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Research Article

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The Urinary Concentrating Defect in the Gunn Strain of Rat

ROLE OF BILIRUBIN

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Abstract The role of high serum and tissue levels of unconjugated bilirubin in the pathogenesis of the impaired urinary concentrating ability was investigated in homozygous (*jj*) Gunn rats with the congenital absence of hepatic glucuronyl transferase. Continuous phototherapy with blue fluorescent lights at a wave length of 460 nm or oral cholestyramine feeding or both reduced serum levels of unconjugated bilirubin to levels consistently below 3.0 mg/100 ml for several weeks in both weanling and adult *jj* Gunn rats. The renal concentrating defect was already present in weanling *jj* Gunn rats by 21 days of age. In treated weanling *jj* animals, maximum concentrating ability and the concentration of urea and nonurea solutes in the papilla and medulla, determined after 24 h of fluid deprivation, were normal when compared to unaffected heterozygous (*Jj*) littermates. Solute-free water reabsorption which is reduced in jaundiced *jj* Gunn rats was restored to normal in treated weanling *jj* rats. The tissue concentration of unconjugated bilirubin was reduced throughout the papilla and inner and outer medulla in the treated *jj* rats in comparison with untreated *jj* littermates. The defect in urinary concentrating ability was only partially reversible and sometimes irreversible in adult *jj* rats, probably because of permanent renal parenchymal damage occurring secondary to massive crystalline deposits in the papilla and medulla. It is concluded that unconjugated bilirubin is directly involved in the pathogenesis of the concentrating defect in jaundiced *jj* Gunn rats.

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INTRODUCTION

The chronically-jaundiced homozygous Gunn rat (*jj*), a mutant of the normal Wistar strain, has a persistent unconjugated hyperbilirubinemia secondary to the congenital absence of the hepatic enzyme glucuronyl transferase (1–4). Urine concentration at both high and low rates of osmolar clearance has been found to be impaired in these animals (5, 6). A causal relationship between the hyperbilirubinemia and the renal defect has been postulated and several observations indirectly support such a relationship. In those animals with impaired urine concentrating ability, tissue concentrations of bilirubin are markedly elevated throughout the kidney, especially in the papilla (4–7). In the adult *jj* animals, massive crystalline deposits of bilirubin are found in the renal papilla and are often associated with papillary necrosis (8). Concentrations of urea and nonurea solutes are greatly reduced in the medulla of adult *jj* rats when compared to normal controls (5). Both free water clearance (C_{H_2O})[1] and solute-free water reabsorption ($T^c_{H_2O}$) are reduced in adult *jj* animals when compared to normal controls (5, 6). This indirect evidence has been used to support the hypothesis that unconjugated bilirubin damages the transport capacity of the ascending limb of Henle, thereby altering the countercurrent system, thus leading to the observed defect in urine concentration (5, 6).

[1] *Abbreviations used in this paper:* C_{H_2O}, free water clearance; C_{osm}, osmolar clearance; $T^c_{H_2O}$, solute-free water reabsorption; U_{max}, maximum urine concentrating ability.

The purpose of the present study was to critically examine the role of unconjugated bilirubin in the pathogenesis of the urine concentrating defect in the homozygous *jj* Gunn rat. Serum levels of unconjugated bilirubin were lowered to near normal levels in both weanling and adult animals for prolonged periods of time through the combined use of phototherapy (9–11) and oral cholestyramine administration (12). In the treated *jj* rats, maximum urine concentrating ability and the concentration of urea and nonurea solutes in the medulla, determined after 24 h of fluid deprivation, were normal. $T^c_{H_2O}$ was restored to normal and the concentration of bilirubin was markedly reduced throughout the renal medulla. Thus, according to all measured parameters, the defect in urine concentrating ability was completely reversed with reduction in serum and tissue levels of bilirubin, directly implicating unconjugated bilirubin as the nephrotoxic agent in this experimental animal model.

METHODS

Offspring of *jj* male and heterozygous (*Jj*) female Gunn rats obtained from our own breeding colony were employed in all experiments. The animals ranged in age from 21 days to 8 mo. Except where otherwise indicated, the animals were allowed free access to standard rat chow (Ralston Purina Co., St. Louis, Mo.) and tap water. Animals treated with phototherapy were shaved and placed in special cages equipped with overhead blue fluorescent lights (F20T12/BB, Westinghouse Electric Corp., Fairmont, W. Va.) with an illumination of 1,000 $\mu W/cm^2$ at a wavelength of 460 nm. The animals were illuminated continuously day and night throughout the period of experimentation. Oral cholestyramine (Questran, Meade Johnson Laboratories, Evansville, Ind.) was fed to certain animals to lower the serum bilirubin concentration. The animals received standard rat chow ground to a fine powder to which cholestyramine was added in a concentration of 5% by weight. Other animals received both modalities of therapy simultaneously. Base-line measurements of serum bilirubin[2] and maximum urine concentrating ability (U_{max}), the latter determined after 24 h of fluid deprivation, were obtained in all animals upon their entry into the study. In most animals, repeat determinations of serum bilirubin and U_{max} were obtained weekly throughout the duration of the study, unless otherwise indicated.

Effect of phototherapy on urine concentrating ability in weanling jj Gunn rats. The ability of phototherapy to reverse the unconjugated hyperbilirubinemia and prevent the defect in urine concentration was studied in male and female weanling *jj* Gunn rats. All of the offspring from five pairings of *jj* Gunn males and *Jj* Gunn females were weaned at 21 days and base-line U_{max} and serum bilirubin concentrations were determined. The *jj* animals from each litter were randomly divided into two groups; one group ($n = 9$) was subjected to continuous phototherapy while the second group ($n = 14$) served as untreated *jj* control animals. Ten *Jj* littermates which have normal serum bilirubin concentrations and do not exhibit hyposthenuria were maintained as normal *Jj* control animals. Weekly measurements of serum bilirubin and U_{max} were obtained in the two groups of *jj* rats and U_{max} only in the *Jj* animals for a period of 20 wk.

Effect of cholestyramine on urine concentrating ability in weanling jj Gunn rats. The ability of oral cholestyramine to reverse the unconjugated hyperbilirubinemia and the urine concentrating defect was next evaluated in four weanling *jj* Gunn rats. Base-line determinations of serum bilirubin and U_{max} were obtained and the animals were then allowed free access to standard rat chow containing 5% cholestyramine by weight. Measurements of serum bilirubin and U_{max} were obtained weekly for a period of 20 wk.

Effect of phototherapy and cholestyramine on urine concentrating ability in weanling jj Gunn rats. The effectiveness and feasibility of combined therapy was studied in 23 weanling *jj* Gunn rats subjected to both phototherapy and oral cholestyramine feeding for a period of 20 wk. 22 *jj* Gunn rats served as untreated controls and 23 *Jj* rats as normal controls. The usual pretreatment and periodic measurements of serum bilirubin and U_{max} were determined in the three groups of animals.

Effect of phototherapy and cholestyramine on urine concentrating ability in adult jj Gunn rats. To determine the reversibility of the urine concentrating defect in adult *jj* Gunn rats, six chronically jaundiced *jj* female rats were chosen at random from the colony and subjected to 20 wk of continuous phototherapy and oral cholestyramine feeding. Weekly measurements of serum bilirubin concentration and U_{max} were obtained for comparison with similar pretreatment values. The animals were sacrificed at the conclusion of the period of observation by stunning and the kidneys were examined grossly.

Measurement of tissue solute and bilirubin concentration. To determine the completeness of the correction of the urine concentrating defect in the weanling *jj* Gunn rat, urea and nonurea solute concentrations were determined in the kidneys of 11 weanling *jj* Gunn rats treated with either phototherapy alone or both phototherapy and oral cholestyramine. Ten weanling *jj* Gunn littermates served as untreated controls and eight *Jj* littermates served as normal controls. Weekly measurements of serum bilirubin and U_{max} were obtained in the two groups of *jj* Gunn rats and U_{max} only in the *Jj* animals. After 24 h of fluid deprivation and the attainment of a urine sample, each animal was sacrificed by stunning. The left kidney was quickly excised and samples of tissue were immediately removed from the papilla, inner medulla, outer medulla, and cortex and placed in thin preweighed polyethylene dishes which were precooled to −80°C on dry ice. Sample size was determined by tare weight. The frozen tissue was then transferred into a small known volume of cold deionized distilled water and homogenized in preparation for measurement of sodium, potassium, urea, and NH_3 content. Small samples of tissue were also removed from the same regions of the kidney in some animals for the determination of tissue water content. The samples were dried at 110°F for 1 wk and weighed by tare to constant weight.

Tissue bilirubin content was determined on the contra-

[2] Initially, total and direct reacting serum bilirubin concentrations were determined in the *jj* rats. The difference between the two measurements represents the fraction of unconjugated bilirubin present in the serum. However, since direct reacting bilirubin represented less than 2% of the total serum bilirubin concentration, the total serum bilirubin concentration was used as a measure of unconjugated bilirubin in all subsequent experiments unless otherwise indicated.

lateral kidney of six untreated *jj* Gunn rats and seven treated *jj* Gunn rats previously utilized for tissue solute analysis. The kidney was bisected and small pieces of tissue were removed from the papilla, inner medulla, outer medulla, and cortex. Sample size was determined by tare weight. The samples were then homogenized for approximately 2 min in a solution containing 2 ml of chloroform and 1 ml of 0.01 N HCl. The sample was then centrifuged at 3,000 rpm for 10 min and the bilirubin content was measured in the supernatant layer.

Determination of $T^{c}_{H_2O}$ in jj and Jj rats. $T^{c}_{H_2O}$ was measured in four treated *jj* Gunn rats, five untreated *jj* Gunn rats, and three normal *Jj* littermates. Upon introduction into the study, all animals were 21 days old. The treated *jj* animals received continuous phototherapy and oral cholestyramine as described above. All *jj* rats were followed with weekly measurements of serum bilirubin concentration and U_{max}. U_{max} only was determined just before acute experiments in the *Jj* rats. All rats were deprived of food, but allowed free access to water during the night preceding the study. On the evening preceding the study, each animal received a subcutaneous injection of 1 U of vasopressin (Pitressin tannate in oil; Parke, Davis & Company, Detroit, Mich.). The rats were lightly anesthetized with sodium pentobarbital (32 mg/kg body wt), and polyethylene catheters were inserted into the femoral artery and vein. A polyethylene 160 catheter was inserted through a small suprapubic incision into the urinary bladder. Estimated surgical blood loss was replaced with isotonic saline (approximately 2 ml). The animals were then placed in a restraining cage and allowed to awaken. Mean arterial pressure was monitored throughout the entire procedure with a Sanborn 267 BC pressure transducer connected to a Sanborn recorder (Hewlett-Packard Co., Waltham Div., Waltham, Mass.). After completion of surgery, each animal received an i.v. inulin prime sufficient to achieve a plasma concentration of approximately 50 mg/100 ml. During the initial hour of the experiment, each animal received two separate i.v. infusions: a 1.2% saline solution administered initially at a rate of 0.02 ml/min and a 0.85% saline infusion administered at a constant rate ranging between 0.0123 and 0.0185 ml/min depending on the weight of the animal. The latter infusion contained aqueous vasopressin (25 mU/ml) and inulin sufficient to maintain a blood level of approximately 50 mg/100 ml. After a 60-min period of equilibration, the 1.2% saline infusion was increased to 0.1 ml/min and three 20-min urine specimens and midpoint blood samples (200 μl/sample) were collected for determination of inulin clearance, urine flow, and urine osmolality. The infusion of 1.2% saline was gradually increased in a stepwise manner to levels as high as 2.3 ml/min so that the infusion rate always exceeded urine flow. Two or more urine and blood collections were obtained at each level of infusion.

Analytical methods. Inulin concentrations in plasma and urine were measured by the anthrone method of Führ, Kaczmarczk, and Krüttgen (13). The concentration of sodium and potassium in urine, blood, and tissue homogenates was determined by standard flame photometry. All osmolalities were determined by the method of freezing-point depression with an Advanced osmometer (Advanced Instruments, Inc., Needham Heights, Mass.). Urine volumes were determined gravimetrically. Serum concentrations of bilirubin were determined with an ultramicro analytical system (model 150, Beckman Instruments, Inc., Fullerton, Calif.) adapted from the method of Malloy and Evelyn (14). Bilirubin measurements in supernates of tissue homogenates were also performed with a modification of the method of Malloy and Evelyn (14). Urea and ammonia measurements were determined with a Uni-test kit (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.), which employs a modification of a method described by Chaney and Marbach (15).

Statistical methods. Where applicable, data have been analyzed by Student's *t* test. Data are expressed as mean ±standard error unless indicated otherwise and *P* values greater than 0.05 are considered to be nonsignificant.

RESULTS

Effect of phototherapy on U_{max} in weanling jj Gunn rats. The effects of phototherapy alone on reducing the concentration of serum bilirubin and increasing U_{max} in *jj* Gunn rats are depicted in Table I and Fig. 1. The average pretreatment serum bilirubin concentration was 11.2±0.8 mg/100 ml in treated *jj* rats ($n = 9$), 12.3±0.4 mg/100 ml in untreated *jj* rats ($n = 14$), and 0.46±0.02 mg/100 ml in the *jj* littermates ($n = 10$). The difference in serum bilirubin concentrations between *Jj* and *jj* animals was highly significant ($P < 0.001$). The mean maximum urine osmolality obtained after 24 h of fluid deprivation in the same three groups of animals was 1,467±108, 1,506±152, and 2,498±129 mosmol/kg H_2O, respectively. Again differences between *Jj* and *jj* rats were highly significant ($P < 0.001$). Thus, contrary to a previous report (7), the jaundiced weanling *jj* rats already had a defect in maximum urine concentrating ability at 21 days of age when compared to their normal heterozygous littermates. The average serum bilirubin concentration throughout the 20-wk period of observation fell to 2.1±0.06 mg/100 ml in the treated *jj* rats and remained elevated at 7.0±0.10 mg/100 ml in the untreated *jj* littermates ($P < 0.001$). In the treated *jj* animals the mean maximum urine osmolality over the 20-wk period of observation was 2,531 ±46 mosmol/kg H_2O, which compared quite favorably with a mean value of 2,765±52 mosmol/kg H_2O achieved by the *Jj* littermates. U_{max} in the untreated *jj* littermates fell slightly from the initial values of 1,506±152 to 1,322±32 mosmol/kg H_2O. Fig. 1 depicts the typical course of the treated and untreated *jj* rats during the 20 wk of observation. Within 1 wk after initiation of phototherapy, the elevated serum bilirubin concentration had fallen to less than 2.5 mg/100 ml, but the defect in U_{max} was only partially corrected. After 2 wk of continuous phototherapy, however, the treated *jj* rats could attain a U_{max} within the normal range. In contrast, U_{max} in the untreated *jj* rats continued to slowly decline over the 20-wk period. The average serum bilirubin concentration in the untreated *jj* rats declined early to approximately 7.0 mg/100 ml where it remained.

Effect of cholestyramine on urine concentrating abil-

TABLE I

*Response of Weanling jj Gunn Rats to Phototherapy, Cholestyramine, or Combined Therapy**

Therapy	No. of animals	Serum bilirubin concentration		Urine osmolality‡	
		Before therapy	During therapy	Before therapy	During therapy
		mg/100 ml		*mosmol/kg H_2O*	
Phototherapy					
jj treated	9	11.2 ±0.8	2.1 ±0.06 $P < 0.001$¶	1,467±108	2,531±46 $P < 0.001$¶
jj untreated	14	12.3 ±0.4 NS§	7.0 ±0.10 $P < 0.001$§	1,506±152 NS§	1,322±32 $P < 0.001$§
Jj control	10	0.46±0.02 $P < 0.001$§	0.63±0.04 $P < 0.001$§	2,498±129 $P < 0.001$§	2,765±52 $P < 0.01$§
Cholestyramine					
jj treated	4	10.8 ±0.3 NS§	3.3 ±0.1 $P < 0.001$§,||,¶	1,474±124 NS§	2,330±86 $P < 0.05$§,||; $P < 0.01$¶
Combined therapy					
jj treated	23	11.3 ±0.7	1.6 ±0.02 $P < 0.001$§,¶	1,380±64	2,635±69 NS§; $P < 0.001$¶
jj untreated	22	10.5 ±0.2 NS||	6.7 ±0.3 $P < 0.001$||	1,363±88 NS||	1,099±49 $P < 0.01$||
Jj control	23	0.45±0.09 $P < 0.001$||	0.48±0.03 $P < 0.001$||	2,445±161 $P < 0.001$||	2,552±135 NS||

* ±Standard error of the mean.

‡ Determined after 24 h of fluid deprivation.

§ Compared to treated *jj* rats receiving phototherapy only.

|| Compared to treated *jj* rats receiving combined therapy.

¶ Compared to pretreatment value.

ity in weanling jj Gunn rats. With initiation of oral cholestyramine therapy, the mean serum bilirubin concentration fell from a pretreatment level of 10.8±0.3 mg/100 ml to a mean value of 3.3±0.1 mg/100 ml over a 20-wk period ($P < 0.001$) (Table I). With lowering of the serum bilirubin concentration, mean U_{max} rose from a pretreatment value of 1,474±124 mosmol/kg H_2O to a mean value of 2,330±86 mosmol/kg H_2O over the same period of time ($P < 0.01$). In comparison with phototherapy alone, significant differences in the response of the serum bilirubin level ($P < 0.001$) and U_{max} ($P < 0.05$) were noted during cholestyramine feeding, despite the fact that pretreatment values for serum bilirubin and U_{max} were not significantly different in the two groups. Thus, oral cholestyramine administered as 5% of the total weight of the dietary intake was not as effective as continuous phototherapy in reducing the serum bilirubin concentration and reversing the defect in urine concentration at low rates of osmolar clearance.

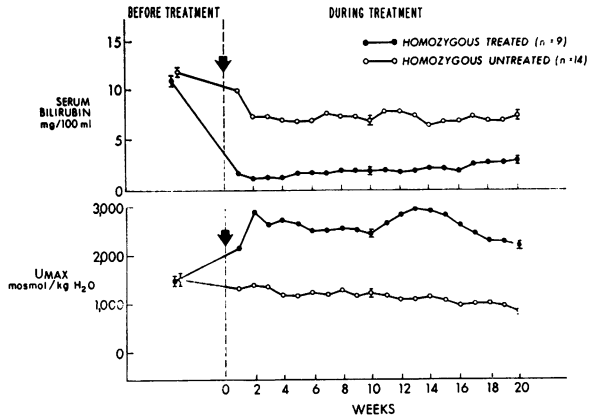

FIGURE 1 Serial measurements of unconjugated serum bilirubin and U_{max} in treated and untreated weanling *jj* Gunn rats over a period of 20 wk. Treated animals were exposed to continuous phototherapy. Brackets indicate ±SE of the group.

Effect of phototherapy and cholestyramine on urine concentrating ability in weanling jj Gunn rats. The combination of oral cholestyramine and phototherapy proved to be more effective than either modality alone in maintaining a reduced level of unconjugated serum bilirubin in the weanling *jj* rats (Table I). Serum levels of unconjugated bilirubin averaged 1.6±0.02

TABLE II

*Response of Adult jj Gunn Rats to Cholestyramine and Phototherapy**

Animal no.	Age at start of therapy	Serum bilirubin concentration		Urine osmolality‡		
		Before therapy	During therapy	Before therapy	During therapy	
	mo	*mg/100 ml*		*mosmol/kg H_2O*		
48	8	7.4±0.2	2.4±0.1	777±38	527±22	$P < 0.001$§
49	8	9.0±0.6	2.9±0.2	917±43	1,011±31	$P < 0.05$§
68	4	7.6±0.1	2.9±0.2	1,240±61	1,560±61	$P < 0.005$§
69	4	9.3±0.3	2.9±0.2	1,093±191	2,074±89	$P < 0.001$§
70	4	8.0±0.4	2.9±0.2	1,306±152	1,958±113	$P < 0.005$§
75	3	8.1±0.4	3.0±0.1	1,240±201	1,715±68	$P < 0.025$§
		$P < 0.001$§				

* ±Standard error of the mean.

‡ Determined after 24 h of fluid deprivation.

§ Compared with pretreatment values.

mg/100 ml in rats receiving combined therapy versus 2.1±0.06 mg/100 ml in rats receiving phototherapy alone ($P < 0.001$) and 3.3±0.1 mg/100 ml in rats given cholestyramine alone ($P < 0.001$). There was no significant difference between U_{max} achieved in animals on combined therapy versus phototherapy alone (P = NS); however, a significant differences in U_{max} was observed between animals receiving combined therapy and oral cholestyramine alone ($P < 0.05$).

Effect of phototherapy and cholestyramine in adult jj Gunn rats (*Table II*). The pretreatment serum bilirubin concentration in the six adult *jj* rats ranged between 7.4±0.2 and 9.3±0.3 mg/100 ml. The pretreatment U_{max} in the four animals that were 3–4 mo of age varied between 1,093±191 and 1,306±152 mosmol/kg H_2O compared to 777±38 and 917±43 mosmol/kg H_2O in the two animals that were 8 mo of age. After initiation of therapy all six animals showed significant decreases in serum bilirubin levels ($P < 0.001$), which were comparable in degree, but a modest improvement in U_{max} was noted in only one of the two older animals, while all four of the younger animals demonstrated significant though partial improvement from their pretreatment values (see Table II). One of the older animals (no. 48) actually demonstrated a decline in U_{max} over the period of therapy (from 777 ±38 to 527±22 mosmol/kg, $P < 0.001$).

Gross examination of the kidneys in these animals provided a possible explanation for the difference in response. Despite treatment, bilirubin deposition in the papillae of both of the older animals was still quite evident and was associated with varying degrees of destruction of the papilla and inner medulla. In addition, mild to moderate hydronephrosis was also evident in the kidneys of one of the older animals and was associated with a calculus (Fig. 2). Marked interstitial scarring which often extended from the medulla to the cortical surface was also present. In contrast, crystalline bilirubin deposits were much less obvious in

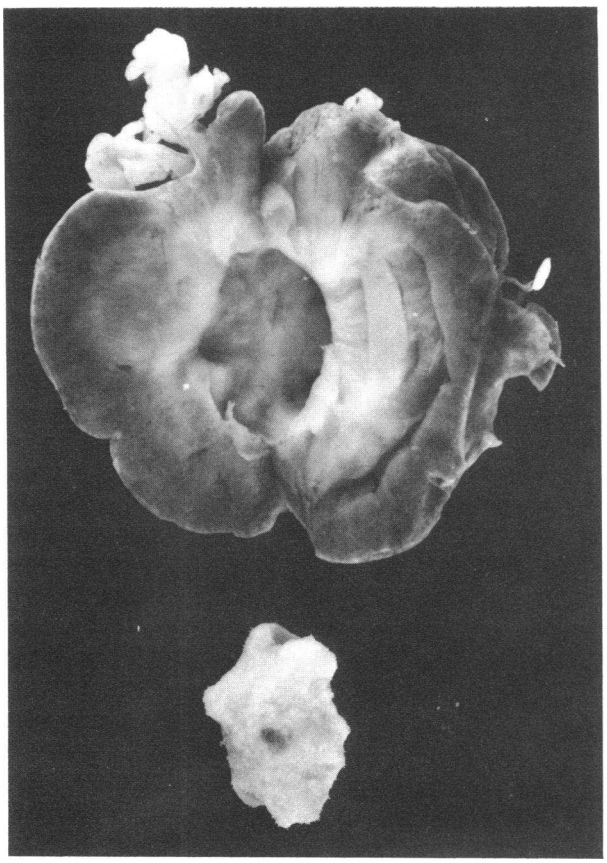

FIGURE 2 Gross appearance of the left kidney of an 8-mo-old *jj* Gunn rat (no. 48) demonstrating severe destruction of the renal papilla and inner medulla and deep cortical scarring. A large calculus (below) filled the entire renal pelvis. Magnification × 3.5.

TABLE III

*Tissue Solute Analysis in Treated and Untreated jj Gunn Rats and Control Jj Littermates**

	Sodium			Potassium			Ammonia			Urea			Total osmolality‡		
	Untreated	Treated	Control	Untreated	Treated	Control	Untreated	Treated	Control	Untreated	Treated	Control	Untreated	Treated	Control
	mmol/kg H_2O			*mmol/kg H_2O*			*mmol/kg/ H_2O*			*mmol/kg H_2O*			*mosmol/kg H_2O*		
Papilla	191±23	464±37	372±31	82±4	89±6	81±4	25±3	35±4	32±6	381±50	1,074±74	898±82	977±99	2,250±148	1,868±129
	$P<0.001$	NS		NS	NS		$P<0.05$	NS		$P<0.001$	NS		$P<0.001$	NS	
		$P<0.001$‖			NS‖			NS‖			$P<0.001$‖			$P<0.001$‖	
Inner medulla	126±10	234±14	202±12	67±3	70±3	68±3	14±3	17±2	18±3	290±35	683±48	664±54	704±57	1,325±70	1,240±69
	$P<0.001$	NS		NS	NS		NS	NS		$P<0.001$	NS		$P<0.001$	NS	
		$P<0.001$‖			NS‖			NS‖			$P<0.001$‖			$P<0.001$‖	
Outer medulla	111±7	145±9	101±4	80±5	85±2	83±3	19±3	20±3	17±2	94±7	171±13	117±10	514±36	671±44	519±12
	$P<0.01$	$P<0.001$		NS	NS		NS	NS		$P<0.001$	$P<0.01$		$P<0.01$	$P<0.001$	
		NS‖			NS‖			NS‖			$P<0.05$‖			NS‖	
Cortex	72±1	75±2	65±4	93±2	100±3	81±5	22±3	23±3	32±7	42±3	38±4	32±7	416±7	434±9	388±19
	NS	$P<0.05$		$P<0.05$	$P<0.01$		NS	NS		NS	NS		NS	$P<0.05$	
		NS‖			$P<0.05$‖			$P<0.05$‖			$P<0.05$‖			NS‖	
Urine	82±12	103±16	94±21	129±7	229±21	294±29	39±8	86±11	84±14	513±90	1,387±100	1,433±132	1,013±63	2,363±116	2,377±148
	NS	NS		$P<0.001$	NS		$P<0.005$	NS		$P<0.001$	NS		$P<0.001$	NS	
													(1,098±91)§	(2,645±117)	(2,662±151)

The untreated group comprised ten *jj* Gunn rats, the treated group eleven *jj* Gunn rats, and eight heterozygous *Jj* littermates served as normal control animals.

* ±Standard error of the mean.

‡ Calculated as the standard formula $2[Na^+ + K^+ + NH_3] +$ urea.

§ Actual measured urine osmolality.

‖ Control *Jj* vs. untreated *jj* Gunn rats.

TABLE IV

*Concentration of Tissue Bilirubin in Treated and Untreated jj Gunn Rats**

Group	Bilirubin concentration			
	Papilla	Inner medulla	Outer medula	Cortex
		μg/mg tissue		
Untreated *jj* rats (n = 6)	5.19±2.50	0.12±0.07	0.13±0.06	0.04±0.005
Treated *jj* rats (n = 7)	0.32±0.15 $P < 0.05$	0.04±0.01 $P < 0.10$	0.03±0.004 $P < 0.10$	0.04±0.010 $P < 0.30$

* Mean±standard error.

the younger adult animals receiving treatment and structural abnormalities in the cortex and medulla were less severe than in the older animals. Thus, it would appear that permanent structural damage occurs after prolonged elevation of serum and tissue concentrations of bilirubin in adult animals which becomes increasingly severe with age, finally reaching a point at which the defect in U_{max} is no longer reversible despite prolonged lowering of the serum bilirubin concentration.

Tissue urea and nonurea solute concentrations. The results of the tissue solute analyses are shown in Table III. Total tissue osmolality (calculated as $2[Na^+ + K^+ + NH_3] +$ urea) was significantly reduced in the renal papilla and inner medulla of untreated *jj* Gunn rats when compared to values obtained in normal *Jj* littermates ($P < 0.001$). The decreased tissue osmolality was due to a significant reduction in the concentration of both sodium and urea ($P < 0.001$). Highly significant differences in total tissue osmolality and sodium and urea concentrations were also noted between untreated and treated weanling *jj* rats in the papilla, inner medulla, and outer medulla ($P < 0.01$ or greater). With treatment the reduction in the concentration of sodium and urea and total osmolality in the papilla and inner medulla of *jj* rats was restored to those levels obtained in the normal *Jj* littermates (treated *jj* vs. *Jj* littermates; $P =$ NS). Thus, the reduction in the serum concentration of unconjugated bilirubin with the resultant correction of the defect in U_{max} during treatment was accompanied by a normalization of the urea and nonurea solute profile in the papilla and inner medulla of the *jj* Gunn rats when compared to their normal *Jj* littermates. The failure to demonstrate a difference in total tissue omolality between untreated *jj* rats and their *Jj* littermates in the outer medulla may reflect a sampling error. In both untreated and treated *jj* Gunn rats the anatomical boundaries of the outer and inner medulla are less distinct than in *Jj* rats. It is quite possible that samples from the outer medulla actually included small amounts of tissue from the inner medulla as well, sufficient to raise the concentration of sodium and urea and hence the total tissue osmolality in both treated and untreated *jj* rats. It should be noted, however, that a significant difference in total tissue osmolality and sodium and urea concentrations did exist in the outer medulla of treated versus untreated *jj* rats ($P < 0.01$ or greater), thus demonstrating the effectiveness of reducing the serum and tissue concentration of bilirubin in these animals. The concentrations of NH_3 and potassium in the papilla, inner medulla, and outer medulla were comparable in the three groups of animals ($P =$ NS).

Tissue bilirubin concentration. The bilirubin concentrations measured in the papilla, inner and outer medulla, and cortex in seven treated and six untreated *jj* rats are shown in Table IV. In all regions of the kidney except the cortex there was a reduction in the concentration of bilirubin in the treated animals; however, only in the papilla where the mean concentration was 5.2 μg/mg of wet tissue in untreated rats versus 0.32 μg/mg of wet tissue in treated animals did the differences become statistically significant ($P < 0.05$). The wide range of values obtained in these analyses, especially in the papilla and inner medulla of the untreated rats was due in part to the problem of loss of bilirubin deposits during tissue harvesting. The large aggregates of bilirubin in the papilla and inner medulla were easily dislodged from the tissue and when this occurred the fragments of bilirubin were not included in the analyses, thus falsely lowering the final values.

$T^c_{H_2O}$ formation in treated and untreated jj Gunn rats and Jj littermates (Table V and Fig. 3). The relationship between $T^c_{H_2O}$ and osmolar clearance (C_{osm}) in the three groups of animals is depicted in Fig. 3. Within all ranges of C_{osm}, $T^c_{H_2O}$ was significantly less in untreated *jj* rats when compared to their normal *Jj* littermates. In contrast, no significant difference was noted in $T^c_{H_2O}$ at any level of C_{osm} between treated *jj* rats and their normal *Jj* littermates.

Glomerular filtration rate determined during maxi-

TABLE V

*T^cH_2O Formation in Treated and Untreated jj Gunn Rats and Jj Littermates**

Group	Range of C_{osm} (μl/min) 0–200		200–400		400–600		600–800	
	C_{osm}	T^cH_2O	C_{osm}	T^cH_2O	C_{osm}	T^cH_2O	C_{osm}	T^cH_2O
	μl/min		*μl/min*		*μl/min*		*μl/min*	
Normal *Jj* rats (n = 3)	124.0±18.6	95.0±12.4 (n = 6)	303.1±23.1	169.0±9.9 (n = 9)	509.6±14.1	200.8±6.2 (n = 13)	686.3±16.3	218.3±13.8 (n = 7)
Untreated *jj* rats (n = 5)	94.0±16.6	52.7±8.8 (n = 15)	278.0±14.1	113.5±7.3 (n = 15)	494.7±13.3	148.7±10.0 (n = 17)	680.9±13.9	149.3±12.6 (n = 13)
	NS‡	$P < 0.01$‡	NS‡	$P < 0.0005$‡	NS‡	$P < 0.0005$‡	NS‡	$P < 0.0005$‡
Treated *jj* rats (n = 4)	152.1±13.5	109.7±8.5 (n = 7)	305.6±16.2	160.4±4.8 (n = 19)	513.9±18.9	194.9±4.2 (n = 13)	703.8±4.2	202.6±3.8 (n = 12)
	NS‡	NS‡	NS‡	NS‡	NS‡	NS‡	NS‡	NS‡

* Mean±standard error.
‡ Compared to normal *Jj* rats.

mum T^cH_2O formation, that is at C_{osm} of 600–800 μl/min, was similar in the three groups of animals and averaged 2.60±0.11 ml/min in *Jj* rats versus 2.43±0.17 ml/min in treated *jj* rats (P = NS) and 1.84±0.16 ml/min in untreated *jj* rats (treated versus untreated *jj* rats, $P < 0.05$). The lower GFR in untreated *jj* rats can probably be ascribed to the loss of renal parenchyma secondary to severe cortical and medullary scarring which was observed on gross inspection of the kidneys of the untreated *jj* animals.

DISCUSSION

Four findings of significance can be derived from the data compiled in the present study. First, as previously suspected, unconjugated bilirubin can be directly implicated in the urine concentrating defect previously described in the Gunn strain of rat. Second, the defect in urine concentration is only partially reversible and sometimes irreversible in adult *jj* Gunn rats, probably because of secondary parenchymal destruction of the renal medulla. Third, the urine concentrating defect is already present in weanling jj Gunn rats by 21 days of age, contrary to a previously published report (7). Fourth, a combination of phototherapy using blue fluorescent lights and oral cholestyramine feeding is the most effective means presently known to lower serum and tissue concentrations of unconjugated bilirubin in the jaundiced *jj* Gunn rat.

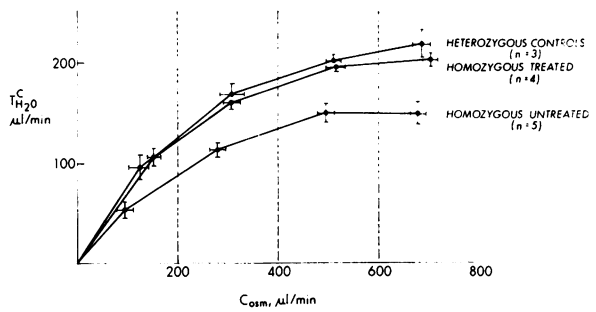

FIGURE 3 Comparison of the relationship between T^cH_2O and C_{osm} in heterozygous *Jj* Gunn rats and treated and untreated *jj* Gunn rats. Each point represents the mean of all values for T^cH_2O and C_{osm} that fell within each of the four ranges of C_{osm} as demarcated by the broken vertical lines. The vertical and horizontal bars through the points represent ±1 SE of mean T^cH_2O and C_{osm}, respectively. Data used to derive the curves are presented in Table V. At all ranges of C_{osm}, T^cH_2O was depressed in untreated *jj* Gunn rats (lower curve). With treatment (middle curve), T^cH_2O was comparable to that measured in normal heterozygous littermates (upper curve).

The results of the present study provide direct evidence that increased concentrations of unconjugated bilirubin in the serum and tissue are causally related to the defect in urine concentration in the *jj* Gunn rat. Although earlier studies had documented an association between hyperbilirubinemia and the renal impairment, a direct cause-and-effect relationship had not been established (2, 5, 6). In the present study, a prolonged reduction in the serum bilirubin level in weanling *jj* Gunn rats, induced through the use of phototherapy or oral cholestyramine or both, was associated with reversal of the impairment in urine concentration and T^cH_2O formation. The reduced concentrations of urea and nonurea solutes in the papilla and inner and outer medulla of untreated *jj* Gunn rats were restored to normal in treated *jj* littermates. The lowering of the hyperbilirubinemia with the attendant normalization of renal function was associated with a marked reduction in the concentration of bilirubin in the papilla and medulla. Thus, a direct cause-and-effect relationship appears well established.

The defect in urine concentrating ability was only partially reversible in young adult *jj* Gunn rats and es-

sentially irrevessible in older adult *jj* animals despite the fact that the concentration of serum bilirubin was lowered to levels comparable to the weanling *jj* rats in which the defect was totally reversible (Table II). Despite therapy, gross examination of the kidneys revealed persistent deposits of bilirubin in the papilla and inner medulla which were associated with varying degrees of parenchymal damage. Often the damage was so severe, especially in the oldest animals, that hydronephrosis of one or both kidneys was evident as well as extensive scarring which extended from the medulla to the cortex. Investigators have previously described extensive bilirubin deposition in untreated adult *jj* rats which was frequently associated with papillary necrosis (8). In the 3- and 4-mo-old animals in which only partial correction in the renal impairment was observed, definite, albeit less extensive parenchymal destruction and bilirubin deposition was evident. Thus, at some point in time between 21 days and 3–4 mo of age irreversible parenchymal damage occurs and appears to be slowly progressive in nature in the absence of therapy directed toward the reduction of serum and tissue concentrations of bilirubin.

In the present study it was observed that the jaundiced weanling *jj* rats already had a significant impairment in U_{max} at 21 days of age. After 24 h of fluid deprivation, the *jj* animals only attained a U_{max} of approximately 1,500 mosmol/kg H_2O, while their normal *Jj* littermates without the defect achieved a U_{max} of approximately 2,500 mosmol/kg H_2O (see Table I). These results conflict with previously published observations of Odell (7), who reported finding no significant difference in the urea and nonurea solute concentrations of the inner medulla in weanling *jj* and *Jj* rats and concluded that jaundiced weanling *jj* rats up to 21 days of age have normal medullary function. However, Odell (7) did not determine urine osmolality in the two groups of animals, which would have been a more sensitive method of estimating urine concentrating ability, and this may explain, at least in part, his failure to detect a defect in U_{max} in the jaundiced weanling *jj* rats. Possibly of equal importance in explaining the discrepancy in results between the two studies is the fact that the solute analyses were performed on the entire inner medulla, not just the papillary tip (7). As a result the average values for urea and nonurea solute concentrations that obtained undoubtedly underestimated the maximum solute concentration that existed at the papillary tip. Finally, in the present study the presence of a significant impairment in U_{max} in jaundiced weanling *jj* rats was further supported by the finding that *jj* rats receiving treatment to lower the concentration of unconjugated serum bilirubin increased their U_{max} by at least 1,000 mosmol/kg H_2O over a period of 2 wk.

The use of both phototherapy and oral cholestyramine to lower the serum concentration of unconjugated bilirubin in the jaundiced *jj* Gunn rats proved to be more effective than either modality alone (Table I). The use of phototherapy alone has been employed in several studies involving *jj* Gunn rats (10, 11, 16–18), but renal functional parameters were never determined. Phototherapy has also been used extensively in many neonatal intensive care units to lower the concentration of unconjugated serum bilirubin in newborn and especially premature infants (19), thereby minimizing the chances for the development of kernicterus with attendant brain damage. When the light source is of the proper wavelength, photodegradation of bilirubin in the skin and cutaneous capillaries occurs probably by a process of photo-oxidation resulting in the formation of water-soluble substances which are excreted principally in the bile, but to a lesser extent in the urine (18). Ostrow (18, 20) has demonstrated that in the *jj* Gunn rat subjected to phototherapy, the water-soluble bilirubin derivatives are chromatographically identical with those normally found in the bile under normal fluorescent lighting conditions, although the exact nature of all of the photoderivatives still remains in doubt. The effectiveness of the therapy depends, at least in part, on the flux of the radiant energy incident on the sample (20, 21). Blue fluorescent lamps with wavelength emission from 420 to 475 nm are the most effective light source and the degree of photodegradation is proportional to the energy flux emitted in the blue region of the visible spectrum (20, 21). It has also been noticed that during phototherapy there is a marked increase in the biliary excretion of unconjugated bilirubin, an amount which accounts for nearly half of the bilirubin that is catabolized during phototherapy (18, 20). Previous studies have demonstrated that cholestyramine, when ingested orally, is also capable of lowering unconjugated bilirubin by binding the pigment in the intestine, thereby preventing its reabsorption (12). These two pieces of evidence formed the basis for utilizing the combination of phototherapy and cholestyramine feeding to maintain lowered bilirubin levels in our animals. If previous observations were correct it was anticipated that the increased unconjugated bilirubin excreted by the biliary system in response to phototherapy should be bound by the cholestyramine in the gut, thus preventing its reabsorption. Although the fecal excretion of bilirubin was not measured, the two modalities were more effective than either one alone in reducing the level of serum bilirubin suggesting that cholestyramine did, in

fact, increase total fecal excretion of unconjugated bilirubin.

The present study was not primarily designed to examine either the mechanism whereby unconjugated bilirubin alters renal concentrating ability or the site of the defect. However, previous studies in adult *jj* Gunn rats have documented the presence of alterations in both C_{H_2O} and $T^c_{H_2O}$ (6), suggesting that the primary renal defect probably involves the ascending limb of Henle. As previously suggested (5, 6) bilirubin could interfere directly with active solute transport out of the ascending limb of Henle or alter the permeability of the segment to water. Either mechanism could explain the impairment in C_{H_2O} and $T^c_{H_2O}$. In vitro data (22, 23) do suggest that unconjugated bilirubin in concentrations comparable to those that have been measured in the papilla of the *jj* Gunn rat (6) are capable of uncoupling oxidative phosphorylation. However, whether the concentration of bilirubin in the outer medulla, where active solute transport occurs, is sufficient to uncouple oxidative phosphorylation is open to question. Diamond and Schmid (24) were unable to demonstrate uncoupling of oxidative phosphorylation in mitochondria isolated from the brains of guinea pigs in which the total tissue concentration of exogenous ^{14}C-labeled bilirubin infused into the animals was 10.6 μg/g of tissue. In the present study the concentration of unconjugated bilirubin was 130 μg/g of tissue in the outer medulla of untreated *jj* Gunn rats and fell to 30 μg/g of tissue with treatment. In addition, Martinez-Maldonado, Suki, and Schenker (6) were unable to demonstrate diminished concentrations of ATP in the medulla of jaundiced adult *jj* Gunn rats, suggesting that if bilirubin does interfere with solute transport in the renal medulla, the mechanism may not be dependent on ATP. Whatever the more acute and potentially reversible effect(s) of bilirubin may be, it is evident from the results of the present study that massive deposits of crystalline bilirubin which develop with time within the papilla and outer medulla result in irreversible parenchymal destruction including papillary necrosis, hydronephrosis, and severe medullary and cortical fibrosis. In such a setting the defect in urine concentration is no longer reversible.

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