Mechanisms of Blood Pressure Elevation in Pyelonephritic Rats After Sodium Loading[1] (37449)

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The relationships of pyelonephritis to hypertension have been studied in this laboratory in a model of experimental pyelonephritis in rats. These studies have indicated that hypertension rarely occurs with chronic pyelonephritis even when the animals develop significant renal failure. On the other hand rats who are made hypertensive by a variety of acute or chronic methods are rendered more susceptible to acute pyelonephritis (1–4), and this previously existing hypertension can be exacerbated by infection (4, 5).

In normotensive rats with chronic pyelonephritis, the administration of DOCA and 1% saline as drinking fluid, induces a greater elevation of blood pressure than in control animals (6). However, the mechanism by which this develops is not clear. In a study we reported in 1969 (7), pyelonephritic rats placed on a high salt diet and allowed access to water *ad libitum* tolerated marked amounts of sodium (up to 30 mEq/day). The animals with the more severe degrees of pyelonephritis became hypertensive but did not retain more sodium. In fact by drinking amounts of water almost equal to their body weight per day they were able to rid themselves of the excess sodium as well as did rats with normal kidney function (7).

In this study, the data suggested that water was retained in excess of salt and accordingly, a series of experiments were undertaken to manipulate several variables and to accumulate more exact data concerning water and sodium balance. Placing the animals on a low sodium intake lowered the blood pressure to normal but particularly in the rats with the more severe grades of pyelonephritis led shortly to sodium loss, azotemia and death. When the water intake of pyelonephritis rats was matched with that of normal rats, hypertension failed to develop but food (and thus sodium) intake similarly fell and marked weight loss occurred in the experimental animals. Further reduction of renal mass by removal of one kidney from the pyelonephritis rats potentiated the hypertension but did not otherwise clarify the problem.

In summary, these several experimentals confirmed the fact that exposure to a high sodium intake with unlimited access to fluid in pyelonephritic rats resulted in an elevation of blood pressure but the balance studies produced equivocal results and did not confirm the impression from the pilot study that water was retained in excess of salt. Accordingly, the present study was undertaken to determine directly the components of body composition of rats with chronic pyelonephritis fed a high sodium intake.

Methods and Materials. Chronic pyelonephritis was induced in female Long Evans hooded rats (Rockland Farms) weighing 150 to 200 g, by intravenous injection of 0.5 ml of tryptose broth culture of *Proteus mirabilis* and then 3 wk later by injection with *Streptococcus fecalis*. Each injection was followed immediately by bilateral renal massage (8). Control rats also were massaged but did not receive the bacteria. All rats were kept in colony cages and received regular rat chow and tap water *ad libitum* for periods up to 6 mo. A left nephrectomy then was per-

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formed to further decrease functioning kidney mass as well as to check on the existence of pyelonephritis at that time. Left kidneys of the infected animals showed macroscopic and microscopic lesions of pyelonephritis as previously described (8) and when cultured revealed either both or one of the two organisms indicating that the chronic lesions usually were still active. Osmolal concentration of the urine after 24 hr of dehydration was reduced approximately 50% in the pyelonephritic rats. The left kidneys removed from control animals showed no pyelonephritic lesions and their cultures were sterile.

One week after nephrectomy rats were isolated in individual metabolic cages and started on a sodium-free powdered rat chow to which was added 16% sodium chloride. When directly tested in our laboratory, sodium content in the different batches of chow varied between 2.5 and 2.8 mEq sodium/g. Both pyelonephritic and normal rats were placed on this diet while an additional group of normal rats was kept on the same diet with the addition of only 0.25 to 0.35 mEq/g.

Body weight and systolic blood pressure measurements, the latter by the tail blood pressure method (4), were obtained weekly. When pyelonephritic rats had a blood pressure of 140 mm Hg or greater on at least two consecutive occasions, they were sacrificed along with at least one control animal on a high sodium diet. Pyelonephritic rats which did not become hypertensive were sacrificed along with control rats 9 to 12 wk after they were started on the high sodium intake.

At sacrifice the blood urea nitrogen (BUN) and serum sodium and potassium concentrations were measured in each rat and the right kidney was scored for gross lesions on a 1 to 4 plus scale as previously described (8). Direct measurements of body composition determined at this time, were body weight, extracellular volume by the ^{82}Br space, total body water, and total body sodium and potassium.

The ^{82}Br space was determined by intraperitoneal injection of 0.3 μCi of ^{82}Br as $NH_4{}^{82}Br$ in isotonic saline and counting of the bromine activity in the dose and in plasma obtained by heart puncture, 1 hr later (9). For correction of urinary loss of ^{82}Br, urine was collected during this hour of equilibration, during which time the animal was deprived of food but had free access to water to maintain his usual fluid balance.

Total body water was measured by drying the carcass to a constant weight in a ventilated oven at a temperature of 105° and subtracting the weight of the dried carcass from the body weight obtained just before sacrifice. The dry carcass was then homogenized, and lipids were extracted with a mixture of equal amounts of petroleum and ethyl ethers. After drying and reweighing the difference between this lean body weight and the dried carcass represented the total body fat. To determine sodium and potassium, a known weight of approximately 5 g of fat-free carcass was completely digested in 70% nitric acid for 49 to 96 hr and the sodium and potassium concentrations in the digestate were determined in a flame photometer. From these data the total body sodium and potassium were calculated.

Rats were divided into four groups as follows:

Group 1: Rats with pyelonephritis and left nephrectomy which received high sodium intake and were hypertensive (*i.e.*, a blood pressure of 140 mm Hg or greater on two consecutive weekly determinations prior to sacrifice).

Group 2: All other rats with pyelonephritis receiving the high sodium diet which did not reach these criteria of blood pressure elevation.

Group 3: Unilaterally nephrectomized rats without pyelonephritis receiving high sodium diet and sacrificed at periods corresponding with that of the hypertensive rats.

Group 4: Unilaterally nephrectomized rats without pyelonephritis receiving a normal salt intake throughout the course of the study and sacrificed at equivalent periods to those in the above mentioned groups.

All chemical determinations were done in duplicate. The data were handled statistically by analysis of variance for four groups with t tests between groups where F values were significant (10).

Results. There were a total of 16 rats in Group 1, 10 rats in Group 2, 12 rats in Group 3 and 8 rats in Group 4. Six additional pyelonephritic rats died spontaneously during the course of the study. The cause of death in two of them was pneumonia whereas three of the other four had quite severe pyelonephritis and probably died of renal failure. All these rats lost weight markedly before death and none were hypertensive. Similarly, 4 of 16 control rats started on the high sodium diet died during the course of the study; three had pneumonia and in one the cause of death was not determined. These rats had normal appearing kidneys at autopsy.

Balance data as obtained in the previous studies were not collected in these animals but urine output of the pyelonephritic rats averaged 157 ml/24 hr and that of controls 135 ml/24 hr; this is approximately 10 to 15 times that of rats on normal sodium intake. The range of urine output in the pyelonephritic rats was considerably greater than that in the controls and related to the degree of renal damage from the infection.

Terminal data are shown in Tables I and II. Both the last blood pressure recorded as well as average blood pressure for the 3 wk prior to sacrifice were higher in the two pyelonephritic groups and in the control group on high salt, than in the control group receiving a normal sodium intake. Group 1, obviously had significantly higher blood pressures than either Group 2 or 3, but there was no significant difference between Groups 2 and 3. The heart weights in all three groups receiving high sodium diets were greater than those in the group receiving a normal sodium diet but in spite of the increased blood pressure in Group 1, their slightly heavier hearts were not significantly increased over Groups 2 and 3.

No significant differences were noted between the body weights of the four groups of rats. However, the data on the body composition indicate clearly that the rats receiving a high sodium diet had a reduction of body fat. For reasons that are not apparent, the body fat loss was greater in the nonhypertensive group of pyelonephritic rats than in the hypertensive group, perhaps because they

TABLE I.

	Group	Weeks of study	Body wt (g)	Water (ml)	Fat (g)	Lean body mass (g)	ECF (ml)	ICF (ml)	As % body wt				
									Water	Fat	LBM	ECF	ICF
I	Pyn. and hyp.	9.4	264	179	25.6	59.1	71.8	107.2	67.9	9.6	22.4	27.3	40.6
II	Pyn. and nor.	10.6	254	180	17.8	56.6	74.0	106.3	70.8	6.9	22.3	29.1	41.9
III	Control	8.7	262	178	25.0	59.5	71.6	105.5	67.8	9.6	22.6	27.6	40.4
IV	Control–reg. diet	12.5	263	170	38.3	54.7	65.8	103.8	64.5	14.6	20.9	25.6	39.3
	Analysis of variance		NS	NS	.01	NS	NS	NS	.01	.01	.01	.05	NS
	Significant group comparisons (*)												
	I × II				*				*	*			
	I × III								*	*	*	*	
	I × IV				*								
	II × III				*				*	*			
	II × IV				*				*	*	*	*	
	III × IV				*				*	*	*	*	

TABLE II.

Group	Total body Na (mEq)	Total body K (mEq)	Total body Na (% LBM)	Total body K (% LBM)	Serum (mEq/liter) Na	Serum (mEq/liter) K	Hematocrit	BUN (mg %)	Final BP (mm Hg)	MBP (last 3 wk) (mm Hg)	Heart wt (mg)	Heart wt (mg % BW)
I	15.4	14.9	26.1	25.3	142	4.44	39.6	16.0	152	151	778	297
II	15.7	15.0	27.8	27.8	145	4.56	40.8	15.4	120	118	756	302
III	15.5	15.1	26.0	25.5	145	4.51	42.0	12.0	123	123	731	280
IV	13.5	15.0	24.8	27.4	141	5.03	40.5	23.2	100	104	664	254
Analysis of variance	NS	NS	NS	.05	NS	NS	NS	.01	.01	.01	.01	.01
Significant group comparisons (*):												
I × II									*	*		
I × III								*	*	*		
I × IV				*				*	*	*	*	*
II × III												
II × IV								*	*	*	*	*
III × IV				*				*	*	*	*	*

were on the high sodium diet slightly longer. Total body water (TBW) and its partition into ECF and ICF were not significantly different among the four groups. However, when total body water was expressed as a percentage of body weight, although the body weights were not different a slight increase was noted in the three groups receiving high sodium intake compared to the group which received a normal diet. Nevertheless, in contrast with what we had suspected from the earlier study there was minimal if any difference in body water between the three groups on high salt diet; in fact it would appear that in the normotensive group the TBW was slightly above that of the control group and the hypertensive group. ECF as a function of total body weight also was slightly but significantly increased in the three groups receiving high sodium intake, but not between these three groups.

The data indicate no difference between the groups in total body sodium or potassium although when expressed as percentage of lean body mass, Groups 1 and 3 had a slightly lower potassium level than did Group 4. No differences in serum sodium or potassium concentrations and no differences in the hematocrits among the four groups of animals were noted. Interestingly, the BUN was actually lower in the markedly diuresing rats on high sodium intake than in the group of unilaterally nephrectomized controls receiving a normal sodium intake.

The lesions of the right kidney in the pyelonephritic rats when rated on the 1 to 4+ scale, averaged 2.2 in the rats which became hypertensive, while in those which were not hypertensive, the average was 1.7. This is in keeping with previous observations in intact rats where a total of over 4 (on a potential total score of 8 from 2 kidneys) results in higher pressures in such rats when exposed to the sodium load than in rats with a total score of lesions less than 4 (7).

Discussion. The present data again confirm that pyelonephritic rats when exposed to a sodium load will develop hypertension, and that the development of this hypertension relates to the severity of the pyelonephritic lesions. What was striking in this study was

the marked efficiency of the animals in excreting the excess sodium essentially by diuresis of their remaining nephrons. Thus sodium retention did not occur nor were body fluid volumes expanded at the time of sacrifice. A previous pilot study using balance techniques had suggested that in spite of the failure to retain salt there was evidence of a retention of water in excess of salt (10) but this was not confirmed in the present experiment which involved direct analysis of the composition of the body tissues and fluids.

If the rats were nevertheless hypertensive in spite of failure to expand fluid volumes, the alternative explanation for the rise in blood pressure must be an increase in peripheral resistance. Previous studies from this laboratory have indicated that experimental pyelonephritis is not associated with any increase in pressor material in the kidneys of rats nor increased sensitivity to pressor agents (11). Thus the blood pressure rise is most compatible with an increase in peripheral resistance which has occurred as a consequence of autoregulation as described in precise studies by Coleman and Guyton (12), Ledingham and Cohen (13). When this phenomenon occurs in the course of the development of the hypertension is by no means clear and the data from our present study do not provide an answer. It may be that early in the course of the development of the hypertension there is an expansion of body fluids, as the data of Ledingham and Cohen and Coleman and Guyton indicate, which we could not measure by the sequences followed in the present study. Additionally, it is possible that changes develop in the electrolyte composition of arterioles to increase vascular resistance, as has been suggested by Tobian (14), which could not be detected by the gross measurements of sodium and potassium utilized in our study.

In any case the data may account for those special situations in clinical medicine where in spite of control of volumes by dialysis in patients with parenchymal renal disease and terminal renal failure, blood pressure control still remains a problem and hypertension persists. Some of this continued blood pressure elevation in clinical situations is due to an elevation of renin which occurs during the course of decreasing the volume in the over-expanded subject (15–17). However, this may not always be the case; in particular it is worth noting that such patients after nephrectomy will sometimes maintain elevated blood pressures possibly due to changes in peripheral resistance from autoregulation (18).

Summary. Rats with experimental chronic pyelonephritis were given a high sodium diet with fluid *ad libitum* and their body composition was determined. The rats with more severe pyelonephritis developed mild to moderate hypertension but analysis of fluid volumes and body electrolyte composition revealed no significant differences from normotensive pyelonephritic rats and nonpyelonephritic rats given the same diet. The results suggest that the elevation of blood pressure which develops in these rats is a consequence of an eventual increase in peripheral resistance, which results from processes of autoregulation following exposure to the increased sodium and fluid load.

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