1. Campo, R. D., Dziewiatkowski, D. D., J. Biol. Chem., 1962, v237, 2729.
2. ———, J. Cell. Biol., 1963, v18, 19.
3. Campo, R. D., Wengert, P. A., Jr., Tourtellotte, C. D., Kirsch, M. A., Biochim. Biophys. Acta, 1966, v124, 101.
4. Joftes, D. L., Lab. Invest., 1959, v8, 131.
5. Dziewiatkowski, D. D., J. Exp. Med., 1951, v93, 451.
6. Pearse, A. G. E., Histochemistry, Little, Brown & Co., 1960, p917.

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Renal Function Studies in the Early Stage of Salt Hypertension in Rats.* (32135)

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The evidence for a genetic component in the pathogenesis of human essential hypertension suggests that a prehypertensive state must exist and if so, that it may be possible to recognize the potentially hypertensive individual prior to the development of clinical hypertension. In experimental hypertension a genetic factor is manifested by the fact that we have developed two strains of rats, one of which is susceptible to, whereas the other is resistant to, manipulations commonly used to provoke experimental hypertension(1,2,3). We are exploring physiological parameters that may differentiate these two strains of rats before the onset of hypertension, in the hope that such differences will also be applicable in establishing the diagnosis of a "prehypertensive state" in man.

Previous studies have shown that rats of the strain prone to hypertension (the S strain) manifested an increased vascular reactivity in response to vasopressor agents(4) and had a relative aversion for NaCl when offered a free choice between water and saline(5) compared to those of the other (the R strain). On the other hand, rats of both strains responded similarly to acute salt loading(6) and had comparable values for total body sodium and exchangeable sodium(7).

The present report of renal function studies in the Sensitive strain was undertaken as part of a more general investigation of the role of the kidney in experimental hypertension. This study showed no evidence of gross renal dysfunction either before or during the early development of experimental hypertension from salt ingestion.

Material and methods. Thirty-one female weanling rats of the Sensitive (S) strain were maintained on a low salt chow (0.38% NaCl) and tap water until the age of 6 weeks, when they were randomly divided into 2 groups. At this time, half of the animals, the "high salt" group, were fed a diet containing 8% NaCl whereas the other half remained on the low salt chow, and served as controls. The first renal function studies were performed on 14 animals (7 from each group) after 2 weeks on this regimen; a second set of tests was performed on the remaining 17 animals (9 "high salt" and 8 controls)—after 6 weeks on the regimen.

The studies were carried out in unanesthetized, undisturbed rats, by a modification of the method of Kleinman *et al*‡(8). Two to three days before the clearance studies, polyethylene cannulae were introduced under ether anesthesia in the jugular vein, the abdominal aorta, and the dome of the bladder. The tubes were led subcutaneously to the back of the neck, exteriorized and shielded by a plastic tube attached to the skin. Thereafter the jugular and aortic cannulae were

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TABLE I. Renal Function Studies in Young Female Rats from a Strain Genetically Prone to Hypertension (S-rats).

Time on different regimen	(A) 2 Weeks		(B) 6 Weeks	
Diet	High salt	Low salt	High salt	Low salt
No. of rats	7	7	9	8
Wt (g)	191 ± 13	187 ± 14	211 ± 5	226 ± 6
B.P. (mm Hg)	143 ± 5	114 ± 12	167 ± 19	121 ± 6
GFR (Cin) (ml/kg/min.)	8.7 ± 1.1	9.6 ± 1.2	9.2 ± 1.8	8.3 ± .94
RPF (C_{PAH}) (ml/kg/min.)	24 ± 3	23 ± 2.6	27 ± 2	24 ± 5
F F (%)	37 ± 4	41 ± 6	35 ± 6	35 ± 3

The high salt group received 8% NaCl in their food for 2 or 6 weeks. The low salt group was fed a chow containing 0.38% NaCl.

Values are averages ± S.D.

The difference in blood pressure between animals on different diets was highly significant in each set of experiments ($p<0.01$).

flushed daily with 0.1 ml of heparin-saline (100 mg%). Systolic blood pressure was measured before surgery by the microphonic method of Friedman and Freed(9). The animals had free access to food and water until the start of the clearance studies which were usually performed in the morning.

Glomerular filtration was estimated by inulin clearance and renal plasma flow by the clearance of para-aminohippurate. A primary intravenous dose of 1 μc carboxyl-labeled inulin C^{14} and 20 mg PAH was followed by a sustaining infusion administered at the rate of 1.2 ml/hr, which delivered 1.3-2 μc inulin, and 15-20 mg PAH per hour. Two hours were allowed for equilibration followed by 3 consecutive 30-minute clearance periods. Aortic blood samples (.5 ml whole blood) were obtained at the beginning of the first, the middle of the second, and the end of the test periods, and mid-point values were calculated by interpolation. Urine was collected in glass plates or plastic bags placed below the cage. At the end of each collection period the bladder was rinsed with 3 ml of distilled water, which the animal excreted through the urethra. The containers were thoroughly rinsed with distilled water and their contents diluted to 100 ml. Radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb, model 314 A), on aliquots of 25 μl plasma and 100 μl diluted urine. The degree of quenching was found to be less than 5% and no corrections were made for this. Chemical analysis of Inulin was not performed; Inulin concentration was considered proportional to specific activity of C^{14}. According to the manufacturer,§ the preparation used in this study had an average molecular weight of 5,300 and with a relatively tight distribution curve. Even after prolonged storage in solution the activity was eluted as a single peak from a column of Sephadex G-10. Recovery from the column was 95%. However, calculations of distribution volume in our animals gave results larger than those reported in the literature for "Inulin space"; and after a single injection in a human subject only 93% of the activity was recovered in urine. The values for Inulin clearance reported here may therefore be slightly off from the true glomerular filtration rate in the rat which may account for the high values of calculated filtration fraction. However, the values compare favorably with those reported by Peters and obtained with a chemical method(11). Since the present study is comparative, we consider the method to be suitable for our purpose. PAH was determined by the method of Smith *et al*(10), but 1:10 trichloracetic acid filtrate was used instead of $CdSO_4$-NaOH recommended by these authors. Recovery from pooled rat plasma of added PAH was 100.1% ± .71 in 12 samples at three different levels of concentration. Statistical significance of the difference between the means was calculated by Student's t test.

Results. Table I-A summarizes the first set of observations in 14 rats. The group of 7 animals maintained on the high salt regi-

§ New England Nuclear Corp., Boston, Mass.

men for 2 weeks had a modest but significant elevation in B.P. compared with the group of 7 controls ($p<0.001$); weights were similar ($p>0.1$). Clearance studies revealed no significant difference in GFR and RPF between the mildly hypertensive and the normotensive rats. Table I-B summarizes results of the second set of experiments on 17 rats, of which 9 had been fed the high salt diet for 6 weeks. As expected, all the high salt animals had developed frank hypertension, while the controls remained normotensive. In spite of the marked difference in B.P. ($p<0.001$), the data revealed no significant difference in the measured parameters between hypertensive and normotensive rats.

Discussion. In this study, the values for GFR and RPF obtained in rats from a strain genetically prone to high blood pressure were comparable to those reported in normal unanesthetized animals(8,11). During the early phase of *salt* hypertension, no evidence was obtained of an impairment in these functions. In a recent study, Peters *et al*(12) also found no evidence of decreased kidney function in intact *renal* hypertensive rats in spite of B.P. readings above 160 mm Hg. Earlier studies by Koletsky(13) indicated that hypertension often preceded histological changes in kidney and blood vessels occurring in rats fed excess salt. Our findings are thus consonant with these studies and provide additional physiological evidence in support of Koletsky's morphological studies in salt hypertension. They are also in agreement with studies in human beings in which normal renal function tests were found in the early phase of essential hypertension(14).

In patients with essential hypertension, a reduction of TM_{PAH} and renal plasma flow and an elevation in the filtration fraction were described as evidence of incipient renal dysfunction(15). In our study, a relatively high filtration fraction was commonly present in both hypertensive and normotensive animals. Other studies in normal unanesthetized rats similarly have described a wide variation in renal plasma flow and a high filtration fraction, which are so far unexplained(11).

In this report we have not included function studies on animals from the Resistant strain, since it was assumed that abnormalities if present would be found in the animals susceptible to hypertension rather than in those resistant to it. However, unpublished studies in R rats have given renal clearance values similar to those reported here.

These results do not exclude, of course, the possibility that in spite of normal values for GFR and RPF animals from the Sensitive strain may have more discrete abnormalities in kidney functions.

Summary. Renal function studies were performed in 2 groups of young rats from a strain genetically prone to hypertension. The animals receiving a high NaCl diet rapidly developed moderate hypertension, while those maintained on a low NaCl regimen remained normotensive. GFR and RPF were comparable in both groups, 2 and 6 weeks after initiation of the high salt regimen, in spite of significant differences in blood pressure. It was concluded that the development of salt hypertension in these rats was *not* associated with evidence of impaired glomerular filtration rate or renal plasma flow during the early phases of the disease.

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1. Dahl, L. K., Heine, M., Tassinari, L., J. Exp. Med., 1962, v115, 1173.
2. ———, ibid., 1963, v118, 605.
3. ———, ibid., 1965, v122, 533.
4. ———, Circulation, Supp. II, 1964, 11.
5. Wolf, G., Dahl, L. K., Miller, N. E., Proc. Soc. Exp. Biol. & Med., 1965, v120, 301.
6. Ben-Ishay, D., Dahl, L. K., ibid., 1966, v123, 304.
7. Schackow, E., Dahl, L. K., ibid., 1966, v122, 952.
8. Kleinman, L. I., Radford, E. P., Jr., Torreli, G., Am. J. Physiol., 1965, v208, 578.
9. Friedman, M., Freed, S. C., Proc. Soc. Exp. Biol. & Med., 1949, v70, 670.
10. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., Graber, M., J. Clin. Invest., 1945, v24, 388.
11. Peters, G., Am. J. Physiol., 1963, v205, 1042.
12. Peters, G., Brunner, H., Gross, F., Nephron, 1964, v1, 295.
13. Koletsky, S., Arch. Path., 1959, v68, 11.
14. Baldwin, D. S., Hulet, W. H., Biggs, A. W., Gombos, E. A., Chasis, H., J. Clin. Invest., 1960,

v39, 395.

15. Goldring, W., Chasis, H., Ranges, H. A., Smith, H. W., ibid., 1941, v20, 637.

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Mechanical Responses of Intestinal Smooth Muscle in a Calcium-Free Medium.* (32136)

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Investigations carried out to determine the role of calcium in smooth muscle function have led to the postulate that calcium is bound to more than one site in the smooth muscle fiber. Part of the calcium in the muscle appears to be complexed with superficial loci. The calcium at this site equilibrates rapidly with extracellular calcium ions and is involved in the regulation of membrane permeability. In addition, calcium bound to superficial loci or present in the extracellular fluid may, under appropriate conditions, migrate into the cytoplasm and activate contractile proteins. Another part of the fiber calcium appears to be combined with loci that are less accessible to the external milieu. The calcium at this site equilibrates more slowly with extracellular calcium ions, does not seem to affect membrane permeability, and can also be mobilized to activate contractile elements (1,2,3,4).

The verification and extension of these concepts are important but still incomplete. In this study we used an experimental procedure which conditions an intestinal smooth muscle preparation to exhibit a high degree of mechanical activity in a calcium-free environment. The tests subsequently performed gave some indication of the ways in which extracellular calcium ions, a high potassium medium, and acetylcholine affect the accumulation and/or release of calcium at a poorly accessible site in the muscle.

Methods. The tissue preparation used was the isolated longitudinal muscle from guinea pig ileum. A segment approximately 3 cm long was suspended in a muscle bath which contained 10 ml of a physiological salt solution. The temperature of the bath was maintained at 31-32°C. Isotonic contractions of the muscle were recorded on a standard kymograph. Tension on the muscle was approximately 0.35 g.

The physiological salt solution had the following composition: NaCl, 125 mM; KCl, 2.7 mM; $CaCl_2$, 1.8 mM; glucose, 11 mM; and tris [tris (hydroxymethyl) aminomethane, Sigma-trizma base] buffer, 23.8 mM. The solution was adjusted to pH 7.5 with 6 N HCl and was saturated with 100% oxygen. The composition of the high potassium medium was identical with that of the physiological salt solution except that all the NaCl was replaced by an equimolar quantity of KCl.

Calcium content of the muscle was measured by the flame photometric method described by Geyer and Bowie(5).

Modifications in the efflux of calcium ions from the longitudinal muscle fibers were estimated from measurements of the rate of outflow of Ca^{45} from the tissue. Ca^{45} was counted in a liquid scintillation counter.

Results. The isolated longitudinal muscle undergoes changes in muscle tone if the physiological salt solution in which it is immersed is exchanged for a bathing medium lacking in calcium ions. The magnitudes of the changes induced are dependent upon the level of calcium to which the tissue is exposed prior to the introduction of the calcium-free medium. These effects were demonstrated in the following manner. A muscle was incubated in the physiological salt solution for

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