Intrarenal Mechanisms of Salt Retention after Bile Duct Ligation in Rats

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ABSTRACT In order to study renal salt-retaining mechanisms during the early stages of ascites formation, rats were subjected to bile duct ligation. After this procedure, plasma volumes were found to be reduced and hematocrits slightly increased. The whole-kidney glomerular filtration rate and plasma flows were reduced to 59 and 57% of control values, but the filtration fraction was unchanged. Absolute sodium excretion, as well as the fraction of the filtered sodium load excreted, was also significantly reduced.

When micropuncture techniques were used to examine the function of single superficial nephrons, the glomerular filtration rate in these nephrons was found to be reduced to 70% of controlled values, and fractional reabsorption was found to be increased at all accessible sites along the nephron. Filtration by intermediate and juxtamedullary nephrons, determined by Hanssen's technique, was reduced to 55 and 48% of control values.

By the use of radioactive microspheres, it was demonstrated that blood flow to superficial, intermediate, and juxtamedullary nephrons was reduced to 49, 59, and 73% of control values. Filtration by superficial nephrons decreased much more than plasma flow—a finding which suggests that the measured increase in fractional reabsorption was associated with an increase in the superficial nephron filtration fraction.

From this study, it appears that two factors play an important part in the sodium retention observed in the initial stages of ascites formation following bile duct ligation in rats: (*a*) a decrease in the filtered sodium load and (*b*) increased fractional reabsorption by the superficial nephrons—the nephrons which show the *least* decrease in filtration.

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INTRODUCTION

The causes of altered sodium excretion have been examined under a number of experimental conditions that lead to positive sodium balance (1–11). A great many possible causes of sodium retention have been identified, but a common unifying hypothesis has not yet been advanced. The variability observed among differing experimental models may be due, at least in part, to fundamental mechanistic differences in the models themselves or to the differing stages of renal sodium retention at which experimental observations are made. Plasma volume, for example, is one factor which may change during the course of sodium retention; and the net impact of the changes in plasma volume upon renal function may vary at different stages (8–11).

It is doubtful that a single mechanism will be found as the basis for abnormal sodium retention in all conditions. Perhaps a more logical hypothesis is that the kidney responds to the net effect of interplay between several different factors which change with time.

Three phenomena thought to play a major role in altering the renal excretion of sodium are (*a*) changes in the glomerular filtration rate, (*b*) changes in the endogenous secretion of mineralocorticoids (3), and (*c*) renal hemodynamic adjustments, including disturbances in the intrarenal distribution of blood flow (5, 6) and alterations of Starling forces within the peritubular circulation (12–15). In the past, the possible interrelationship between the effects of these two hemodynamic adjustments has not been emphasized. Several investigators, however, have recently suggested that, in the con-

TABLE I

Numbers and Types of Rats Studied by Various Methods

Technique	BDL	S-BDL	Controls		
			Sham	Pair-fed	Unoperated
GFR	46 (4–14)*	0	18 (4–14)	12 (4–7)	17
RPF	20 (4–10)	0	0	12 (4–7)	9
Micropuncture	14 (4–11)	0	10 (4–11)	4 (5–7)	0
Microspheres	14 (4–10)	0	0	4 (5–7)	8
Hanssen's	12 (4–10)	0	0	4 (4–7)	8
Plasma volumes	10 (4–9)	0	0	0	9
Balance studies	56 (4–14)	7 (7)	18 (4–14)	12 (4–7)	0
Totals	56	7	18	12	25

* Numbers outside parentheses refer to the number of rats studied; numbers within parenthesis, the number of days elapsing after surgery before the studies were performed or, in the case of balance studies, completed.

verse condition of sodium diuresis, changes in the intrarenal distribution of blood flow and glomerular filtration may be uneven and may alter the peritubular environment of some nephrons in a way which facilitates sodium excretion (16, 17). It would seem equally possible that, in other circumstances, the net effect of interplay between blood flow and glomerular filtration distribution may alter peritubular capillary physical factors in a way that favors sodium retention.

The purpose of the present study was to evaluate the role of several possible causes of decreased sodium excretion after ligation of the common bile duct in rats. Since our observations were purposely limited to the early stage of ascites formation, when sodium balance first becomes positive, it is possible that they are not applicable to later stages of sodium retention. Particular attention was directed to the following questions:

(*a*) Is the renal tubular reabsorption of sodium increased and, if so, at which site(s) along the nephron?

(*b*) Is the appearance of positive sodium balance attended by changes in the intrarenal distribution of blood flow and glomerular filtration? If so, are the changes in a direction that might be expected to facilitate sodium retention?

(*c*) Do changes in extrarenal factors such as plasma volume and hematocrit contribute to sodium retention?

The results of this study suggest that sodium excretion is decreased by a reduction in filtration and a concomitant increase in fractional reabsorption on the part of the superficial nephrons. Increased fractional reabsorption by these nephrons appears to be related, at least in part, to alterations in the filtration fraction and a consequent modification of the peritubular environment. These alterations result because blood flow to the superficial cortex is diminished at a time when the filtration rate is only slightly reduced. Extrarenal factors which may play a contributory role are (*a*) a decrease in the plasma volume and (*b*) a slight increase in the arterial hematocrit.

METHODS

One or more of the following studies were performed in 63 male Sprague-Dawley rats subjected to ligation of the common bile duct: renal clearance and micropuncture studies, determination of the intrarenal distribution of blood flow and glomerular filtration, and metabolic balance studies. Table I gives a complete list of the studies performed on these animals and on three control groups: 18 *sham rats*[1]

[1] *Abbreviations used in this paper:* BDL rats, rats drinking tap water after ligation of the bile duct; C, control rats (combined group of sham, pair-fed, and unoperated rats); C_{Na}/GFR, the fraction of the filtered sodium load excreted in the urine; *E*, renal extraction ratio; FF, filtration fraction; GFR, whole-kidney glomerular filtration rate; PAH, *p*-aminohippuric acid; pair-fed rats, rats who were subjected to sham surgery and whose intakes of food and water were matched to those of individual BDL rats; RPF, whole-kidney renal plasma flow; S-BDL, BDL rats who drank water containing 1% sodium chloride and 5% dextrose; sham rats, rats subjected to sham surgery (laparotomy with placement of a ligature through the duodenal mesentery), eating and drinking ad libitum; SNGFR, single superficial nephron glomerular filtration rate determined by micropuncture techniques; TF/P inulin, tubular fluid to plasma inulin ratio; U_{Na}, concentration of sodium in the urine; $U_{Na}V$, absolute rate of urinary sodium excretion; U/P_{osm}, ratio of urine to plasma osmolality; *V*, tubular fluid flow rate.

In the following abbreviations, S or s = superficial, I or i = intermediate, and J or j = juxtamedullary (nephrons or cortices): F_s, F_i, F_j, fractional distribution of nephrons in the Hanssen technique studies; FBF_s, FBF_i, FBF_j, fractional blood flow to the areas of the cortex; SPF, IPF, JPF, plasma flow to the nephrons; SGFR, IGFR, JGFR, glomerular filtration rates of the nephrons, determined in the Hanssen studies; I/S, J/S, ratio of glomerular filtration rate (reflected by precipitated [^{14}C]ferrocyanide) in intermediate or juxtamedullary nephrons to that of superficial nephrons in the Hanssen studies.

(rats subjected to sham surgery and then allowed rat chow and water ad libitum); 12 *pair-fed rats* (rats subjected to sham surgery and then pair-fed with 12 individual BDL rats); and 25 healthy *unoperated* rats.

Methods of bile duct ligation and sham surgery. After ether anesthesia and surgical preparation of the skin, a small upper abdominal midline incision was made in 93 rats. In 63 rats, the common bile duct was doubly ligated; in the 30 rats subjected to sham surgery, the ligatures were passed through the duodenal mesentery but not around the bile duct. All the operated rats were given, i.m., 40,000 U of potassium penicillin G and 50 mg of streptomycin immediately after operation and on alternate days thereafter.

Balance studies and preliminary blood studies. Beginning on the day of operation, balance studies were performed for periods varying from 4 to 14 days. After surgery, these animals were placed in rodent metabolism cages. All rats subjected to ligation of the common bile duct and the 18 sham rats were allowed free access to water and to ground rat chow (Purina Rat Chow, Ralston Purina Co., St. Louis, Mo.). Each of the pair-fed rats was given the amounts of food and water consumed by its BDL "twin." 56 of the rats whose bile duct had been ligated (hereafter referred to as "BDL rats") and all sham-operated rats drank tap water. The other seven rats subjected to bile duct ligation (hereafter referred to as "S-BDL rats") drank water containing 1% sodium chloride and 5% dextrose.

The following measurements were made every day in order to determine the daily intake and output of water, sodium, and potassium; (*a*) intake of food and water, (*b*) output of urine and feces, and (*c*) concentrations of sodium and potassium in the urine and in an acid extract of the feces (18). The rat chow was known to contain 182 μeq of sodium and 238 μeq of potassium per gram, and the drinking water of the S-BDL rats contained approximately 165 meq of sodium per liter.

In 14 BDL, 7 S-BDL, and 11 sham rats, blood was taken from the cut end of the tail on the day of surgery and on the 3rd, 5th, and 7th days thereafter. This blood was used for determinations of arterial hematocrit and plasma creatinine concentration.

The other studies described below were conducted on the BDL rats shortly after sodium balance became clearly positive. This event coincided with the earliest phase of ascites development and generally occurred 4–7 days after bile duct ligation. Only balance studies and preliminary blood studies were performed on S-BDL rats.

Clearance studies. In 32 BDL, 8 sham, 8 pair-fed, and 17 unoperated rats, clearance studies were performed without micropuncture. The anesthetic employed for these studies was Inactin (Promonta-Hamburg, Hamburg, West Germany). In the BDL rats, the initial dose of anesthetic was 50 mg/kg, supplemental doses being given as required. The sham, pair-fed, and unoperated rats received a single dose of 100 mg/kg. The total dose given was comparable in all groups. The preparation of these animals for clearance studies has been described elsewhere (19). 16 BDL, 8 pair-fed, and 8 unoperated rats received [*methoxy*-^{3}H]inulin (International Chemical and Nuclear Corp., Chemical and Radioisotopes Division, Irvine, Calif.) in priming and sustaining amounts of 1.0 μCi/100 g body weight and 2 μCi/100 g per 100 min. 16 BDL, 8 sham, and 9 unoperated rats received [*carboxyl*-^{14}C]inulin (New England Nuclear, Boston, Mass.) and [^{3}H]*p*-aminohippuric acid (PAH, New England Nuclear) in priming doses of 1.0 and 3.0 μCi/100 g body weight and maintenance doses of 2.0 and 20.0 μCi/100 g per 100 min. All maintenance infusions included vasopressin (Pitressin, Parke, Davis & Co., Detroit, Mich.), 50 mU/kg per h, and were contained in Ringer's lactate solution given at the rate of 0.02 ml/min.

To calculate inulin and PAH clearances aliquots of urine specimens and plasma samples (drawn at the midpoint of each urine collection) were counted with quenched reference standards in an Iso/Cap 300 liquid scintillation system (Nuclear Chicago, Searle Analytic, Inc., Des Plaines, Ill.). For counting, each sample was added to 5 ml of a scintillation solution composed of 1 liter of toluene, 42 ml of Liquifluor (New England Nuclear), and 100 ml of Bio-Solv BBS-3 (Beckman Instruments, Inc., Fullerton, Calif.). In 20 BDL, 12-pair-fed, and 9 unoperated rats blood was collected from the renal vein by venipuncture with a 30-gauge needle. These renal vein samples and simultaneously collected arterial specimens were used to determine the renal extraction ratio of PAH or inulin.

Micropuncture studies. 14 BDL, 10 sham, and 4 pair-fed rats were studied by renal micropuncture techniques. The method used to prepared these rats for study has been described elsewhere (19). End-proximal and distal tubules were identified by their morphological characteristics (20) and by injecting the jugular vein with 0.05-ml boluses of 1% saline containing filtered 10% lissamine green dye and then following the progress of the dye through the tubules.

8 BDL, 10 sham, and 4 pair-fed rats were given [*methoxy*-^{3}H] inulin. After a priming dose, maintenance infusions were given as needed to keep the isotope level in the blood high enough to yield tubular fluid samples containing radioactivity at least three times background. Another group of six BDL rats received [*carboxyl*-^{14}C]inulin and [^{3}H]PAH. The priming and sustaining doses were regulated so that the ^{14}C counts in the tubular fluid samples were at least three times background, and the plasma ^{3}H were at least two to three times as high as plasma ^{14}C levels.

Beginning 45 min after injection of the priming solution, several samples of urine, tubular fluid, and arterial blood samples were collected to be counted for radioactivity. Tubular fluids were counted for 100 min. The total number of counts on a single sample of tubular fluid ranged from 4,000 to 10,000.

Samples of tubular fluid from proximal and distal tubules were collected for a minimum of 3 min after insertion of a distal oil block which was at least 5 tubular diameters in length. The samples of tubular fluid were measured in a calibrated glass microcapillary tube before being counted.

After each collection of tubular fluid from a distal tubule, the tubule was injected with liquid latex (latex injection compound, General Biological Supply House, Inc., Chicago, Ill.) and later microdissected and measured by the methods of Windhager (21). Samples of fluid obtained from the first half of the distal tubule are referred to as "early distal"; the remaining samples from the distal tubules are referred to as "late distal."

Studies using radioactive microspheres. The intrarenal distribution of blood flow was studied in 14 BDL, 4 pair-fed, and 8 unoperated rats. Approximately 40,000 ^{51}Cr-labeled microspheres (3M Co., St. Paul, Minn.) 15±5 μm in diameter (30 mCi/g, sp act) were injected into the left ventricle, according to the method of Mendell and Hollenberg (22). At the conclusion of the study, 1.0 ml of India ink was injected intraarterially. The animals were then killed by an i.v. injection of potassium chloride.

Both kidneys were removed through an abdominal incision, and the kidneys from 10 BDL and all 8 unoperated rats were fixed overnight in a 10% solution of buffered

formalin, to which sodium chloride had been added in an amount sufficient to render it isotonic with plasma. The kidneys from the other four BDL and four pair-fed rats were fixed overnight in 20% ethanol in saline. After fixation, each kidney was cut so as to obtain a median slice that included both poles and the hilum. The cortex of the slice, identified by India ink in the glomeruli, was divided, as previously described (23), into three roughly equal strips corresponding to the superficial, intermediate, and juxtamedullary cortices. These slices were blotted and weighed in tared tubes. The radioactivity of each slice was counted in a Beckman Biogamma three-channel counting system (Beckman Instruments, Inc., Fullerton, Calif.).

Studies using Hanssen's technique. The intrarenal distribution of the glomerular filtration rate was determined by the [^{14}C]ferrocyanide precipitation technique originally described by Hanssen (24) and modified by Coelho et al. (25). 12 BDL, 4 pair-fed and 8 unoperated rats were prepared as for micropuncture, except that two ligatures were placed loosely around the left renal pedicle. After the performance of base-line [*methoxy*-^{3}H]inulin clearance studies, a Harvard syringe pump (Harvard Apparatus Co., Inc., Millis, Mass.) was used to inject 0.4 ml of a solution containing (by weight) 7% nonradioactive sodium ferrocyanide and 50–100 μCi of [^{14}C]sodium ferrocyanide (International Chemical and Nuclear Corp.). This solution was injected into the jugular vein over a period of 12 s. At the end of the injection, the ligatures around the renal pedicle were tied and the kidney was rapidly removed and frozen in acetone chilled to -65°C with dry ice.

The frozen kidneys were fragmented into pieces less than 2.0 mm thick, which were immediately placed in a solution of alcoholic ferric chloride (30 g of anhydrous ferric chloride, 95 ml of absolute ethanol, and 5.0 ml of fuming hydrochloric acid). After being stored in this solution at -20°C for 12–24 h, the kidney fragments were macerated in 20% hydrochloric acid for 15 h at room temperature or for 85 min at 39°C. After maceration the fragments were washed and stored in a 1% solution of acetic acid containing ferric chloride (100 mg/100 ml).

Individual nephrons (including their glomeruli and proximal tubules) were dissected from superficial, intermediate, and juxtamedullary regions. As defined previously (25), superficial nephrons have at least two loops touching the renal capsule. Juxtamedullary nephrons are found in the subcortex, frequently under an arcuate artery (26); their efferent arterioles divide into vasa recta and their proximal tubules do not have definite pars recta (25). All other nephrons are classified as intermediate. Coelho et al. (25) have determined the fractional distribution of superficial (s), intermediate (i), and juxtamedullary (j) nephrons as follows: $F_s = 0.49$, $F_i = 0.36$, and $F_j = 0.15$.

After microdissection each individual tubule was removed from the dissecting dish and placed on a cover slip in a droplet of the acetic acid storage fluid mixed with a small amount of albumin fixative (Harleco, Gibbstown, N. J.). After most of this fluid had been suctioned off, the tubule was straightened out and any remaining peritubular capillaries were removed. The cover slip was then placed on a hemocytometer and the outline of the tubule was traced on calibrated graph paper or drawn with a camera lucida (Wild Heerbrugg Ltd., Heerbrugg, Switzerland), so that its length could be measured with a map measurer (Tacro, Switzerland). After being washed with distilled water, the tubule was transferred by a curved needle to a counting vial containing the scintillation counting fluid previously described. The counting vial was agitated, and the fluid was counted for radioactivity by liquid scintillation techniques.

To determine extraluminal radioactive contamination, sections of distal convoluted tubules and cortical collecting ducts were measured and counted in a similar fashion (25).

Plasma volumes. Plasma volumes were determined in 10 BDL and 9 unoperated rats. After inulin and PAH clearances had been determined in the manner described earlier, carefully measured amounts (10–20 μCi) of radioiodinated (^{131}I) human serum albumin (Abbott Laboratories, North Chicago, Ill.) were injected into the jugular vein. 60 min later, an arterial blood sample was drawn and the activity of 10 μl of plasma was determined by liquid scintillation techniques, in an Iso/Cap 300 liquid scintillation system, with appropriate quenched standards and the scintillation solution described above.

Blood and urine chemistry studies. Sodium and potassium concentrations in blood and urine were determined by lithium internal standard flame photometry (Instrumentation Laboratory, Inc., Lexington, Mass.). Plasma and urine osmolality was determined by freezing point depression (Advanced Instruments osmometer, Needham Heights, Mass.) or by vapor pressure depression (model 5100 vapor pressure osmometer, Wescor, Inc., Logan, Utah). Plasma creatinine concentration was measured by a micro-adaptation of the method of Owen et al. (27). Qualitative urine bilirubin was estimated by the use of Ictotest reagent tablets (Ames Co., Elkhart, Ind.).

Calculations. In micropuncture experiments the reabsorption of water along the nephron was estimated from the ratio of inulin in tubular fluid to that in plasma (TF/P inulin). The glomerular filtration rate of single superficial nephrons (SNGFR) was calculated as follows:

$$\text{SNGFR} = \text{TF/P inulin} \times V,$$

where V equals tubular fluid flow rate. The whole-kidney glomerular filtration rate (GFR) was estimated from the inulin clearance.

The renal extraction ratio (E) of inulin or PAH was determined as the difference between the arterial (a) and the renal venous (v) concentration divided by the arterial concentration as follows:

$$E = (a - v)/a.$$

Renal plasma flow (RPF) was calculated from the clearance of either inulin or PAH divided by the renal extraction ratio for that drug as follows:

$$\text{RPF} = \text{clearance/extraction ratio}.$$

In microsphere experiments, the fractional distribution of renal blood flow to the superficial, intermediate, and juxtamedullary zones of the cortex was calculated by the method of McNay and Abe (28). The mean fractional volume of each zone determined in 19 rats and expressed as a percentage of whole kidney volume was as follows: superficial, 12.96%; intermediate, 11.79%; and juxtamedullary, 10.86%. The fractional distribution of glomeruli among these three zones was determined in four BDL, four pair-fed, and eight normal, healthy rats not included in any of the other studies. Expressed as percentages of the total number of glomeruli (29), the values for the three zones were as follows: superficial, 43%; intermediate, 40%; and juxtamedullary, 17%. (The slight differences between these percentages and those reported in studies utilizing Hanssen's technique (25) are due to differences in selection criteria in these two types of experiments.)

In order to determine the ratio between the mean filtration rates of superficial nephrons in BDL rats and control rats ($SGFR_{BDL}/SGFR_C$), the data from the Hanssen experiments were used in the following equation:

$$\frac{SGFR_{BDL}}{SGFR_C} = \frac{[F_s + (I/S)_{BDL} \times F_i + (J/S)_{BDL} \times F_j]}{[F_s + (I/S)_C \times F_i + (J/S)_C \times F_j]} \times \frac{GFR_C}{GFR_{BDL}},$$

where I/S and J/S equal the ratio of precipitated [^{14}C]-ferrocyanide in intermediate and juxtamedullary nephrons to that in superficial nephrons; and F_s, F_i, and F_j equal the fractional distribution of nephrons reported in studies utilizing Hanssen's technique (25).

The ratios between BDL and control rats for the glomerular filtration rate of intermediate (IGFR) and juxtamedullary (JGFR) nephrons were determined by the following expressions:

$$IGFR_{BDL}/IGFR_C = [(I/S)_{BDL}/(I/S)_C]/(SGFR_{BDL}/SGFR_C),$$

$$JGFR_{BDL}/JGFR_C = [(J/S)_{BDL}/(J/S)_C]/(SGFR_{BDL}/SGFR_C).$$

In order to determine the ratios of superficial (SPF), intermediate (IPF), and juxtamedullary (JPF) nephron plasma flow in BDL rats to those in control (C) rats the following equations were used:

$$SPF_{BDL}/SPF_C = (RPF \times FBF_s)_{BDL}/(RPF \times FBF_s)_C,$$

$$IPF_{BDL}/IPF_C = (RPF \times FBF_i)_{BDL}/(RPF \times FBF_i)_C,$$

$$JPF_{BDL}/JPF_C = (RPF \times FBF_j)_{BDL}/(RPF \times FBF_j)_C,$$

where FBF_s, FBF_i, and FBF_j represent the fractional blood flow to the superficial, intermediate, and juxtamedullary cortex (determined in microsphere experiments).

Comparison of the results obtained in the three different subgroups of control rats (sham-operated spontaneously fed, sham-operated pair-fed, and unoperated) showed no significant differences except where noted; therefore the three subgroups were combined and are referred to as "control" (C) rats.

Data are expressed as mean±standard error. Differences between means were determined by Student's t test (30); when P was greater than 0.05, the difference was considered nonsignificant.

RESULTS

Balance studies. BDL, sham, and pair-fed rats all lost weight immediately after surgery, but in the sham rats weight soon reached or exceeded the preoperative level. In the BDL and pair-fed rats weight loss continued, averaging 41 and 24 g at the time balance studies were discontinued and the other studies (described below) were performed. Although there were no overall differences in water intake, the food intake of the BDL rats was only about two-thirds that of the sham rats. Nevertheless, both groups of animals were in slight positive sodium balance when studied. The mean sodium balance of the BDL rats (+783±134 μeq) was almost twice that of the sham group (+375±78 μeq), and the difference between the BDL (1,048±133 μeq) and the pair-fed rats (327±63 μeq) was statistically significant ($P < 0.01$).

It should be noted that these balance data in the BDL rats refer to the *final* values, recorded as early as 4 days and as late as 14 days after bile duct ligation. Preliminary studies had revealed that marked ascites eventually develops in almost *all* BDL rats. Because we feared that opening the abdomen of animals with massive ascites might cause a change in renal function, we did not want to study rats in this condition. In order to restrict our experiments to the very early phase of ascites formation, balance studies were discontinued when sodium balance in BDL rats became demonstrably positive, and at that point the other studies listed below were performed. This explanation accounts for the small amount of ascitic fluid found in the BDL rats (8.1±2.7 ml). Although the degree of positive balance in the BDL and sham rats did not differ significantly ($P > 0.05$), it was associated with marked weight loss in the former group and slight weight gain in the latter. BDL rats also demonstrated significant potassium loss (−4,636±524 μeq) corresponding to this weight loss. Potassium balance in sham rats did not differ from zero ($P > 0.05$), but in pair-fed rats it averaged −2,144±633 μeq.

Clearance studies. The GFR in the BDL rats was reduced to 59% of the value for control rats (Table II). RPF was also reduced to 57.5% of the value for control rats, but filtration fraction (FF) was not altered. Absolute sodium excretion ($U_{Na}V$) was significantly reduced, partly because of the decrease in filtered load and partly because of increased fractional reabsorption (evidenced by the decreased fractional sodium clearance, C_{Na}/GFR). Although the ratio of urine to plasma osmolality (U/P_{osm}) was slightly reduced in the BDL rats, they were still able to produce urine with a very low sodium concentration (U_{Na})—evidence that other "distal" capabilities were intact.

TABLE II

Whole-Kidney Clearance and Excretory Data

Measurement	BDL	Control	P
GFR, *ml/min*	0.681±0.057 (46)*	1.149±0.042 (47)	<0.001
RPF, *ml/min*	1.864±0.220 (20)	3.245±0.128 (21)	<0.001
FF	0.335±0.019 (20)	0.336±0.016 (21)	NS
$U_{Na}V$, *μeq/min*	0.131±0.030 (46)	0.290±0.052 (47)	<0.01
C_{Na}/GFR, %	0.071±0.019 (46)	0.190±0.029 (47)	<0.001
U/P_{osm}	4.305±0.255 (46)	5.573±0.370 (47)	<0.01
U_{Na}, *meq/liter*	14.0±1.5 (46)	51.7±6.8 (47)	<0.001

* Mean±SE (number of rats).

TABLE III
Micropuncture Data from Single Superficial Nephrons

Measurement	BDL	Control	P
SNGFR, *nl/min*	29.6±3.4 (14)*	42.2±3.0 (14)	<0.02
End-proximal TF/P inulin	3.86±0.39 (14)	2.32±0.10 (14)	<0.001
Early distal TF/P inulin	9.04±0.63 (6)	4.47±0.29 (9)	<0.001
Late distal TF/P inulin	26.23±6.25 (5)	9.86±1.03 (10)	<0.01

* Mean±SE (number of rats).

Micropuncture studies. Micropuncture data from BDL and control rats are shown in Table III. The glomerular filtration rate in single superficial nephrons (SNGFR) of BDL rats was reduced by 30%; the whole-kidney GFR, by 41%. The fraction of the glomerular filtrate reabsorbed, indicated by the TF/P inulin, was increased at all accessible sites along these superficial nephrons.

Studies using radioactive microspheres. Previous workers have suggested that the distribution of intrarenal blood flow is altered in sodium-retaining conditions (5, 6). Exploring this possibility by the use of radioactive microspheres, we found that the fraction of whole-kidney blood flow delivered to the superficial cortex of the BDL rat was significantly decreased, while the fraction delivered to the juxtamedullary cortex was increased (Table IV). It should be emphasized that these data refer only to changes in *fractional* blood flow, not absolute blood flow.

Studies using Hanssen's technique. Although the blood flow to superficial nephrons was disproportionately reduced in BDL rats, the data shown in Tables II and III do not suggest that filtration was affected to the same extent. In an effort to explore the extent of this discrepancy, the intrarenal distribution of filtration was determined by Hanssen's technique, in which filtered [^{14}C]ferrocyanide is precipitated as insoluble ferric ferrocyanide (prussian blue). The data are shown in Table V.

The ^{14}C activity of an individual nephron, corrected for background and extraluminal contamination (25), is proportional to the filtration rate of that nephron. The ratios of ^{14}C activity in intermediate and juxtamedullary nephrons to ^{14}C activity in superficial nephrons (I/S and J/S) were used to compare results between rats. In control animals the filtration rates of both intermediate and juxtamedullary nephrons were greater than the filtration rate of superficial nephrons (as reflected by mean I/S and J/S ratios greater than one). In BDL rats, however, the filtration rates of both I and J nephrons were less than the filtration rate of S nephrons (mean I/S and J/S ratios less than one).

Influence of extrarenal factors. In order to determine whether the changes in renal function of BDL rats could be attributed to changes in extrarenal factors, the initial weights, arterial blood pressures, plasma sodium concentrations, and hematocrits were examined in all rats (Table VI). Of these factors, only arterial hematocrits showed any significant difference between BDL and control groups. This increase in hematocrit suggests that a contraction of the plasma volume may have occurred in the BDL rats.

To test this assumption, plasma volume and arterial hematocrit were measured in 10 BDL and 9 control rats (Table VII). The increase in hematocrit observed in these BDL rats was accompanied by a significant decrease in plasma volume, as well as in the ratio of plasma volume to body weight. This ratio was significantly reduced in the BDL rats, whether the weight used as a basis for calculation was that recorded before ligation of the bile duct or that observed on the day of the experiment.

In order to determine whether this contraction of plasma volume was related to the decreased filtration rate, we measured the hematocrit (an indicator of plasma volume) and the plasma creatinine concentration (a crude indicator of GFR) (Tables VIII and IX). For these measurements, we included the S-BDL animals in which contraction of the plasma volume after bile duct ligation was prevented by adding 1% sodium chloride and 5% dextrose to the drinking water.

Neither the hematocrit nor the plasma creatinine concentration was altered in the sham or S-BDL rats. In the BDL rats, however, the hematocrit rose progressively —a finding compatible with contraction of the plasma volume—and the plasma creatinine concentration ap-

TABLE IV
Intrarenal Blood Flow Distribution Determined by the Use of Radioactive Microspheres

Area of cortex	Fractional blood flow		P
	BDL	Control	
Superficial	35.0±0.9 (14)*	41.0±2.1 (12)	<0.01
Intermediate	38.8±1.4 (14)	38.1±1.6 (12)	NS
Juxtamedullary	26.8±0.8 (14)	20.9±1.9 (12)	<0.02

* Mean±SE (number of rats).

TABLE V

[^{14}C]*Ferrocyanide Precipitated in Superficial (S),*

Rats with bile duct ligation					
GFR	S	I	J	I/S	J/S
ml/min	*cpm*	*cpm*	*cpm*		
1.168	153.1±7.5 (10)*	—‡	144.8±9.9 (10)	—	0.946
0.068	165.1±5.5 (6)	143.7±19.3 (6)	151.8±17.3 (6)	0.870	0.919
1.077	138.0±9.3 (5)	114.2±10.5 (5)	142.9±12.8 (5)	0.828	1.036
0.241	101.6±3.3 (16)	83.7±3.1 (14)	80.5±11.6 (14)	0.824	0.792
0.300	45.8±2.5 (14)	41.3±3.0 (13)	39.2±2.8 (14)	0.902	0.856
0.756	76.1±9.1 (19)	65.3±10.7 (14)	79.9±14.6 (14)	0.859	1.050
1.200	70.2±4.9 (12)	67.9±4.4 (12)	76.7±6.1 (12)	0.967	1.092
1.007	115.5±3.0 (18)	113.9±4.1 (15)	113.6±6.1 (15)	0.986	0.984
0.430§	69.3±4.1 (15)	73.4±3.4 (15)	71.2±6.5 (15)	1.061	1.027
1.138§	53.3±1.9 (15)	58.1±1.7 (15)	57.2±1.2 (15)	1.090	1.074
0.206§	96.2±1.3 (15)	95.6±1.5 (15)	91.2±1.3 (15)	0.994	0.948
1.093§	84.4±2.0 (15)	87.9±1.6 (15)	92.2±1.3 (15)	1.041	1.092
Mean 0.800				0.947	0.979
SE ±0.114				±0.029	±0.026

* Average cpm of precipitated [^{14}C]ferrocyanide corrected for background and extraluminal contamination ±SE (number of nephrons).

‡ No intermediate nephrons were dissected in these experiments.

§ Rats with bile duct ligation and their pair-fed controls.

proximately doubled. This increase in plasma creatinine concentration is commensurate with the 41% reduction in GFR observed in these rats. Because the lean body mass of the BDL and S-BDL rats was probably decreasing, some caution is necessary in interpreting the values for plasma creatinine. Nevertheless, it seems reasonable to conclude that the GFR was not greatly altered in the S-BDL animals.

DISCUSSION

Overall effect of bile duct ligation in rats. Among the effects of bile duct ligation in rats demonstrated by the present study are: (*a*) ascites, (*b*) positive sodium balance, (*c*) contraction of the plasma volume, and (*d*) increase in the arterial hematocrit. These findings suggest that, in this experimental model, ascites may accumulate partially at the expense of plasma volume.

The GFR and the filtered sodium load were decreased, while fractional reabsorption by the superficial nephrons was increased. The decrease in GFR occurred primarily in the inner cortical nephrons and was least marked in the superficial nephrons. Conversely, the reduction in plasma flow was most marked in the superficial nephrons and was relatively slight in the inner cortical nephrons. As a result of these disproportionate changes in the distribution of blood flow and glomerular filtration, the filtration fraction of the superficial nephrons was probably increased, while the filtration fraction of inner cortical nephrons was probably reduced. In this experimental model, the net effect of all the above changes—together, perhaps, with changes in other unmeasured factors—was to decrease renal sodium excretion.

Site of increased reabsorption. Our micropuncture results demonstrate that the TF/P inulin ratio was significantly increased at the end of the proximal convoluted tubule and at the beginning and end of the distal tubule. Fractional reabsorption was thus increased all along the length of superficial nephrons (Table X). Because the decrease in the filtered sodium load, however, was greater than the increase in proximal fractional reabsorption, *absolute* proximal reabsorption by BDL rats was actually decreased. The same conclusion must obtain for the kidney as a whole, inasmuch as the control animals reabsorbed more sodium (165.8 μeq/min) than the entire amount filtered by the BDL rats (96.7 μeq/min). In assessing the importance of this increase in fractional reabsorption, one can only conclude that less sodium would have been retained by the BDL rats if glomerulo-tubular balance (fractional reabsorption) had not changed.

Intrarenal distribution of blood flow and glomerular filtration and their relation to sodium excretion. The distribution of blood flow within the cortex of our BDL rats differed quite markedly from the distribution of glomerular filtration. Unfortunately, our data shed no light on the basic mechanisms underlying these intra-

Intermediate (I), and Juxtamedullary (J) Nephrons

Control rats					
GFR	S	I	J	I/S	J/S
ml/min	*cpm*	*cpm*	*cpm*		
1.320	122.4±5.1 (10)	—‡	171.4±6.2 (10)	—	1.374
1.350	90.6±5.8 (13)	113.3±4.0 (12)	132.5±6.6 (10)	1.251	1.463
1.197	58.8±2.4 (10)	70.0±1.5 (13)	83.7±4.3 (8)	1.191	1.434
1.284	85.3±8.4 (12)	104.5±3.7 (11)	124.2±7.2 (12)	1.225	1.458
1.135	61.3±3.4 (17)	68.0±3.9 (15)	86.5±5.3 (15)	1.109	1.411
1.621	88.7±2.5 (15)	95.6±3.5 (15)	105.6±3.1 (15)	1.110	1.190
1.177	93.0±5.2 (13)	109.3±4.8 (19)	126.7±5.3 (13)	1.164	1.349
1.516	63.1±1.9 (18)	73.7±1.0 (24)	82.5±1.2 (20)	1.168	1.309
0.659§	45.2±2.4 (15)	46.6±1.9 (15)	52.3±1.8 (15)	1.031	1.167
1.172§	66.0±2.4 (15)	85.9±1.9 (15)	102.3±1.3 (15)	1.301	1.548
1.042§	40.1±1.7 (15)	50.9±1.2 (15)	59.3±0.8 (15)	1.269	1.479
1.201§	52.4±1.3 (15)	58.9±1.0 (15)	76.5±1.2 (15)	1.124	1.460
1.224				1.176	1.386
±0.070				±0.024	±0.033

renal hemodynamic adjustments. They might be hormonal (31, 32) or neurological (33, 34) or might have still another basis (23).

It is pertinent to ask whether the altered intrarenal distribution of glomerular filtration and blood flow in BDL rats is in any way related to the increase in the fractional reabsorption rate. To answer this question, we expressed the filtration rate and plasma flow of nephrons in the superficial, intermediate, and juxtamedullary areas of the cortex in BDL rats as fractions of the values for control rats (Table XI). Since the decrease in plasma flow in the superficial nephrons was obviously greater than the decrease in glomerular filtration rate, it seems reasonable to conclude that the filtration fraction of the superficial nephrons was probably increased in the BDL rats.

For two reasons, it is difficult to assess these changes quantitatively. First, the studies on which these data are based (microsphere and Hanssen's) were performed in two different groups of rats. Second, as was noted in the Methods section, the nephron groups measured by these two techniques are somewhat different. Specifically, part of what is called *superficial cortex* in microsphere studies was aglomerular; and in the Hanssen studies some nephrons of the superficial type were found in the intermediate cortex. Even with these reservations, however, it seems certain that superficial filtration fractions were increased after bile duct ligation.

These alterations in the superficial filtration fraction undoubtedly affected peritubular capillary physical forces in BDL rats. Several recent studies (12–15) have shown that changes in peritubular physical factors can signifi-

TABLE VI

Extrarenal Factors Capable of Affecting Renal Function and Sodium Excretion

	BDL	Control	*P*
Initial weight, *kg*	0.272±0.005 (56)*	0.268±0.007 (55)	NS
Blood pressure, *mm Hg*	131±3 (56)	127±2 (55)	NS
Plasma sodium, *meq/liter*	142.0±0.8 (56)	144.6±0.7 (55)	NS
Hematocrit, %	53±3 (56)	45±2 (55)	<0.05

* Mean±SE (number of rats).

TABLE VII
Plasma Volume and Hematocrit

	BDL (n = 10)	Control (n = 9)	P
Original weight, *g*	287.7±10.5*	285.6±11.6	NS
Plasma volume, *ml*	8.84±0.75	12.44±0.77	<0.01
$\frac{\text{Plasma volume}}{\text{Original weight,}}$ %	2.96±0.18	4.33±0.12	<0.001
$\frac{\text{Plasma volume}}{\text{Experimental weight,}}$ %	3.50±0.22	4.33±0.12	<0.01
Hematocrit	53.2±1.9	46.1±1.2	<0.01

* Mean±SE.

cantly alter sodium reabsorption in the proximal tubule; hence, it is possible that this alteration of filtration fraction represents one mechanism whereby sodium excretion is decreased after bile duct ligation. One might reasonably ask whether the apparent *decrease* in the filtration fraction of the inner cortical nephrons might not vitiate the antinatriuretic effects of the increased filtration fraction in the outer cortex. In reply to this question, it can be said that the effect of this decrease will probably be minimal, because (*a*) the inner cortical nephrons represent a relatively small fraction of the total nephron population, (*b*) their filtered sodium load is decreased more than that of any other nephrons, and (*c*) the antinatriuretic effects of an increased hematocrit (35) will tend to increase their fractional reabsorption rate. Finally, Kawamura et al. (36) have recently demonstrated that intrinsic differences exist in the pars recta of superficial nephrons as contrasted to pars recta of juxtamedullary nephrons. It is possible that changes in filtration rate, blood flow, and filtration fraction to the superficial versus juxtamedullary nephrons may effect total renal salt and water balance by virtue of different alterations in the peritubular environment in a subset of tubules with intrinsically different transport characteristics.

Role of ascites and other extrarenal factors in bile duct ligation. Alterations in extrarenal factors such as ascites, plasma volume, and arterial hematocrit may influence the renal excretion of sodium both directly and indirectly.

The mechanism responsible for ascites formation in our rats was not identified, but studies on BDL dogs

TABLE VIII
Changes in Hematocrit during Balance Studies

Group	Hct (Day 1)	Change in hematocrit from day 1		
		Day 3	Day 5	Day 7
BDL	46.5±0.5 (14)*	4.5±0.5 (14)	9.2±0.9 (11)‡	11.9±2.0 (6)‡
Sham	46.6±0.6 (11)	0.4±0.4 (11)	0.8±0.7 (10)‡	1.8±0.8 (7)‡
S-BDL	45.7±0.7 (7)	1.7±1.0 (7)	1.0±0.9 (7)	−0.6±0.7 (7)
P (sham vs. BDL)	NS	<0.001	<0.001	<0.001
P (sham vs. S-BDL)	NS	NS	NS	NS

* Mean±SE (number of rats).
‡ The number of rats in the BDL and sham groups decreased as animals in positive sodium balance were withdrawn for other studies.

TABLE IX
Changes in Plasma Creatinine during Balance Studies

Group	Initial plasma creatinine (Day 1)	Change in plasma creatinine from day 1		
		Day 3	Day 5	Day 7
	mg/dl		*mg/dl*	
BDL	0.80±0.50 (14)*	+0.29±0.03 (14)	+0.56±0.08 (11)‡	+0.93±0.19 (6)‡
Sham	0.81±0.07 (11)	+0.08±0.05 (11)	+0.04±0.07 (11)‡	+0.11±0.07 (7)‡
S-BDL	0.93±0.07 (7)	−0.02±0.06 (7)	+0.03±0.07 (7)	−0.16±0.05 (7)
P (sham vs. BDL)	NS	<0.001	<0.001	<0.001
P (sham vs. S-BDL)	NS	NS	NS	NS

* Mean±SE (number of rats).
‡ The number of rats in the BDL and sham groups decreased as animals in positive sodium balance were withdrawn for other studies.

(2) have suggested that a partial explanation may lie in an elevation of portal venous pressure and a decrease in the size of the hepatic venous bed.

Concomitantly with the accumulation of ascites after bile duct ligation, the plasma volume was reduced by mechanisms yet unknown. This contraction of plasma volume was probably instrumental in producing the increase in hematocrit observed in our BDL rats. Burke et al. (35) have shown that an increase in the arterial hematocrit is associated with a significant increase in proximal tubular reabsorption. The reduction of plasma volume, by affecting the secretion of mineralocorticoids and the distribution of blood within the kidney, may also influence sodium excretion indirectly. In more advanced stages of the ascites that follows bile duct ligation and occurs in other conditions leading to sodium retention, the plasma volumes may be normal or expanded as sodium is progressively retained. In dogs studied by Levy (11) after ligation of the thoracic inferior vena cava, the plasma volume was expanded. He and others have suggested that differences in plasma volume may be one factor responsible for the great variability of proximal tubular function which has been reported in various experimental models associated with sodium retention (1, 7–11).

Clinical connotations. The relevance of the present study to the complete spectrum of clinical conditions associated with sodium retention may be limited. It reemphasizes the importance of changes in the glomerular filtration rate as a determinant of sodium retention. It is true that alterations in the intrarenal distribution of glomerular filtration and blood flow and changes in the peritubular environment all act to increase sodium reabsorption; yet in our experimental model at least, sodium retention is due more to a decrease in the filtered load than to the increased reabsorption. The present study showed that, during the early stages of ascites formation, a relationship exists between decreased filtration, increased sodium reabsorption, and plasma volume contraction. This finding suggests that a similar relationship may exist in human subjects at various stages of sodium retention. It suggests further that sodium retention in such patients may be increased if the extracellular fluid volume is altered by the use of diuretics or other procedures (paracentesis, for example) which cause contraction of the plasma volume.

Histological examination of the livers of our BDL rats demonstrated them to be essentially normal. Thus, in spite of some similarities between the effects of bile duct ligation in rats and the hepatorenal syndrome in man (ascites, renal failure, and reduced plasma volumes [37]), there is no histological evidence to suggest that liver failure, the central feature of the hepatorenal syndrome, occurs in BDL rats.

TABLE X

Fractional Reabsorption by the Superficial Nephrons

Segment	BDL	Control
	% SNGFR*	
Proximal tubule	74.5	56.9
Loop of Henle	88.9	77.6
Distal tubule	96.2	89.9
	% DV‡	
Proximal tubule	74.1	56.9
Loop of Henle	57.3	48.1
Distal tubule	65.5	54.7

* % SNGFR, percentage of the glomerular filtrate reabsorbed at the end (n) of the segments of superficial nephrons $= (1 - P/TF_n \text{ inulin}) \times 100$, where P/TF_n is the reciprocal of TF/P inulin shown in Table III.

‡ % DV, percentage of the volume delivered to each segment that was reabsorbed by that segment $= (P/TF_{n-1} - P/TF_n)/(P/TF_{n-1})$, where P/TF is the ratio of the inulin concentration in plasma to that at the beginning ($n - 1$) or the end (n) of the segment.

TABLE XI

Glomerular Filtration Rate and Plasma Flow of Superficial, Intermediate, and Juxtamedullary Nephrons: Ratio of BDL Rats to Control Rats

Nephron type	Glomerular filtration rate*	Plasma flow‡
Superficial	0.680§	0.490
Intermediate	0.547	0.589
Juxtamedullary	0.480	0.737

* Glomerular filtration rate determined from Hanssen's technique data and mean GFR.

‡ Plasma flow, determined from microsphere data and mean RPF.

§ This figure is comparable to the ratio of $SNGFR_{BDL}$ to $SNGFR_C$ determined in micropuncture experiments (0.701).

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