Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 124 MARCH, 1967 No. 3

SECTION MEETINGS

CLEVELAND, OHIO
Western Reserve University — February 20, 1967

IOWA
University of Iowa — February 28, 1967
March 28, 1967

# Renal Content of Renin-Like Material and Pressor Reactivity of Rats with Chronic Pyelonephritis.* (31824)

HENRI E. TURRIAN† AND ALVIN P. SHAPIRO
*Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Intravenous administration of bacteria with subsequent massage of the kidneys induces a pyelonephritis in rats which can be made chronic by repetition of the procedure(1). The renal lesion is characterized by inflammatory changes followed by scarring and distortion of the kidneys. Functional studies demonstrate marked diminution of the concentrating ability of such kidneys but only minimal development of proteinuria and glomerular damage late in the course of the disease.

Several authors(2,3) have reported development of hypertension in experimental pyelonephritis, but in the "renal massage model" where no kidney damage but that which is secondary to the infection is present, this has been a rare finding, particularly when *E. coli* or enterococci are the infecting organisms. With proteus strains, which produce the most destructive lesions, slight elevation of blood pressure has been noted, but again usually not to hypertensive levels(4). Failure of hypertension to develop consistently in the rat with pyelonephritis is in keeping with the irregularity of its coexistence with pyelonephritis clinically. Since renal hypertension generally is considered a consequence of vascular lesions with ischemia, the fact that pyelonephritis is primarily a parenchymal disease has been suggested to explain the low incidence of hypertension in experimental pyelonephritis *per se*(5).

On the other hand pyelonephritic rats are more sensitive to the hypertensive effect of

* Supported by grants from American and Allegheny Mountain Heart Assn. and USPHS HE-5711.

† Trainee in Clinical Pharmacology, USPHS HE-5467; Present address: Medizinisch-Biologische Forschungsabteilung der Sandoz AG, Basel.

DOCA and salt-loading(6) and also pyelonephritis aggravates pre-existing hypertensive disease in the rat and presumably in man (7,8). These observations, plus the increased susceptibility of hypertensive rats to pyelonephritis(6,7,9), might account for the increased incidence of pyelonephritis in hypertensive patients, without invoking a direct cause and effect relationship.

Several questions concerning absence of such a direct relationship seemed to need elucidation and the purpose of this study was therefore to examine the following points: 1) Are changes in the renin content of the kidneys produced by pyelonephritis? 2) Is there an increase of pressor reactivity to various vasoconstrictor agents in pyelonephritic animals?

*Materials and methods.* Chronic pyelonephritis was produced in rats by intravenous injection of 0.5 ml of an 18-hour tryptose broth culture of *Proteus mirabilis* and massage of both kidneys through the intact body wall. A second injection was given 3-4 weeks after the first administration, this time utilizing a strain of *Streptococcus zymogenes*. The details of the techniques have been described(1,4). In all experiments female albino rats of an initial weight of 150-200 g (Carworth) were used. Systolic blood pressure was measured at 2-week intervals by the tail phonomicrophone technique(7). The blood pressures indicated in the various Tables represent the average of the last determinations before sacrifice, unless stated otherwise. Impairment of renal function was observed by studying the changes in blood urea nitrogen (BUN) and/or in urine osmolality. The BUN was measured in whole blood, collected by intracardiac puncture at the time of sacrifice. Urine osmolality was determined after quantitative collection from each rat during a 24-hour period of fasting and thirsting, performed 3-5 weeks before sacrifice.

*A) Content of pressor material in pyelonephritic kidneys.* Renin-like pressor material in the kidneys was determined according to the method described by Gross *et al*(21) in which extracts of the kidney are prepared with 2 ml 0.9% NaCl per g renal tissue. The pressor effect of 0.1 ml of a 1:5 dilution of these extracts was measured directly from the carotid artery of intact assay rats, anesthetized (pentobarbital 40-60 mg/kg) and pretreated with pentolinium (2.5 mg/100 g), using a Statham strain guage. In each experiment the activity of 0.03 and 0.1 unit renin (Nutritional Biochemicals Corp., Cleveland) injected in a volume of 0.1 ml was determined. The mean blood pressure elevation after 0.03 unit of renin was 10.8 $\pm$ 1.5 ($n = 25$) and after 0.1 unit, 30.0 $\pm$ 2.2 mm Hg ($n = 25$). All determinations of pressor material were from animals sacrificed 3 to 4 months after the second bacterial injection.

Two separate experiments were performed. The first study included 7 experimental animals and the same number of controls. From each of the pyelonephritic rats the more severely damaged kidney was taken and frozen until the time of the bioassay. Pressor material of each pyelonephritic and control kidney was compared on 2 test animals. In the second study both kidneys of 11 pyelonephritic and 11 control animals were compared.

*B) Blood pressure reactivity of pyelonephritic rats.* Acute pressor responses to intravenous injections of angiotensin‡ and phenyllysine vasopressin (PLV-2)‡ in pyelonephritic rats and controls were determined indirectly on the tail by the phonomicrophone technique as described by Gaskell *et al*(18).

Three separate experiments were performed: a) Blood pressure effects of 0.1 $\mu$g/kg angiotensin and 0.02 u/kg PLV-2 were examined during the development of the chronic pyelonephritis, *i.e.*, 10, 20, 40 and 80 days after the second bacterial injection. Results were obtained with 5 to 7 animals per dose for angiotensin and with 5 to 13 animals per dose for PLV-2. b) Blood pressure effects of 0.3 $\mu$g/kg of angiotensin and 0.1 u/kg of PLV-2 were investigated on 10 pyelonephritic and 10 control animals, 3 months after the second bacterial injection, on 2 subsequent weeks. The first week, half of the animals received angiotensin,

‡ Angiotensin and phenyllysine vasopressin were kindly supplied respectively by Ciba Pharmaceutical Co., Summit, N. J., and Sandoz Pharmaceutical Co., Hanover, N. J.

TABLE I. Pressor Material in Kidneys of Pyelonephritic Rats (Mean ± Standard Error).

| | | Pyelonephritics (n=7) | p values | Controls (n=7) |
|---|---|---|---|---|
| Macroscopic lesions | (Grade)* | 3+ | | 0 |
| Pressor activity (.1 ml of 1:5 extract) | (mm Hg) | 24.4 ± 3.2 | >.05 | 29.1 ± 3.4 |
| Final systolic blood pressure | (mm Hg) | 119.4 ± 2.6 | .05† | 101.6 ± 3.9 |
| BUN | (mg/100 ml) | 24.3 ± 2.6 | .05† | 15.7 ± 2.2 |
| Osmolality | (mosm/kg) | 1090 ±168 | .05† | 2135 ±312 |

* Graded according to scale of Shapiro *et al*(4). † Significant differences.

the other half PLV-2; the following week the order of the injections was reversed. c) Dose-response curves with PLV-2 were investigated further. Assays were performed 3 months after the second bacterial injection on 6 pyelonephritic and 6 control animals. The 3 doses of 0.03, 0.1 and 0.3 u/kg were administered in the same bioassay and the determinations were performed alternatively with a control and a pyelonephritic animal. The regression was calculated according to the usual method (22) adding 2.0 to the logarithm of the dosage.

Sensitivity to repeated renin administration was tested on 8 animals. After control studies of a duration of 2 days, renin was started. The preparation was a purified hog renin, prepared by Nutritional Biochemicals Corp., Cleveland, Ohio, and containing 1 Goldblatt unit per mg. It was given as a solution in peanut oil (125 mg/ml), after homogenization. Each animal received 3 subcutaneous injections of 25 mg daily for 5 days while the control animals were given a corresponding amount of peanut oil. The blood pressure was registered daily, prior to the renin injection. During the experiment urine was collected for several periods of 12 hours, during which the animals were fasted and thirsted. The animals were observed for 24 hours after the last renin administration and then sacrificed.

*Results. 1) Content of renin-like material in the kidneys.* Results of the 2 studies are summarized separately in Table I and II. In the first experiment the infected animals had a higher BUN than the controls and they displayed a marked diminution of their concentrating ability. The pyelonephritic rats had a slightly higher blood pressure than the controls although not to hypertensive levels, but there was no significant difference in the content of pressor material in their kidneys.

The determinations performed in the second study showed essentially the same results. The left and the right kidney both were analyzed and although the right organ displayed more severe lesions than the left, no difference in the renin-like material in their kidneys was apparent (Table II). Even if one considers the total pressor material contained in both kidneys there is no significant difference between pyelonephritic animals (33.3 ± 6.5 mm Hg) and controls (42.3 ± 8.9 mm Hg).

*2) Pressor response to angiotensin and phenyllysine vasopressin (PLV-2).* While the pressor effect of angiotensin (0.1 μg/kg) remained unchanged, the response to PLV-2 (0.02 u/kg) was diminished 10 days after the second bacterial injection (Table III). Eighty days later the reaction to PLV-2 was slightly greater in pyelonephritic than in control animals, but the difference was just outside the limit of significance (p = 0.10). In view of this equivocal result studies were repeated

TABLE II. Effect of Lesions of Different Intensity on Pressor Material (Mean ± Standard Error).

| | | Pyelonephritics (n=11) | | | Controls (n=11) | |
|---|---|---|---|---|---|---|
| | | Left | Right | p values | Left | Right |
| Macroscopic lesions | (Grade)* | 2+ | 3+ | | 0 | 0 |
| Pressor activity (.1 ml of 1:5 extract) | (mm Hg) | 17.1 ± 3.5 | 16.2 ± 3.6 | >.05 >.05 | 22.9 ± 5.1 | 19.5 ± 4.1 |
| Final systolic blood pressure | (mm Hg) | 118.2 ± 5.4 | | >.05 | 110.4 ± 4.9 | |
| BUN | (mg/100 ml) | 20.8 ± 1.2 | | .05† | 17.2 ± .8 | |

* Graded according to scale of Shapiro *et al*(4). † Significant differences.

TABLE III. Maximum Systolic Blood Pressure Response to Vasopressors During Development of Chronic Pyelonephritis (mm Hg; Mean* ± Standard Error).

| Time elapsed after infection (days) | Angiotensin (.1 μg/kg) | | Phenyllysine vasopressin (.02 unit/kg) | |
|---|---|---|---|---|
| | Pyelonephritics | Controls | Pyelonephritics | Controls |
| 10 | 13.4 ± 2.1 | 17.5 ± 2.5 | 9.8 ± 1.5 | 16.3 ± .9† |
| 20 | 18.1 ± 3.8 | 19.2 ± 2.5 | 15.6 ± 2.6 | 16.1 ± 2.1 |
| 40 | 15.8 ± 2.4 | 13.5 ± 2.2 | 13.8 ± 2.6 | 13.5 ± 2.1 |
| 80 | — | — | 18.6 ± 4.6 | 13.9 ± 1.1 |

* Determinations on 5 to 13 rats per period.
† Significant at .05 level; other pairings not significantly different.

TABLE IV. Maximum Systolic Response to Larger Dose of Vasopressors in Established* Chronic Pyelonephritis.

| | | Pyelonephritics (n = 10) | p values | Controls (n = 10) |
|---|---|---|---|---|
| Response to angiotensin (.3 μg/kg) | (mm Hg) | 24.0 ± 3.1 | >.05 | 24.5 ± 3.8 |
| Response to PLV-2 (.1 unit/kg) | (mm Hg) | 29.6 ± 4.9 | >.05 | 24.9 ± 3.8 |
| Final systolic blood pressure | (mm Hg) | 107.9 ± 4.7 | >.05 | 112.0 ± 4.7 |
| BUN | (mg/100 ml) | 18.4 ± 4.7 | >.05 | 14.9 ± 1.8 |
| Osmolality | (mosm/kg) | 1182 ± 39 | .01† | 2500 ± 245 |

* 3 months after infection.
† Significant difference.

with a higher dosage of PLV-2 (0.1 u/kg) and a dose response curve was established. At the same time the effect of 0.3 μg/kg angiotensin in animals with a 3-month-old pyelonephritis was examined. The results, summarized in Table IV and Fig. 1, reveal no difference between pyelonephritic and control animals, either for PLV-2 or for angiotensin. The average blood pressure of the pyelonephritic animals used for the dose-response curve was 116.1 ± 3.9 mm Hg, their BUN was 24.8 ± 3.4 mg%; corresponding values for the controls were 108.8 ± 2.2 mm Hg and 19.6 ± 1.4 mg%.

*3) Sensitivity to renin.* Chronic administration of renin caused weight diminution and a reduction of blood pressure in the treated animals (Table V). These effects were probably due to a diminution of the circulating blood volume and of the body sodium, as the known diuretic effect of renin(11) was particularly marked. Even when measured 2 hours after i.v. injection of renin a blood pressure elevation was not observed. An eclampsia-like syndrome as described by Masson *et al*(12) did not occur.

*Discussion.* The fact that pyelonephritic lesions did not modify the content of pressor material in the kidneys is in agreement with the findings that the glomerulus and the periglomerular region are seldom involved in this disease. The data also confirm the results of Tribe and Heptinstall(13), who found in rats that pyelonephritic scarring did not cause any substantial damage to the juxtaglomerular apparatus. Turgeon and Sommers(14) showed that in man also there were no changes in the juxtaglomerular cell count in cases of pyelonephritis with or without hypertension; and Fitz *et al*(15) reported normal

TABLE V. Effect of Repeated Renin Administration on Pyelonephritic Rats.

| | | Pyelonephritics (n = 8) | | | Controls (n = 8) | | |
|---|---|---|---|---|---|---|---|
| | | Before | p value | After | Before | p value | After |
| Systolic blood pressure | (mm Hg) | 114.9 ± 1.8 | .01* | 100.0 ± 5.0 | 109.8 ± 4.1 | >.05 | 108.6 ± 4.6 |
| Urine vol | (ml/12 hr) | 5.9 ± 1.0 | .01* | 19.7 ± 2.3 | 4.1 ± .7 | >.05 | 4.9 ± 1.3 |
| Wt | (g) | 223 ± 1.8 | .05* | 217 ± 2.0 | 212 ±7.1 | >.05 | 220 ± 9.5 |

* Significant differences.

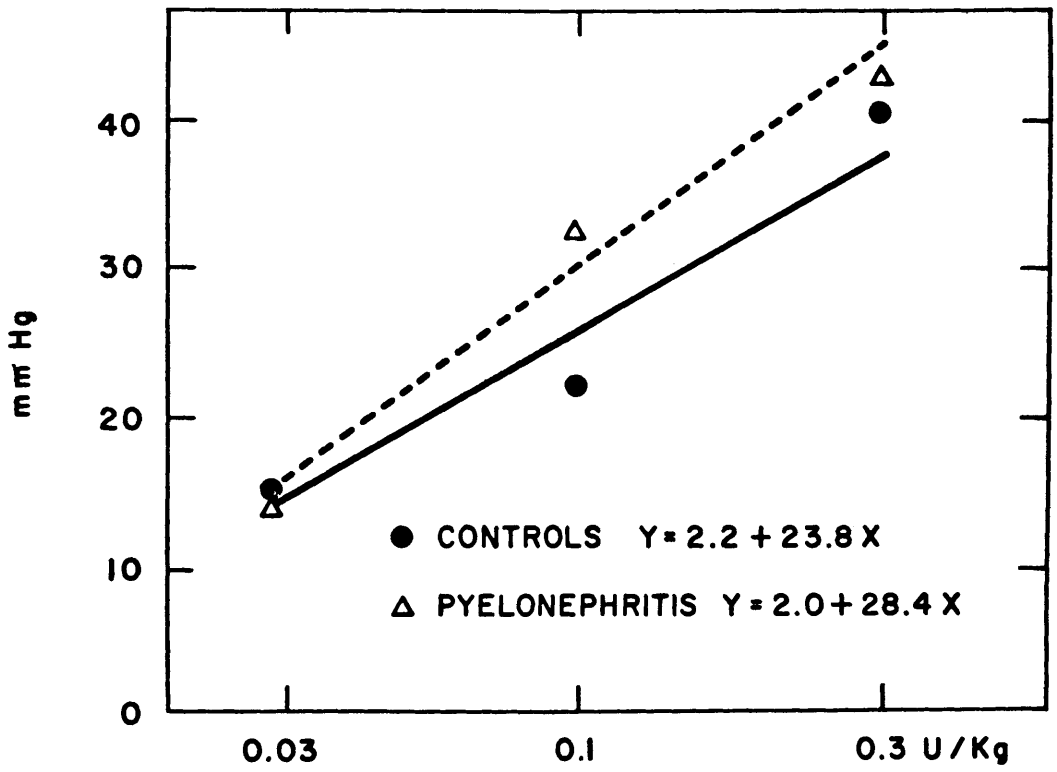


FIG. 1. Dose response curve of PLV-2 in pyelonephritic and control animals. Each point represents the average of determinations of 6 animals. Abscissa: Dose PLV-2 on log scale. Ordinate: Maximum blood pressure response in mm Hg.

plasma renin levels in pyelonephritis in man except for 3 cases with accelerated hypertension.

The findings of unchanged responsiveness of pyelonephritic rats to angiotensin—as measured by maximum pressor response—are in agreement with earlier experiments of Jones *et al*(10), who demonstrated that chronic administration of this vasoconstrictor polypeptide had the same effect in pyelonephritic and control animals. The fact that no changes in vascular reactivity can be demonstrated for compounds as different as angiotensin and PLV-2 suggests that lack of hyperreactivity is a general rather than a specific phenomenon. It appears then, that if pyelonephritis is a prehypertensive state, it differs from other similar states, as for example the hereditary tendency to hypertension, in which animals present an enhanced cardiovascular responsiveness(16). Similarly rats with a renal artery stenosis or pretreated with DOCA, even if not hypertensive, are more sensitive than controls when tested with vasopressin, angiotensin, or other vasoconstrictive compounds(17-20). The observation that pyelonephritic rats are not more sensitive to repeated applications of exogenous renin confirms this normal vascular reactivity. In addition, it is in agreement with the absence of changes in the pressor material content of the kidneys as experimental methods which result in a hypersensitivity to renin lead simultaneously to a disappearance of renin from the kidney(21). This is particularly true in unilaterally nephrectomized DOCA rats which show a pressor material content of their kidney almost reduced to zero and yet with small doses of exogenous renin develop an eclampsia-like syndrome with hypertension, edema formation and increased capillary permeability(23).

Our results indicate clearly that pyelonephritic rats do not present an increase of the renin-like material of their kidneys and that they do not show an enhancement

of their reactivity to angiotensin, renin or phenyllysine vasopressin. Therefore the explanation that changes in the renin-angiotensin cycle or an enhanced sensitivity to vasopressor stimuli might play a role in the susceptibility to hypertension in pyelonephritic animals appears unlikely.

Accordingly, our previous data concerning the effect of DOCA and salt on the blood pressure of pyelonephritic rats become pertinent in considering pathogenetic mechanisms to explain the relationships between hypertension and pyelonephritis. In advanced pyelonephritis, salt and water retention may develop which in turn may cause hypertension. This would be in keeping with the defects in renal function which develop in pyelonephritic animals, and in other forms of parenchymal renal disease, which make them both "salt wasters" when too little salt is given, and "salt retainers" when they receive too much for their diminished number of nephrons to handle. This hypothesis offers an alternative etiologic explanation to hypertension in parenchymal, in contrast with ischemic, renal disease which is in keeping with current concepts of defects in sodium and water homeostasis in these situations.

*Summary.* Although rats with chronic pyelonephritis do not develop hypertension directly, they show an increased sensitivity to DOCA hypertension and in addition pyelonephritis aggravates a pre-existing hypertensive disease. The purpose of this experimental study was to explore several possible mechanisms involved in these relationships. The likelihood that changes in the renin content of the kidney of pyelonephritic rats are associated with these phenomena first was investigated and it was demonstrated that extracts of scarred pyelonephritic kidneys contained the same amount of renin-like material as controls. In a separate experiment it was shown that the acute vascular reactivity of pyelonephritic rats to intravenous angiotensin and phenyllysine vasopressin was normal. These findings suggest alternate etiologic linkages between hypertension and chronic pyelonephritis such as changes resulting from impaired sodium and water homeostasis.

Appreciation is extended to Byron Croker for technical assistance.

1. Shapiro, A. P., Braude, A. I., Siemenski, J., Proc. Soc. Exp. Biol. and Med., 1956, v91, 18.

2. Vivaldi, E., Zangwill, D. P., Cotran, R., Kass, E. H., Biology of Pyelonephritis Little, Brown & Co., 1960, p27.

3. Heptinstall, R. M., Brit. J. Exp. Path., 1962, v43, 333.

4. Shapiro, A. P., Braude, A. I., Siemenski, J., J. Clin. Invest., 1959, v38, 1228.

5. Shapiro, A. P., Ann. Int. Med., 1963, v59, 37.

6. Shapiro, A. P., Kobernick, J. L., Circ. Res., 1961, v9, 869.

7. Shapiro, A. P., J. Lab. Clin. Med., 1960, v55, 715.

8. Shapiro, A. P., Moutsos, S. E., Krifcher, E., Sapira, J. D., Am. J. Cardiol, 1966, v17, 638.

9. Jones, R. K., Shapiro, A. P., J. Clin. Invest., 1963, v42, 179.

10. Jones, R. K., Gaskell, J. R., Shapiro, A. P., Invest. Urology, 1964, v1, 561.

11. Peart, W. S., Pharmacol. Rev., 1965, v17, 143.

12. Masson, G. M. C., Corcoran, A. C., Page, J. M., J. Lab. and Clin. Med., 1951, v38, 213.

13. Tribe, C. R., Heptinstall, R. M., Brit. J. Exp. Path., 1965, v46, 339.

14. Turgeon, C., Sommers, S. C., Am. J. Path., 1961, v38, 227.

15. Fitz, A. E., Kirkendall, W., Armstrong, M. L., Circulation, 1965, v32, Suppl. II, 84.

16. Dahl, L. K., Heine, M., Tassinari, L., ibid., 1964, v30, II, 11.

17. Turrian, H., Vitamins & Hormones, 1960, v18, 279.

18. Gaskell, J. R., Jones, R. K., Shapiro, A. P., Proc. Soc. Exp. Biol and Med., 1964, v115, 179.

19. Gardner, D. L., Honore, L. H., Arch. Int. Pharmacodyn., 1964, v150, 492

20. Ostrovsky, D., Cornall, A. G., Canad. Med. Assn. J., 1964, v90, 180.

21. Gross, F., Buschor, O., Zeugin, P., Am. J. Physiol., 1960, v199, 1.

22. Burn, J. H., Biological Standardisation, Oxford Univ. Press, 1952.

23. Gross, F., Zur Pathophysiologie des experimentellen renalen Hochdruckes. Symposium d. Med. Univ. Klinik, Munster (Westfalen), Georg Thieme Verlag, Stuttgart, 1962.

Received November 14, 1966. P.S.E.B.M., 1967, v124.