Unconjugated and Conjugated Bilirubin Transport in Normal and Mutant Corriedale Sheep with Dubin-Johnson Syndrome[1] (34981)

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The Dubin-Johnson (Sprinz-Nelson) syndrome in man is a chronic, nonhemolytic, familial jaundice in which conjugated bilirubin (CB) is elevated in the plasma (1, 2) and a melanin pigment accumulates in the pericanalicular dense bodies of the hepatic cells (3). A nearly identical syndrome has recently been discovered in mutant Corriedale sheep (4). The disease is characterized by an increase in both unconjugated bilirubin (UCB) and CB in the plasma and by hepatic pigmentation similar to that observed in human Dubin-Johnson (DJ) syndrome. Preliminary studies on both human and ovine mutants have indicated that the excretion or hepatic transport maxima (T_m) of organic anions, such as sulfobromophthalein sodium (BSP), can be defective while relative hepatic storage (S) and conjugation of BSP remain normal (4). Arias *et al.* (5) recently reported on a defect in the biliary excretion of tritium-labeled metabolites of 7-^{3}H-epinephrine in mutant Corriedale sheep; they also observed that 7-^{3}H-epinephrine or its metabolite was incorporated in the hepatic pigment. Studies utilizing this ovine mutant have revealed that different hepatic transport mechanisms exist for the excretion of anions such as taurocholic acid and BSP (6).

In this study, ^{14}C-UCB and ^{14}C-CB were used as tracers to estimate the intercompartmental transfer rates and pool sizes of UCB and CB in both normal and mutant Corriedale sheep with the Dubin-Johnson syndrome.

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Materials and Methods. ^{14}C-UCB was prepared and injected intravenously according to methods previously described (7), into four mutant and three normal mature Corriedale sheep. To obtain ^{14}C-CB, an aqueous solution of recrystallized ^{14}C-UCB was intravenously infused into anesthetized rats with cannulated bile ducts. More than 90% of the radioactivity in the rat bile samples containing ^{14}C-CB was in the polar CB fraction as determined by Weber and Schalm separation technique (8). The specific activity of purified bilirubin subsequently crystallized from rat bile was 90% of the specific activity calculated from the total bile radioactivity and the concentration of bilirubin in bile. Rat bile containing ^{14}C-CB was collected in 2 ml of sheep plasma at 0° avoiding exposure to light and immediately injected intravenously into three normal and three mutant sheep through polyethylene tubing inserted into the left jugular vein.

The UCB fraction separated by the Weber and Schalm technique (8) contained 8–10% of the total plasma radioactivity after the injection into sheep of rat bile containing ^{14}C-CB. This suggested that little deconjugation of ^{14}C-CB, if any, had occurred. Much of the radioactivity in the plasma UCB fraction could have resulted from innate error in the separation technique and would not represent deconjugation. These findings ensure validity in the use of bile from rats infused with ^{14}C-UCB as a radioactive tracer for CB in sheep.

Three to five milliliters of heparinized blood were withdrawn from the right jugular vein at timed intervals during the first 7 hr after injecting either ^{14}C-UCB or ^{14}C-CB. Plasma bile pigments were separated into

TABLE I. Plasma Disappearance of Unconjugated Bilirubin-^{14}C and Conjugated Bilirubin-^{14}C Intravenously Injected into Normal and Mutant Corriedale Sheep.[a]

Animal no.	Types of bilirubin	Radioactivity injected (dpm $\times 10^3$/kg)	Extrapolated radioactivity at zero time after injection (dpm $\times 10^3/\mu$g of UCB or CB)		Half-times (min)	
			1st component	2nd component	1st component	2nd component
Normal						
1	UCB-^{14}C	446	6.38	0.370	5.95	119
2	UCB-^{14}C	404	7.94	0.297	5.33	110
3	UCB-^{14}C	1390	27.68	2.113	7.88	84
4	CB-^{14}C	232	17.94	0.440	2.48	112
5	CB-^{14}C	229	25.61	0.363	4.76	92
6	CB-^{14}C	256	21.04	0.474	3.85	185
Mutant						
7	UCB-^{14}C	222	0.776	0.270	18.0	212
8	UCB-^{14}C	370	0.835	0.252	23.8	385
9	UCB-^{14}C	220	0.373	0.285	21.0	196
10	UCB-^{14}C	267	0.497	0.353	21.0	307
10	CB-^{14}C	119	0.237	0.151	14.0	338
11	CB-^{14}C	167	0.286	0.167	21.0	352
12	CB-^{14}C	23.3	0.0198	0.0198	20.0	430

[a] UCB = unconjugated bilirubin; CB = conjugated bilirubin; dpm = disintegrations per minute.

UCB and CB fractions by a modified method of Weber and Schalm, and the UCB fraction was counted for radioactivity as previously described (7).

The CB fraction was prepared for counting by adding 3 ml of solubilizer[3] and 10 ml of counting solution (0.4% PPO and 0.01% POPOP in toluene) to 1 ml of this polar fraction. Concentrations of plasma bilirubin were determined by the method of Malloy and Evelyn (9). The specific activities of UCB and of CB were determined from their plasma concentrations and radioactivity corrected as previously described (7). Intercompartmental transfer rates and pool sizes of UCB and CB were calculated mathematically by analyzing the plasma ^{14}C-UCB and ^{14}C-CB disappearance curves. Calculations were based on a two-compartment model with a mixing pool (MP) and a storage pool (SP). Mathematical equations have been discussed previously (7). The parameters of the best-fit, two-component curve for the disappearance of plasma radioactivity were determined with the aid of a digital computer, the criterion of acceptance being the least-squared fractional deviation between calculated and observed radioactivities.

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Results and Discussion. Data on the disappearance of ^{14}C-UCB and ^{14}C-CB from the plasma of normal and mutant sheep are presented in Table I. Plasma UCB and CB radioactivity disappeared as the sum of two exponential processes in all cases. No additional exponential component was apparent. A model for the transport of both UCB and CB, along with supporting equations for calculating SP of CB, is presented in Fig. 1. According to this model, UCB and CB in the MP (primarily plasma) and the SP (primarily hepatic cell mass) are in constant equilibrium and are subsequently removed irreversibly from the SP. UCB and CB are irreversibly removed by conjugation with glucuronic acid and by excretion into the bile, respectively. Under steady-state conditions in each animal, the quantity of UCB irreversi-

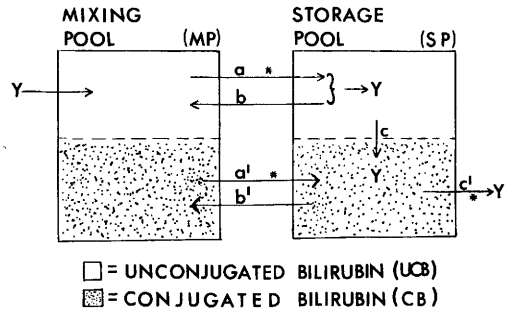

FIG. 1. Diagrammatic presentation of bilirubin transport. a or a′, b or b′, or c or c′ are the fractional transfer rates of UCB or CB from the MP to the SP, the SP to the MP, and the SP to irreversible removal (IR), respectively. Y is the amount of UCB produced per unit-time. An asterisk (*) indicates marked differences in the mutant Dubin–Johnson sheep from normal. At steady state, CB entering the MP must equal its removal. Thus b′ × SP of CB = a′ × MP of CB; therefore, SP of CB = a′ × MP of CB/b′.

bly removed per minute is equal to its production rate or to the rate that CB is excreted. The total quantity of endogenous CB excreted into the bile should equal the irreversible removal (IR) of UCB or CB. In one Dubin-Johnson sheep with a biliary T tube *in situ*, the endogenous bilirubin excretion in bile as measured directly was 92% of the calculated IR of UCB determined by the ^{14}C-UCB technique used in this study.

Fractional transfer rates for the movement of UCB and CB from the MP to the SP in each case are an index of transport efficiency through the hepatic plasma membrane into the cell (10). Fractional transfer rates of UCB and CB from the SP to the MP are correlated inversely to the efficiency in preventing their regurgitation from hepatic cells into plasma. Under steady-state conditions, the amount of UCB transferred from the MP to the SP equals the production rate plus the amount regurgitated from the SP to the MP. Because CB is formed in the SP and not in the MP, its transfer from the MP to the SP under steady-state conditions equals the amount of CB transported from the SP to the MP. The fractional transfer rates for the IR of UCB and CB depend upon the respective efficiencies of glucuronide conjugation of UCB and of hepatic excretion of CB into the bile. A change in any of the three transfer processes will result in an appropriate change in the relative size of the two pools, which, in turn, could affect the remaining transfer processes.

Intercompartmental transfer rates and pool sizes of UCB and CB in normal and mutant sheep are presented in Table II. The MP values of both UCB and CB are increased greatly in mutant sheep as compared with normal sheep, whereas their SP values are not significantly increased. Fractional transfers of UCB and CB from the MP to the SP in mutant sheep were 22% and 12% of the corresponding transfers in normal sheep, respectively. Such a decrease in the fractional transfers of UCB and CB from the MP to the SP in mutant sheep suggested a possible defect in membrane transport of UCB and CB from the plasma into the hepatic cells. The fractional transfer of UCB and CB in the opposite direction (SP to MP) in the mutant sheep was two and three times the corresponding values in the normal sheep. Such an increase in fractional transfers of UCB and CB from the SP to the MP could indicate that hepatic cells in the mutants were less efficient in preventing UCB and CB from re-entering the plasma. Increased regurgitation of UCB and CB could result from a decreased fractional IR of UCB and CB in the mutant sheep. By experimentally increasing the UCB in the SP in normal sheep by bilirubin infusion, an increased fractional transfer of UCB from the SP to the MP (7) resulted, but no increase in the fractional transfer of UCB from the MP to the SP was apparent.

The relative decrease in fractional transfer of UCB from the MP to the SP so enlarged the MP of UCB that the amount of UCB transferred from the MP to the SP equalled its production plus the amount regurgitated from the SP to the MP. Similarly, the MP of CB would have to be enlarged to make the transfer of CB from the MP to the SP equal to its regurgitation from the SP to the MP.

Fractional transfer for IR of CB in the Corriedale mutants was considerably decreased, which suggested defective excretion.

TABLE II. Intercompartmental Pool Sizes and Transfer Rates of Bilirubin in Normal and Dubin-Johnson Sheep.

	Unit	Normal (mean ± SE)	DJ (mean ± SE)
Plasma bilirubin			
UCB	μg/ml	0.8 ± 0.05	4.7 ± 0.08**
CB	μg/ml	0.1 ± 0.02	7.7 ± 0.4 **
Mixing pool (MP)			
UCB	μg/kg	54.7 ± 7.2	300 ±127 **
CB	μg/kg	11.1 ± 1.2	422 ± 86 **
Storage pool (SP)			
UCB	μg/kg	495 ±113	574 ±127
CB	μg/kg	572 ± 39	673 ± 65
Fractional transfer from:			
MP to SP			
UCB	% of MP/min	10.6 ± 1.3	2.3 ± 0.2 **
CB	% of MP/min	19.8 ± 0.4	2.4 ± 0.4 **
SP to MP			
UCB	% of SP/min	0.51 ± 0.04	0.9 ± 0.1 *
CB	% of SP/min	0.41 ± 0.13	1.4 ± 0.2 *
SP to IR			
UCB	% of SP/min	0.72 ± 0.08	0.4 ± 0.07*
CB	% of SP/min	0.57 ± 0.09	0.31 ± 0.13‡
IR of			
UCB	μg/min/kg	3.34 ± 0.48	2.07 ± 0.2
CB	μg/min/kg	3.48 ± 0.9	2.07 ± 0.14
Excretion efficiency[a]	%/min	0.307± 0.03	0.105± 0.01**

[a] Total excretion of CB/min/total pool of UCB and CB.
* $p < .05$
** $p < .001$
‡ $p < .06$

Excretion of CB in relation to the total pool of bilirubin (MP + SP of UCB and CB) in the mutants also decreased significantly, indicating an overall deficiency in bilirubin excretion. The lower fractional transfer rate for IR of UCB in the mutant sheep suggested a lower conjugating efficiency.

Billing *et al.*, (10) in a bilirubin-loading study in Dubin-Johnson and Rotor syndromes in man presented evidence of a defect in transport of UCB from plasma to liver and in conjugation of UCB in patients with high plasma UCB levels (2.5 mg/100 ml). Their evidence supports our observation in Corriedale mutants with this Dubin-Johnson-like syndrome.

A recent study of ^{14}C-UCB turnover in mutant Southdown sheep with congenital hyperbilirubinemia indicated that the condition resembles Gilbert's syndrome in man (7); turnover of ^{14}C-CB also has been studied in one mutant Southdown sheep by the method utilized here. These studies indicated that the mild hyperbilirubinemia (5–10 μg/ml total bilirubin of which 40% is CB) in the mutant Southdown sheep was due primarily to increased regurgitation of UCB and CB from the SP to the MP with normal conjugation and excretion of CB. In contrast, the marked hyperbilirubinemia (10–16 μg/ml with 70% CB) in the mutant Corriedale sheep with Dubin–Johnson syndrome was due to decreased transport of UCB and CB from the MP to the SP, as well as to defective excretion of CB.

Summary. Bilirubin transport was studied

in normal and mutant Corriedale sheep with Dubin-Johnson syndrome using ^{14}C-labeled unconjugated and conjugated bilirubin. Intercompartmental transfer rates and pool sizes of unconjugated bilirubin (UCB) and of conjugated bilirubin (CB) were calculated by observing the rates of disappearance of injected ^{14}C-UCB and ^{14}C-CB from the plasma. The transport efficiencies of UCB and CB from a mixing pool (primarily plasma) to a storage pool (primarily hepatic cells) in the mutants were 22% and 12%, respectively, that of normal sheep. Bilirubin excretion efficiency in mutants was approximately 33% that of normal sheep.

Increased UCB and CB plasma concentrations in the mutant Corriedale sheep apparently result from a decreased transport efficiency of both UCB and CB from plasma to liver as well as the defective excretion of CB by the liver into bile.

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