pounds cannot be assigned from this study, nevertheless it is evident that the trimethylsilyl ether is much more effective than a similar dose of testosterone propionate by 7 days after a single injection.

In an earlier study(2) I reported the prolonged effects of a single injection of 4-estrene-3β,17β-diol 3,17-dipropionate in castrated rats. The maximal response to this dipropionate was achieved by the tenth day as indicated by seminal vesicle weights of 134 mg and ventral prostate weights of 113 mg. With the trimethylsilyl ether, the maximal seminal vesicle weights, 237 mg, were observed 20 days, and the maximal ventral prostate gland weights, 232 mg, 30 days after the single injection.

In somewhat similar tests the peak ventral prostate response to testosterone-17-cyclopentylpropionate was observed at 14 days (Sala and Baldratti(3)). The 3-cyclopentyl enol ether of methyltestosterone produced its maximal effect only a few days later than did methyltestosterone (Meli(4)). Diczfalusy(5) reported peak effects after a single injection of testosterone-17-enanthate at 16 to 28 days, testosterone-17-cyclopentylpropionate at 16 to 28 days while the p-hexophenyl propionic acid ester of testosterone produced its peak response at 42 days. In a review of steroid esters, Junkmann and Witzel(6) indicated a maximal response to the 17-enanthate (14 days) and the cyclopentylpropionate (12 days) only slightly delayed compared to the propionate (11 days).

Interest in the trimethylsilyl ether of testosterone arises from its long duration of action, the magnitude of the ultimate response in the male accessory organs and its unique chemical composition.

Summary. Substitution of a trimethylsilyl ether for the propionic acid moiety in testosterone propionate resulted in a peculiarly long-acting androgenic compound. The maximal response to a single subcutaneous injection in castrated rats was obtained 20 to 30 days after treatment. Not only was the duration of effectiveness of the trimethylsilyl ether much greater than that of the same dose of testosterone propionate, but the ultimate magnitude of the response was also increased.

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Absence of an Exaggerated Renal Response to Acute Salt Loading In Salt-Hypertensive Rats.* (31473)

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Hypertensive individuals generally respond to expansion of their body fluids by a greater natriuresis and diuresis than normotensive subjects. The mechanism for this phenomenon remains unexplained. It was suggested that the enhanced natriuresis was due to the high pressure *per se,* and was consequently a result of the hypertensive state(3,14,16). Other workers have attributed the phenomenon to an abnormal sensitivity of a volume regulating mechanism associated with hypertension(24,26), and possibly preceding the advent of high blood pressure(25).

In man, a basic difficulty in determining whether the exaggerated response to acute salt

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loading precedes, or follows, the onset of hypertension is that the diagnosis of the latter can be made with certainty only *after* elevated pressure has been found. In contrast, by selective inbreeding we have evolved a strain of rats that will invariably develop experimental hypertension, using any one of several standard techniques including salt ingestion(4,5,6). Simultaneously, another strain has been evolved resistant to the manipulations which uniformly produce hypertension in the first strain.

The current report on the response to acute NaCl loading is based on a study of animals from the 2 strains over a period of 3 months before and during the development of salt-induced hypertension in members from the Sensitive (or S) strain; on the same regimen rats from the Resistant (or R) strain remained normotensive. It was found that under the conditions of these experiments an elevated B.P. was not associated with enhanced natriuresis in the rat, but that the response was influenced by the salt content of the diet.

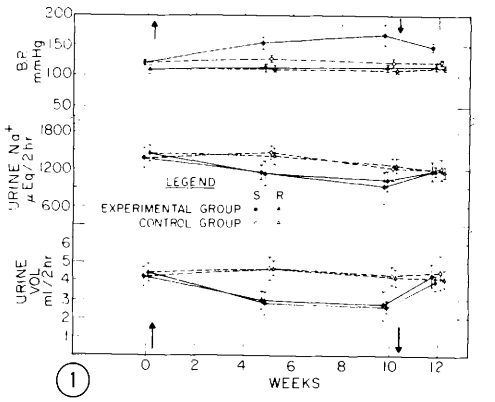
Material and methods. 17 S and 18 R female rats, maintained from weaning at 3 weeks of age on a low salt chow (0.38% NaCl) were first subjected to a standard oral load of hypertonic saline at the age of 9 weeks. The test was slightly modified from that of Friedman(10). The experiments were performed in the morning following an overnight fast of 15-17 hours, during which drinking water was permitted. The bladder was voided by manual compression and the animals placed in individual metabolic cages fitted with sodium-free plastic bags for urine collections. After a 2-hour control period, 3 ml of 0.57 M saline (1700 μEq Na) were instilled into the stomach, and urine was collected for an additional 2-hour period. After this initial loading experiment, the animals were divided into two groups. The experimental group, consisting of 11 S and 12 R rats, received a high salt (8% NaCl) chow for 10 weeks, followed by the low salt (0.38% NaCl) chow for 2 weeks. Loading experiments (performed as described above) were repeated at the middle and end of the period on high salt, and again after the 2 weeks on

TABLE I. Response to a Standard Oral Salt Load in Hypertensive and Normotensive Rats on High and Low Salt Intake.

Weeks	0		5			10			12		
Group	Baseline		Experimental		Control	Experimental		Control	Experimental		Control
Diet	Low salt		High salt		Low salt	High salt		Low salt	Low salt		Low salt
Strain (n)	S (17)	R (18)	S (11)	R (12)	S (6) R (6)	S (11)	R (12)	S (6) R (6)	S (11)	R (12)	S (6) R (6)
B.P., mm Hg	120 ±5	109 ±6	153 ±10	113 ±6	128 111 ±6 ±5	168 ±17	114 ±4	122 111 ±7 ±4	148 ±7	116 ±6	124 115 ±4 ±3
Urine vol, ml/2 hr	4.2 ±.52	4.4 ±.54	3 ±.54	2.8 ±.67	4.7 4.7 ±.66 ±.41	2.7 ±.84	2.6 ±.30	4.3 4.2 ±.47 ±.51	4.3 ±.63	3.9 ±.44	4.5 4.1 ±.87 ±.49
Urine Na, μEq/2 hr	1381 ±168	1452 ±135	1157 ±113	1134 ±187	1482 1425 ±110 ±141	913 ±267	1029 ±164	1214 1276 ±128 ±115	1187 ±158	1187 ±78	1218 1178 ±172 ±87
Urine Na, mEq/l	324	329	384	403	315 303	338	396	282 303	277	305	270 287

The load consisted of 3 ml of .57 M saline (1700 μEq Na). The high salt diet contained 8% NaCl and the low salt diet 0.38% NaCl. Values are mean ± S.D. (n) = number of animals. S = Sensitive strain. R = Resistant strain.

Significance of differences: In all instances, B.P. of S animals in each group exceeded that of corresponding R animals, $p < 0.001$. Urine volumes and Na excretion were similar for S and R animals on the same salt intake, $p > 0.1$. Animals from both strains on high salt excreted less urine and less Na than their controls, $p < 0.005$. After 2 wk on low salt, urine and Na excretion in the experimental group had returned to control levels, $p > 0.1$, and while B.P. of hypertensive (S) animals had decreased as compared with value at 10 wk ($p < 0.05$), B.P. of this group was still significantly elevated compared with other 3 groups in test ($p < 0.001$).



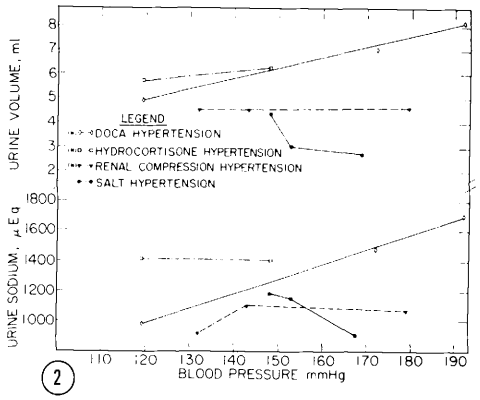


FIG. 1. Response to a standard oral salt load in hypertensive and normotensive rats. At the time of the first load the animals were 9 weeks old, and had been on a low salt diet (0.38% NaCl) for 6 weeks. The load consisted of 3 ml of .57 M saline (1700 μEq Na). ↑ = Experimental group placed on high salt diet (8% NaCl). ↓ = Experimental group placed on low salt diet (0.38% NaCl). Values represent mean ± S.D.

FIG. 2. Relationship between blood pressure and response to a standard oral salt load in 4 types of experimental hypertension in rats. The load consisted of 3 ml. .57 M saline (1700 μEq Na). Friedman's data are average cumulative excretion 150 min after load. Our data are average excretion at 120 min. after load. * = values taken from Friedman *et al*(10).

low salt. The control group, with 6 S and 6 R rats, received the low salt diet continuously throughout these studies and was studied simultaneously with the experimental group.

Systolic blood pressure was measured under light ether anesthesia usually within one day after the test by a modification of the tail microphonic method(9). Urine sodium was determined by flame photometry. Statistical significance of the difference between the means of groups was calculated by Student's t test. In early experiments, urine and sodium outputs were determined during the control period to estimate possible differences in basal excretion: this procedure was discountinued when no significant difference was observed between the groups.

Results. The data summarized in Table I show that, when they were on comparable NaCl intake, S and R animals responded similarly to an acute salt load.

At the time of the first test, at the age of 9 weeks, the rats had never been exposed to a high salt regimen. Yet, the average B.P. in the Sensitive (S) strain, while still within "normal," was significantly higher than in the Resistant (R) strain (Table I, Baseline). This initial difference was amplified by the administration of a high salt diet for 10 weeks to the experimental group. The S rats showed a gradual and highly significant elevation in B.P. whereas that of the R rats remained unchanged. In spite of the marked difference in pressure between the two strains, their response to the loading remained comparable, both at the 5th and the 10th week of the experiment (Table I, Fig. 1). However, animals from both strains on high salt diet, with or without high blood pressure, excreted significantly less water and sodium than those on low salt ($p<0.005$). After 5 weeks on high salt intake the experimental group excreted an average of 38% less water and 21% less Na than the control group. The tendency of the former group on high salt to conserve water, in the presence of a hypertonic load, was reflected in its higher urine sodium concentration (Table I).

When the experimental group was placed on low salt diet and the test repeated after 2 weeks both S and R rats manifested a significant increase in water ($p<.001$) and sodium outputs ($p<.05$) compared to their former response on the high salt diet (Table I). The experimental and control groups were now indistinguishable in their response to the load. The S members of the experimental group remained hypertensive, although some decrease in their blood pressure was recorded. On comparing the results of the two loading tests

which mark the 12-week interval between the beginning and the end of this experiment, all the animals manifested a decreased natriuresis ($p<.005$) but no change in the diuretic response.

Discussion. In this study we found that rats with opposite genetic susceptibility to hypertension responded similarly when challenged with an acute load of hypertonic saline. Elevation of B.P. in the strain predisposed to hypertension (the Sensitive or S strain) had no effect on the salt and water excretion. In these salt-hypertensive rats, there appeared to be no correlation between blood pressure level and response to an acute salt load.

Our results are in accordance with some studies in renal hypertensive rats(8,22) in which an augmented natriuresis was absent, but differ from others in which an exaggerated response was reported(2,10,12). A review of the latter studies suggests, however, that the phenomenon was not an invariable accompaniment of experimental hypertension. Thus, in rats with perinephritic hypertension, exaggerated natriuresis was observed in the earlier, but not in the later, stages of the disease(12); and in rats rendered hypertensive by unilateral clamping of the renal artery, an abnormal response was elicited by an oral, but not by an intravenous, salt load (2,22). In view of these reports, we tested in two separate sets of experiments young hypertensive rats after only 2 weeks on high salt (Avg. B.P. 150 mm Hg) and also subjected other rats with salt hypertension to both intravenous and oral loads: in none of these experiments were we able to elicit an exaggerated response. Further, since uninephrectomy was a standard procedure in some studies where accelerated natriuresis was observed(10,12), 24 animals from the present experiment were uninephrectomized and the studies repeated as in the original experiment: exaggerated natriuretic and diuretic responses still did not occur in hypertensive animals.

Friedman *et al*(10) performed salt loading tests in uninephrectomized rats with 3 types of experimental hypertension. An accelerated response approximately proportional to blood pressure level was reported in the operated animals treated with DOCA-NaCl as well as in those subjected to compression of the remaining kidney, but not in those treated with hydrocortisone. Since their loading procedure was virtually the same as ours, we have plotted the results obtained in these 4 types of experimental hypertension in Fig. 2. In general, there seems to be a correlation between B.P. levels and the magnitude of the diuretic response only in the DOCA-hypertensive rats. The Figure also shows a considerable variability in response among rats with comparable levels of blood pressure induced by different methods: thus DOCA-hypertensive rats (B.P. 172 mm Hg) had a significantly higher natriuresis and diuresis than rats with either salt hypertension (B.P. 168 mm Hg) or renal compression hypertension (B.P. 179 mm Hg). Furthermore, hydrocortisone-treated rats, irrespective of their blood pressure, excreted the same or perhaps more water and sodium than did other animals at comparable B.P. levels. In sum, data summarized in Fig. 2 suggest that in rats an elevated B.P. is not necessarily associated with an exaggerated natriuretic-diuretic response to acute salt loading.

The relatively inconstant occurrence of an accelerated natriuresis in hypertensive rats may be due to the high capacity of the rat kidney to conserve sodium. Micropuncture studies during hypertonic saline loading in normal rats revealed no change in the fractional reabsorption of sodium by the proximal tubule, in the presence of increasing filtered load(11). By contrast, in dogs, isotonic and hypertonic salt loads induced a marked depression in the fractional reabsorption of sodium(7). This propensity of the rat to retain sodium in the presence of increased filtered load, may thus explain not only the generally absent exaggerated natriuresis, but also the high susceptibility of this species, as contrasted with dogs, to develop salt-hypertension.

The variable natriuretic responses observed in different forms of experimental hypertension might be explained if those in which tubular lesions were more prominent evinced the phenomenon and *vice versa*. The experimental evidence is suggestive that this

may be true. Knowlton *et al*(17) have described the striking changes in renal tubular epithelium in rats given DOCA and NaCl. By contrast, in cortisone-hypertension(18), and in the early stages of salt hypertension (19) such tubular changes were absent. According to this interpretation, the phenomenon of exaggerated natriuresis would be a manifestation of the degree of altered tubular structure and presumably would be present in varieties of experimental hypertension in which tubular changes were prominent early.

In our experiments, one factor that clearly modified the response to a salt load was the salt content of the diet: rats from both strains maintained on high salt chow invariably excreted less water and sodium after loading than did rats on low salt. The explanation for this phenomenon is obscure. Possibly it is related to the action of ADH. Radford has reported that a high salt diet increased the concentrating ability of the kidney(23). Later Little and Radford(20) found elevated plasma ADH levels in rats fed a diet containing 6% NaCl on the basis of which they suggested that transient rises in plasma osmotic pressure due to ingestion of highly salted food could stimulate an increase in circulating ADH. In our study, systematic estimates of plasma Na concentration were not made but such measurements on many similar rats fed 8% NaCl chow have not shown *chronic* elevation of plasma Na: however, this is not incompatible with *transient* elevations such as would be predicted from the data of Little and Radford. Serum osmolality has not been measured in our rats. The explanation proposed by Little and Radford accords with our results relative to differences in water excretion on high and low NaCl intakes.

The results in our studies probably cannot be ascribed to renal damage from the salt since reversion to a low salt regimen caused the diuretic and natriuretic responses to return to the levels of the control groups from both strains. Furthermore, in studies now being readied for publication, renal function tests in rats with comparable salt-hypertension revealed normal values for glomerular filtration rate and renal plasma flow.

The evidence at hand suggests that, while hypertensive human subjects are frequently hyperexcretors of an acute salt load, hypertensive rats usually do not manifest this phenomenon. The reason for the disparity between species is not completely understood, but it may be related to the unusual capacity of the rat kidney to conserve sodium. On the other hand, the phenomenon is not universally present in hypertensive patients since only 40% of the individuals in one study were hyperexcretors(13) and patients with renal hypertension were recently reported to be normoexcretors(21). The effect of dietary sodium on the excretion of a salt load also seems to act in opposite direction in man and rat. In human hypertensive subjects a high salt diet was reported to enhance(1,15), whereas in our rats it depressed, the rate of excretion of an acute salt load. Possibly there is a difference in the speed with which aldosterone control becomes effective in the two species; a second speculation is that under the influence of a high salt diet, rats have a greater capacity than man to store sodium in some relatively "inert" pool.

In conclusion, the available evidence in both hypertensive man and rats suggests that the response to acute salt loading cannot be solely related to high blood pressure *per se*, and that in rats the quantity of salt in the diet has a direct effect on the rate at which the load is eliminated.

Summary. The response to a standard oral load of hypertonic saline was studied in two strains of rats with opposite genetic susceptibility to hypertension. Following ingestion of a chow containing 8% NaCl, the B.P. of the strain predisposed to hypertension rose to an average of 168 mm Hg, while that of the strain resistant to developing hypertension remained 114 mm Hg. Control animals of both strains ingesting a 0.38% NaCl chow remained normotensive. The natriuretic and diuretic response was the same in hypertensive and normotensive rats on high salt, but a significant difference was observed between these animals and their controls on low salt in that the latter excreted more water and sodium. Reversal from a high salt to a low salt diet enhanced diuresis and natriuresis comparably in both hypertensive and nor-

motensive rats. Evidence is summarized indicating that an exaggerated natriuresis after an acute salt load in the hypertensive rat is not constantly present, and it was proposed that the phenomenon might be related to altered tubular structure. Evidence from other workers indicates that ADH secretion is enhanced by a high salt diet and this may play a role in the response to a salt load in rats.

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Uremic Serum Inhibition of Renal Paraaminohippurate Transport. (31474)

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The effects of renal insufficiency, including uremia, on various organ systems are well-known(1). Nevertheless, little attention has been paid to the possible effects of renal insufficiency on the kidney itself. The studies described in the report represent an initial exploration of this possibility(2).

White rats, weighing approximately 150 g, had both kidneys removed under ether anesthesia. Forty-eight hours later, they were sacrificed by decapitation. Each series of experiments was performed with a pooled collection of uremic serum, as well as with a pooled collection of normal serum. The BUN of rat uremic serum ranged from 240 to 312 mg%.