Before using disofenin to assess accuracy of biliary secretion measurements, it was necessary to be certain that there was no enterohepatic circulation of this compound. This was accomplished by oral administration of about 500 μCi at 9:00 PM in two subjects. The following morning a sample of gallbladder bile was obtained and analyzed for radioactivity and bilirubin. The ratio of radioactivity/bilirubin was multiplied by the amount of bilirubin produced in the interim 11–12 h determined by V_{CO}. In both subjects this calculation indicated that less than 1.5% of the orally administered dose had been absorbed and secreted into bile.

Fig. 2 shows the result of measurements of biliary disofenin secretion by both marker perfusion and CO methods in subjects receiving a constant intravenous infusion of disofenin. For the nine subjects, mean (± SEM)

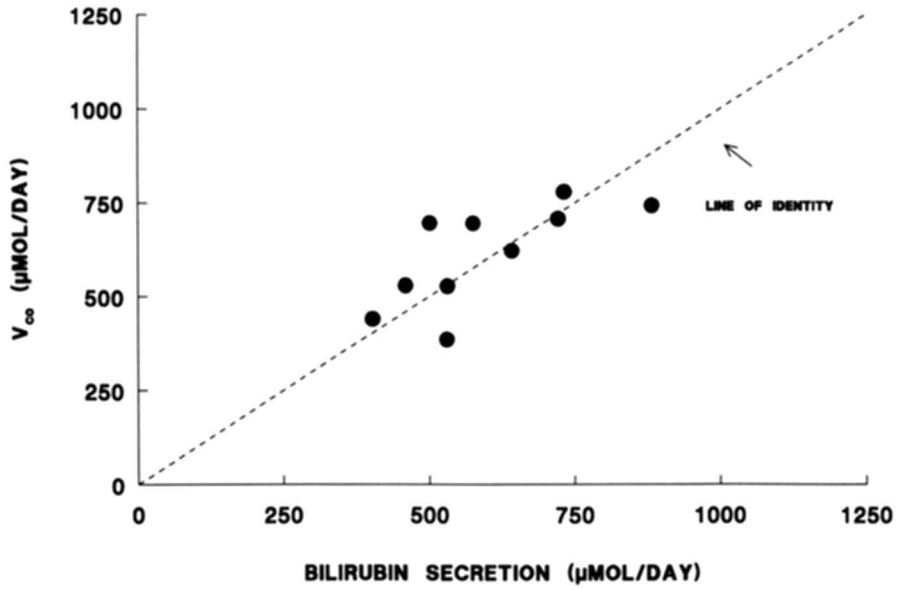

Fig. 1. Relationship between V_{CO} measured by breath sampling to the rate of bilirubin secretion into bile measured by standard marker perfusion in ten subjects. While the correspondence was not perfect, V_{CO} appeared to provide a reasonable estimate of bilirubin secretion rate.

secretion of disofenin measured by marker dilution was 97.8 ± 13.1% of the infusion rate (minus urinary secretion) while average secretion measured by the CO method was 104.2 ± 7.2% of the infusion rate. Thus, both methods provided an accurate measurement of disofenin secretion, but there was more variation in measurements done by marker perfusion.

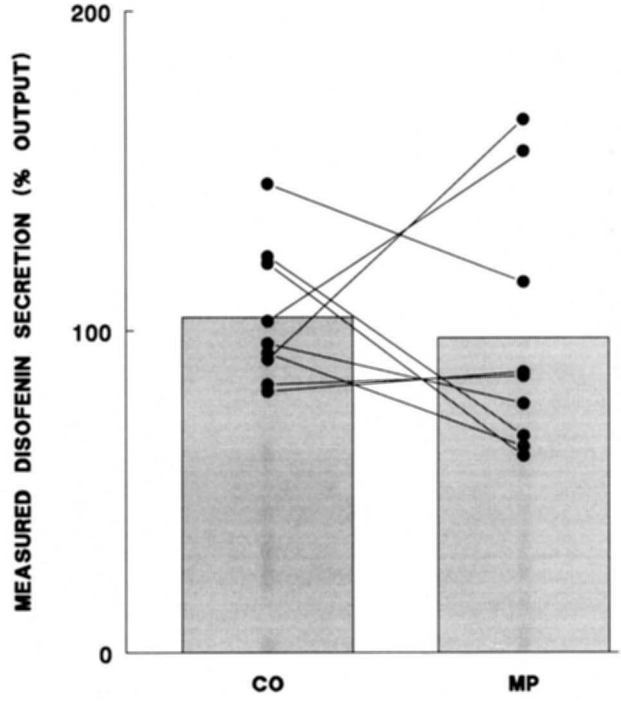

Fig. 2. Secretion rate of ^{99m}Technetium-labeled disofenin during steady-state constant intravenous infusion measured by the CO method (CO) and on a separate occasion by marker perfusion (MP). Secretion is expressed as percentage of actual output into bile, calculated as intravenous infusion rate minus urinary excretion rate.

Table 1 shows secretion rates of biliary lipids measured in ten subjects by both methods. Average cholesterol secretion was about 10% lower by the CO method compared to marker perfusion, but the difference was not statistically significant. Average bile acid secretion was about 17% lower and average lecithin secretion was about 29% lower by the CO method compared to marker perfusion. Both differences were statistically significant.

To assess the ability of the CO method to measure an expected change, cholesterol secretion was measured in six subjects during a control period and again after taking lovastatin, 40 mg b.i.d. for 4 weeks. As shown in **Fig. 3,** the method demonstrated an approximate 25% reduction in cholesterol secretion on lovastatin, about what would be expected from previous work using marker perfusion (7).

DISCUSSION

The idea of using bilirubin as a marker for measurement of biliary secretion of other compounds is not new. Mok, von Bergmann, and Grundy (9) measured output of bilirubin into bile by marker perfusion methods and showed that this output was constant over 24 h. They then used the bilirubin secretion rate and measurements of lipid and bilirubin concentration in fasting bile to calculate biliary lipid secretion during fasting. This approach is identical in principle to that used in the present study, but it still requires prolonged intubation and infusion of liquid formula to measure bilirubin secretion rate.

TABLE 1. Biliary lipid secretion measured by marker perfusion (MP) and carbon monoxide (CO) methods

	Cholesterol		Bile Acid		Lecithin	
	MP	CO	MP	CO	MP	CO
			μmol/h			
	111	118	1249	1180	524	351
	102	115	882	1386	254	313
	105	89	1189	910	419	278
	86	93	1477	1375	350	358
	175	150	1603	1134	642	339
	135	134	1792	1251	421	339
	116	73	1662	863	461	252
	83	81	930	885	282	214
	123	91	1320	1097	505	271
	98	87	1218	1025	274	237
Mean	113	103	1332	1110	413	295
Mean Δ	10.3		222		118	
Paired *t*	1.76		2.01		3.27	
P value	0.112		0.076		0.0097	

To circumvent such arduous perfusion studies we instead used output of CO on the breath as an estimate of bilirubin secretion. It has been known for many years that CO is released stoichiometrically when heme is converted to bilirubin and that V_{CO} is essentially equivalent to bilirubin production (10, 11). Moreover, because there is virtually no enterohepatic circulation of bilirubin (12), V_{CO} should provide a good estimate of bilirubin secretion into bile. Our studies presented in Fig. 1, while not showing perfect correspondence between bilirubin secretion and V_{CO}, confirm this premise and validate the use of V_{CO} to estimate bilirubin secretion into bile.

Once bilirubin secretion rate is known, biliary secretion rate of any compound can be calculated if the biliary concentration ratio of that compound/bilirubin is also known. We chose morning gallbladder bile on which to measure this ratio because gallbladder bile is a mixture of bile secreted in both postprandial and fasting states. We hypothesized that concentration ratios in this bile might provide estimates of secretion approximating 24-h secretion rates. Moreover, gallbladder bile can be obtained relatively quickly and easily with minimal discomfort and inconvenience. We assessed the accuracy of this approach by using the CO method to measure biliary secretion of disofenin. This isotopic compound is excreted almost exclusively in bile (there is a minor component of urinary excretion, for which we corrected) and has virtually no enterohepatic circulation (see Results). We could therefore reasonably assume that after a prolonged constant intravenous infusion, biliary output rate of disofenin was equivalent to its infusion rate (minus urinary output). As shown in Fig. 2, the CO method yielded an average disofenin secretion rate nearly identical to the corrected infusion rate with acceptable variability of individual measurements. The standard marker perfusion method also yielded an average disofenin secretion nearly identical to the corrected infusion rate, but with more variability in individual measurements. These data show that the CO method provides an accurate measure of biliary secretion with acceptable random variation.

We also compared biliary lipid secretion rates measured by the CO method to those measured by marker perfusion. As shown in Table 1, secretion of cholesterol measured by the two methods was similar. Moreover, the data in Fig. 3 show that the CO method can faithfully reflect changes in cholesterol secretion, because the 25% reduction induced by lovastatin is commensurate with previous work using marker perfusion methodology (7). However, secretion of bile acid and lecithin by the CO method was 20–30% lower than that reported by the marker perfusion technique (Table 1). Because the marker perfusion method is believed to provide secretion rates quite close to average daily secretion (8), this finding suggests that morning gallbladder bile used in our determinations contains a disproportionate amount of fasting versus postprandial bile. Such a disproportionate representation would be expected to affect bile acids and phospholipid, whose secretion rates change considerably with fasting, more than cholesterol, whose secretion rate changes relatively less with fasting (8). Indeed reported differences in lipid composition for stimulated hepatic bile versus gallbladder bile are consistent with this suggestion

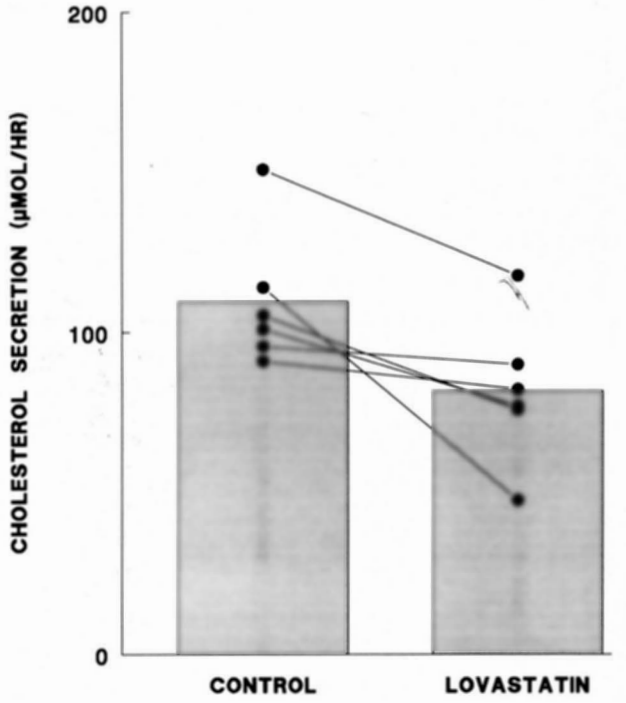

Fig. 3. Biliary secretion of cholesterol measured by the CO method during a control period and following 4 weeks of treatment of lovastatin 40 mg b.i.d. On lovastatin, measured cholesterol secretion was about 25% lower than control ($P < 0.05$).

(4). We conclude that if one aims to estimate actual 24-h secretion of biliary lipid, that can be done using the CO method for cholesterol with minimal inaccuracy. For bile acid and lecithin, however, the CO method can be used only if a 20–30% underestimation is acceptable, which may not be the case in many situations.

In addition to lipids many other substances, both endogenous and exogenous, are secreted into bile. Many of these, such as apoproteins, immunoglobulins, calcium, iron, copper, and a variety of drugs, are relevant to normal or abnormal physiology. The CO method should provide accurate output measurements for any such compound providing its secretion is relatively constant. The method would lend itself particularly well to studies requiring multiple within-subject comparisons because volunteers would more readily accept repeated measurements by this relatively easy, quick, and comfortable technique compared to long marker perfusion measurements.

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