Renal Function Defect in Mutant Southdown Sheep with Congenital Hyperbilirubinemia[1] (35763)

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Southdown (SD) sheep with congenital hyperbilirubinemia and photosensitivity were first described in New Zealand in 1942 (1). Subsequently, the syndrome was determined to be inherited as a single autosomal recessive factor (2). Photosensitivity has been attributed to retention of phylloerythrin (a product of chlorophyll metabolism in the rumen), normally removed from the portal blood by the liver and excreted into the bile (3). Cornelius and Gronwall (4) reported a marked delay in plasma clearance of injected bilirubin, sulfobromophthalein (BSP), rose bengal, sodium cholate, and indocyanine green in mutant SD sheep with no apparent defect in conjugation of bilirubin and BSP. Hyperbilirubinemia in mutant SD sheep has recently been attributed to a decreased net hepatic uptake of unconjugated bilirubin (5).

Renal disorders of varying degrees in man and other animals (6–8) have been associated with various types of jaundice. Although water intolerance was noted in early descriptions of the Southdown mutant syndrome (9), renal function studies in those sheep were not reported.

In this present study, renal clearances of inulin (C_{In}), *para*-aminohippuric acid (C_{PAH}), and endogenous urea (C_{urea}) and some of the physicochemical properties of urine were determined in normal and mutant SD sheep.

Materials and Methods. Renal clearances were studied in 3 adult SD mutant rams, 4 normal adult SD rams and 3 adult Corriedale sheep with an inherited hyperbilirubinemia resembling Dubin-Johnson syndrome in man (10). The sheep received alfalfa hay and water *ad libitum* and were maintained under the same environmental conditions for at least 3 weeks prior to and during the trials. In addition, renal function was studied in one 8-week-old nursing SD mutant lamb and in her normal male twin.

Renal clearances of inulin, *para*-aminohippuric acid (PAH), and endogenous urea were determined simultaneously by standard techniques (11). One hr before inulin and PAH were injected, 30 to 40 ml of water/kg body weight were administered via stomach to each sheep to induce diuresis. Priming doses of 15 to 20 mg of inulin and 0.8 to 1 mg of PAH/kg of body weight were injected intravenously; then 0.8 to 1.0 mg of inulin and 0.2 to 0.3 mg of PAH/min/kg were infused continuously. One hr after infusion was started, six to eight consecutive urine samples of 15- to 30-min duration were collected. Plasma samples were taken for each urine collection. Inulin, PAH, and urea in urine and plasma samples were assayed according to the methods of Galli (12), Harvey and Brothers (13), and Ormsby (14), respectively. Clearance values were calculated as the average of the sample clearances, excluding those more than 15% from the mean (which amounted to no more than 2 samples/animal).

"Twenty-four hr" urine samples were collected for 3 consecutive days according to the procedure described previously (15). Osmolarity and specific gravity of urine were determined using a freezing point osmometer

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and urinometer. Na^+ and K^+ concentrations were determined by flame photometry.

Results. Renal clearances of inulin, PAH, and endogenous urea in normal and mutant sheep are presented in Table I. The C_{In} and C_{PAH} of the normal rams were similar to values reported for normal sheep (16, 17). The C_{In} and C_{PAH} were significantly lower for the SD mutants than for normal sheep. Clearance of endogenous urea was also significantly lower in SD mutants than in normal sheep while urine flow rates during clearance studies were similar in both groups. Renal clearances of inulin, PAH, and endogenous urea were much lower in the 8-week-old mutant lamb than in her normal twin.

Physicochemical properties of 24-hr urine samples from normal and mutant sheep are presented in Table II. Specific gravity and osmolarity of urine were significantly lower from SD mutants than from normal sheep and were accompanied by a nearly proportionate increase in urine volume. Urinary sodium excretion was significantly higher in SD mutants than in normal sheep.

Renal clearances of inulin and PAH in the adult Corriedale mutants with hyperbilirubinemia were not statistically different from those of normal sheep, but the physicochemical properties of their urine more closely resembled those of the SD mutants.

Within 1 hr after water was administered (per os), a marked diuresis began in both normal and Corriedale mutant sheep; in the SD mutants even by 2 hr after water was administered, urine flow increased little, if at all.

Discussion. Glomerular filtration (C_{In}), effective renal plasma flow (C_{PAH}), and the clearance of endogenous urea were reduced significantly in the SD mutant sheep. The reduced glomerular filtration was not solely the result of reduced renal plasma flow as indicated by the significantly lower filtration fraction (C_{In}/C_{PAH}) in these animals (see Table I). The observed decrease in kidney function was consistent with the chronic nephritis characterized by interstitial fibrosis and tubular dilatation, as revealed by histological examination of renal tissue from the SD mutant rams and the 8-week-old mutant lamb (18). Histological and functional changes in the 8-week-old mutant SD lamb indicated that the kidney lesions developed early. Alterations in renal morphology associated with reduced glomerular filtration and renal plasma flow in the SD mutants might not be related to the hepatic dysfunction. Three mutant Corriedale sheep with hepatic anion transport defects, which also results in hyperbilirubinemia and photosensitivity, did not exhibit similar reductions of glomerular filtration and renal plasma flow.

The larger urine volume and sodium excretion of mutant SD sheep suggested impaired sodium resorption despite reduced glomerular filtration. The pattern of increased daily urine volume, increased sodium excretion, and reduced urinary osmolarity and specific gravity was observed in both SD and Corriedale mutants and might reflect some influence of the hepatic dysfunction on kidney function in both mutant strains. Polyuria and decreased glomerular filtration have been reported in Gunn rats (8) with hyperbilirubinemia resulting from a deficiency in bilirubin glucuronyl transferase. Excretion of dilute urine associated with normal renal function has been reported in sheep with experimental hyperbilirubinemia following surgical ligation of the common bile duct (19). Intravenous infusion of homologous bile has been found to increase urine flow and Na^+ excretion in dogs (20).

The mutant SD sheep maximum capacity to concentrate urine was not determined because of the risk involved in subjecting the limited number of mutants to water deprivation. Failure to induce diuresis within 2 hr following water loading in SD mutants confirmed the earlier observations of water intolerance (9).

Urea reabsorption $[1 - (C_{urea}/C_{In})]$ at similar urine flow rates was significantly higher in the adult SD mutants than in the normal SD rams (Table I). Ruminants maintained on rations low in protein conserve urea (21). The extensive renal lesion in the adult SD mutants might allow for significant loss of amino acids via the kidneys, thus, indirectly creating a condition functionally similar to

TABLE I. Renal Clearance of Inulin, PAH and Endogenous Urea in Normal and Mutant Sheep.[a]

	No. of sheep	Urine flow during clearance study (ml/min/kg; $x \pm$ SE)	Renal clearance (ml/min/kg; $x \pm$ SE)			Filtration fraction (%; $x \pm$ SE)	Fractional urea[b] reabsorption (%; $x \pm$ SE)
			PAH	Inulin	Urea		
Normal							
Adult	4	0.12 ± 0.02	14.73 ± 0.89	2.30 ± 0.12	1.35 ± 0.12	15.65 ± 0.30	41.60 ± 3.90
Lamb[d]	1	0.11	12.20	3.50	2.70	28.70	23.00
Mutant (Southdown)							
Adult	3	$0.13 \pm .05$	$6.07^{c} \pm 1.12$	$0.73^{c} \pm 0.95$	$0.23^{c} \pm 0.01$	$12.27^{c} \pm 0.60$	$68.54^{c} \pm 3.75$
Lamb[d]	1	0.09	6.51	1.02	0.85	15.42	15.00
Mutant (Corriedale)							
Adult	3	$0.12 \pm .04$	12.3 ± 0.9	$1.98 \pm .08$	N.D.	16.30 ± 1.3	N.D.

[a] $x \pm$ SE = Mean $\pm$ standard error of means; ND. = not done.
[b] $1 - (C_{urea}/C_{PAH})$.
[c] $p < .001$ compared to normal adults.
[d] Twin lambs.

TABLE II. Physicochemical Properties of Urine in Normal and Mutant Sheep.[a]

Normal	No. of sheep	Vol (ml/day/kg; $x \pm$ SE)	Sp gr ($x \pm$ SE)	Osmolarity (mOsm/liter; $x \pm$ SE)	pH ($x \pm$ SE)	Na^+ (mEq/day/kg; $x \pm$ SE)	K^+ (mEq/day/kg; $x \pm$ SE)
Adult	3	17.8 ± 3.9	1.044 ± 0.004	1189 ± 137	8.7 ± 0.18	0.49 ± 0.25	8.74 ± 2.46
Lamb[e]	1	44.4	1.036	1276	8.0	0.60	21.31
Mutant (Southdown)							
Adult	2	78.2 ± 11.8	1.016 ± 0.009[b]	410 ± 70[c]	8.6 ± 0.10	3.05 ± 0.27[c]	12.23 ± 0.80
Lamb[e]	1	101.2	1.016	581	8.1	2.19	30.30
Mutant (Corriedale)							
Adult	3	55.2 ± 11.1[b]	1.027 ± 0.001[b]	767 ± 53[bd]	8.2 ± 0.30	2.30 ± 0.61[b]	17.42 ± 3.40[b]

[a] $x \pm$ SE = mean ± standard error of means.
[b] Compared to normal adults: $p < 0.5$; [c] $p < 0.01$.
[d] $p < .05$, compared with adult Southdown mutants.
[e] Twin lambs.

low protein intake and enhancing urea retention. Both normal and mutant lambs receiving protein-rich milk had minimal urea reabsorption.

Summary. Mutant Southdown (SD) sheep with inherited hyperbilirubinemia due to a hepatic anion transport deficiency had significantly reduced glomerular filtration (clearance of inulin) and effective renal plasma flow (clearance of p-aminohippuric acid) as compared to normal SD sheep. Mutant SD sheep excreted five times more sodium per day than normal SD sheep on the same free choice diet. The daily urine volume of the mutants was four times as great as that of the normals.

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