J. L., Proc. Nat. Acad. Sci., 1964, v52, 1138.

10. Sabin, A. B., Koch, M. A., ibid., 1964, v52, 1131.

11. Hoggan, M. D., Rowe, W. P., Black, P. H., Huebner, R. J., ibid., 1965, v53, 12.

12. Gilden, R. V., Carp, R. I., Taguchi, F., Defendi, V., ibid., 1965, v53, 684.

13. Melnick, J. L., Stinebaugh, S. E., Rapp, F., J. Exp. Med., 1964, v119, 313.

14. Melnick, J. L., Wenner, H. A., Rosen, L., in Diagnostic Procedures for Viral and Rickettsial Diseases, 3rd ed., Am. Public Health Assn., 1964, p194.

15. Ashkenazi, A., Melnick, J. L., J. Nat. Cancer Inst., 1963, v30, 1227.

16. Melnick, J. L., Khera, K. S., Rapp, F., Virology, 1964, v23, 430.

17. Melnick, J. L., Serology of viruses. In Analytical Serology of Microorganisms, J. B. G. Kwapinski, ed., John Wiley & Sons, New York, 1967, in press.

18. Sabin, A. B., Koch, M. A., Proc. Nat. Acad. Sci., 1963, v50, 407.

19. Geder, L., Sabin, A. B., cited by Sabin, A. B., in Malignant Transformation by Viruses (ed. W. H. Kirsten) Springer-Verlag, New York, 1966, p164.

20. Vonka, V., Zavadova, H., Kutinova, L., Rezacova, D., Proc. Soc. Exp. Biol. & Med., 1967, v125, 790.

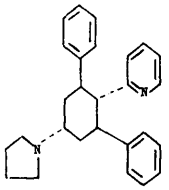
Received April 10, 1967. P.S.E.B.M., 1967, v125.

A New Diuretic Drug Dependent on the Adrenal for its Action. (32209)

ROBERT GAUNT, A. A. RENZI,* NANCY HOWIE, ELVIRA GISOLDI, AND PATRICIA WALDRON

Research Department, CIBA Pharmaceutical Co., Summit, N. J.

In chemical studies of compounds related to lobinaline, Robinson and coworkers(1) of this laboratory synthesized the following substance:



2-[2,6-Diphenyl-4(1-pyrrolidinyl) cyclohexyl]-pyridine

(Su-15049)

Its primary pharmacological action in rats is reported here. It was to induce a natriuresis and water diuresis with little or no kaliuresis. Similar actions in dogs have been shown by Barrett *et al*(6) and Cohen and Cafruny(7).

Methods. Unless otherwise stated, male rats (180-200 g) were fasted overnight but allowed water and then given 0.2% NaCl, 5 ml/100 g, by stomach tube. The animals were then placed in individual metabolism cages and urinary volumes recorded every 30 minutes for 3 hours. Sodium and potassium content of the total urine collections was measured at the end of 3 hours by flame photometry.

Su-15049† was suspended in a vehicle of carboxymethylcellulose and administered by stomach tube at the time of fluid loading. The steroids used were similarly suspended except for the low doses (0.2 and 10 μg) of aldosterone which were in aqueous solution. The steroids were given subcutaneously one hour before fluid loading and dosages indicated in Table I are per rat. Control groups, either intact or adrenalectomized, received the appropriate vehicle or solvent of the drug being tested.

In most of the groups shown in Table I, in which individual drugs or treatments were being compared, equal numbers of treated and control animals were included in each day's run. Series 1, on the other hand, is made up largely of a pool of control animals

* Present address: Dept. of Pharmacology, Dow Chemical Co., Human Health Division, Zionsville, Ind.

† The water-soluble citrate of Su-15049 (designated Su-15049A) is being used by most other groups studying this compound because of its greater convenience. With regard to sodium excretion, the dose-response curve of Su-15049A in rats is identical to that of Su-15049 (Fig. 1), when given in equimolar amounts of base. A slight enhancement of potassium excretion was not of proven signficance.

TABLE I. Effects of Su-15049 on Electrolyte and Water Excretion.

Treatment	No. rats	Excretion: Water, % load	Excretion: Na^+, mEq × 100	Excretion: K^+, mEq × 100	Urine Na/K ratio	Statistical notes
Series 1. Su-15049 (12.5 mg/kg) in adrenalectomized animals						
Vehicle	38	70	11.9	6.4	2.10	Adx. increased Na^+ ($P=.05$) and decreased K^+ ($P=.01$) excretion. Su-15049 reduced water and K^+ excretion ($P=.01$) but had no effect on Na^+ excretion.
Adx. + vehicle	80	65	15.0	4.8	3.34	
Adx. + Su-15049	77	53	13.4	3.7	3.84	
Series 2. Su-15049 (12.5 mg/kg) and aldosterone in adrenalectomized animals						
Adx. + vehicle	35	61	13.4	4.5	3.25	Su-15049 did not antagonize Na-retaining effect of aldosterone except when latter given at .5 mg dose ($P=<.001$). It increased Na/K ratio ($P=<.01$) at 2 higher aldosterone doses.
Aldo., .2 μg	12	66	5.6	6.5	.84	
" " + Su-15049	12	48	8.0	4.2	1.45	
" , 10.0 μg	11	54	1.5	6.7	.22	
" " + Su-15049	11	56	3.6	5.5	.61	
" , .5 mg	12	75	1.7	17.5	.10	
" " + Su-15049	12	77	14.6	17.6	.85	
Series 3. Su-15049 (12.5 mg/kg) and cortisol in adrenalectomized animals						
Adx. + vehicle	30	62	13.4	4.8	3.00	Su-15049 in presence of cortisol exerts natriuretic effect and increases Na/K ratio ($P=<.001$) at both doses. It enhanced water diuresis ($P=<.001$) and kaliuresis ($P=.05$) at .5 mg dose.
Cortisol, .25 mg	12	81	13.4	18.8	.74	
" " + Su-15049	12	99	39.7	21.0	1.91	
" , .5 mg	18	80	9.4	22.1	.42	
" " + Su-15049	18	105	48.2	26.8	1.79	
Series 4. Su-15049 (12.5 mg/kg) + cortisol + aldosterone in adrenalectomized animals						
Adx. + vehicle	11	67	16.5	4.4	3.90	Su-15049 in presence of cortisol antagonized Na-retaining effect of aldosterone ($P=<.001$). It had no effect on K^+ excretion.
Su-15049	11	49	9.9	2.7	3.64	
Cortisol, .5 mg + aldo., 10 μg	11	70	2.9	18.5	.16	
Cortisol + aldo. + Su-15049	11	98	32.4	18.4	1.81	
Series 5. Su-15049 (12.5 mg/kg) and corticosterone in adrenalectomized animals						
Adx. + vehicle	12	72	19.5	5.5	3.45	Su-15049 in presence of corticosterone exerted weak Na-diuretic effect ($P=<.001$) without affecting K^+ excretion.
Su-15049	12	50	12.4	4.0	4.01	
Cortico., .5 mg	12	83	13.7	17.9	.75	
" " + Su-15049	12	86	25.1	16.8	1.59	
Series 6. Su-15049 and hydrochlorothiazide (1.25 mg/kg) in intact animals						
Vehicle	12	65	11.4	7.3	1.94	Su-15049 and HCTZ had sub-additive effects on water and Na^+ excretion ($P=<.001$). Su-15049 partially antagonized kaliuretic action of HCTZ ($P=<.001$).
Su-15049, 12.5 mg/kg	12	123	78.6	11.5	6.96	
HCTZ	12	101	65.8	19.6	3.49	
" + Su-15049	12	124	93.3	15.7	6.14	
Vehicle	11	72	15.7	8.4	1.93	
Su-15049, 25 mg/kg	11	108	76.7	8.3	10.17	
HCTZ	11	93	53.8	14.9	3.70	
" + Su-15049	11	123	99.9	10.2	10.32	

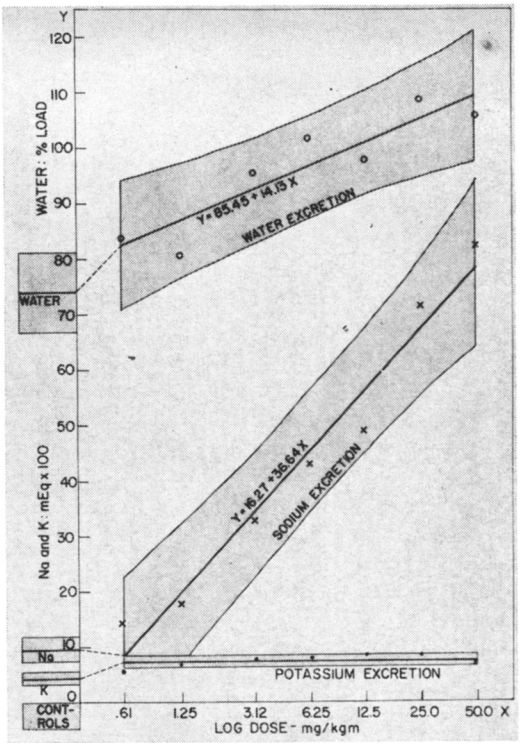


FIG. 1. Effects of Su-15049 on electrolyte and water excretion: log dose-response curves. The calculated straight line dose-response curves for water and sodium include 95% confidence belts. For potassium excretion and all control values, means with 95% confidence limits are shown. Control figures are based on 15 animals and treatment values on 4 animals per dose.

from other groups not necessarily paired as to time of observation.

In Table I, Series 1, statistical values were obtained by analyses of variance and t tests to which multiple comparisons criteria for significance were applied. In other cases when combined effects of two treatments were studied, significance of differences between groups was estimated from 2 × 2 factorial design in randomized blocks. The urinary Na/K ratios were analyzed in logarithms.

Effects on electrolyte and water excretion (Fig. 1). Su-15049 showed a natriuretic action in doses from 0.6 to 50 mg/kg. Results were erratic with doses higher than 50 mg/kg. The dose-response curve for water excretion was flatter than that for sodium, but high doses could cause the excretion of more fluid than was administered. There was, however, only a very slight, but real, effect on potassium excretion—an effect curiously not related to dose and not seen in all circumstances (see below). The urinary Na/K ratio values permit drawing a straight line log dose-response curve ascending from 2.26 to 12.47 (Log Y = 0.4395 + 0.35749X).

Effects of increased sodium loads. In the routine procedure used here the sodium load (0.2% NaCl) was light. When 5 ml/100 g of 0.9% NaCl was given and test methods otherwise identical, the qualitative response to Su-15049 was unchanged. Either 6 or 12 mg/kg approximately doubled urine volume and more than doubled sodium output ($P = < 0.001$) without any significant effect on potassium excretion.

Effects of adrenalectomy (Table I, Series 1). Tests were made in adrenalectomized rats 18 hours after operation. The operated group excreted slightly more sodium and less potassium than intact controls. Had the tests been made later than 18 hours after adrenalectomy or the fluid loads been larger, the well-known deficiencies of adrenal hypofunction in handling excess water and salt(2) presumably would have been apparent.

The effect of adrenalectomy was to block any significant natriuretic response to Su-15049. The drug did, however, reduce the excretion of water and potassium slightly. This is an unusual situation and an attempt was made to determine the replacement therapy needed to permit responsiveness to the drug.

Effect of aldosterone on response to Su-15049 (Table I, Series 2). Aldosterone in various doses produced its expected antinatriuretic and kaliuretic effects in adrenalectomized animals. When given with a standard (12.5 mg/kg) dose of Su-15049, the usual response to relatively high doses of aldosterone (0.2 or 10 μg/rat) was virtually unmodified by Su-15049. Hence, aldosterone does not make adrenalectomized animals responsive to Su-15049 and in a strict sense, Su-15049 cannot be considered an antagonist of aldosterone. Under other conditions, however, exceptions must be made to this statement as shown below.

It is known that massive doses of aldosterone—ones entirely outside physiological limits—can exert some glucocorticoid-like ef-

fects(3). It is of interest, therefore, that 0.5 mg of aldosterone given with Su-15049 permitted, like cortisol (see below), some slight manifestations of the drug's sodium diuretic action. We consider this cortisol-like action the probable cause of the otherwise anomalous fact that Su-15049 antagonized slightly a huge dose of aldosterone but not smaller ones.

Effect of cortisol on response to Su-15049 (Table I, Series 3). In a manner similar to the experiments with aldosterone, cortisol was given to adrenalectomized rats alone and with Su-15049. In the doses used, cortisol alone had little or no effect on water or sodium excretion but did cause a relatively large kaliuresis. Unlike the situation when aldosterone was given, Su-15049 clearly exerted, when cortisol was present, its full water diuretic and natriuretic effects after adrenalectomy. Su-15049, however, did not modify the kaliuresis induced by cortisol. It is probable, therefore, that glucocorticoids are the adrenal factors essential for a natriuretic response to Su-15049.

Effect of aldosterone and cortisol combined on response to Su-15049 (Table I, Series 4). When the intact animal responds to Su-15049 both glucocorticoids and mineralocorticoids are presumably present. Therefore substitution experiments were set up in adrenalectomized animals in which the effect of Su-15049 was observed in the presence of both aldosterone and cortisol. The actions of aldosterone and cortisol alone were previously shown in Table I, Series 2 and 3 respectively.

The effect of aldosterone and cortisol together, without Su-15049, are shown in Table I, Series 4. In the doses used, the effect of aldosterone was entirely dominant: the antinatriuretic action of aldosterone was manifest in the presence of cortisol. The kaliuresis expected from either hormone was present but not additive.

An entirely different picture was seen, however, when Su-15049 was given together with aldosterone and cortisol. A relatively massive natriuresis and water diuresis occurred. The kaliuresis resulting from the corticoids was not definitely modified by Su-15049. Hence, again it is seen that responsiveness to Su-15049 is dependent on a glucocorticoid and, in addition, if a glucocorticoid is present, the drug can antagonize the sodium-retaining actions of aldosterone.

Effects of Su-15049 and corticosterone (Table I, Series 5). The rat normally secretes little, if any, cortisol. Corticosterone is one of its major hormones. Accordingly, the effects of corticosterone on the response to Su-15049 were determined in adrenalectomized animals. In the one dose of corticosterone used (0.5 mg/rat), the response was like that of a weak cortisol. By itself it only enhanced potassium excretion, but did permit Su-15049 to exert a weak but distinct natriuresis.

Effect of Su-15049 and hydrochlorothiazide (Table I, Series 6). The effects of Su-15049 and hydrochlorothiazide were studied alone and in combination. When given together, the water diuretic and natriuretic effects of the two drugs were sub-additive and thiazide-induced potassium excretion inhibited. This combined effect was apparent despite the fact that the 12.5 mg/kg dose of Su-15049 alone had a uniquely strong effect in this particular experiment.

Other pharmacological effects. In a variety of other tests, no distinct pharmacological effects of Su-15049 were seen in rats except that it had definite activity in conventional tests for anti-inflammatory agents. In the upper ranges of the doses used in Fig. 1, Su-15049 inhibited fluid accumulation in Selye granuloma pouches, reduced paw edema after local carrageenin injections and decreased the accumulation of pleural fluid after intrathoracic injection of turpentine. Pending further study, the significance of these findings is uncertain, but it is known that furosemide also has both diuretic and anti-inflammatory properties(4).

Discussion. Su-15049 is a diuretic with an unusual or unique combination of properties. It stimulates sodium excretion markedly with only slight and variable effects on postassium excretion. Although it was not determined in the rat, Barrett *et al*(6) and Cohen and Cafruny(7) have found in the dog that sodium excretion under influence of the drug was accompanied by near equivalent amounts of chloride. The latter investigators have shown that in the dog the major na-

triuretic action of the compound was exerted in the proximal renal tubule but that it also interfered with sodium-potassium exchange in more distal segments. It did not appreciably affect renal hemodynamics.

An unexpected aspect of its action, since it is not a corticoid antagonist in the sense of the spirolactones, is (as least in rats) its complete dependence for natriuretic activity on the presence of the adrenals. The requisite adrenal factor is apparently a modest amount of some glucocorticoid. This seems to be an example of the permissive action of corticoids as that term is used by Ingle(5). The amounts of adrenal hormone required are ones which alone have little, if any, effect on sodium excretion. The drug antagonized the Na-retaining effects of aldosterone only if some glucocorticoid was present, or if aldosterone itself was given in doses (0.5 mg/rat) which themselves have glucocorticoid effects. The possible hypothesis that the glucocorticoids permitted responsiveness to Su-15049 by an effect on glomerular filtration is unlikely, but not ruled out by the fact that under the conditions used, adrenalectomy itself did not delay natriuresis nor did the requisite corticoid replacement therapy enhance it.

There is, however, a puzzling complication in the corticoid substitution experiments. In intact rats, Su-15049 caused natriuresis without consistent or biologically significant kaliuresis. When exogenous glucocorticoid was present in adrenalectomized animals, Su-15049 caused natriuresis, but at the same time the "permissive" corticoid caused kaliuresis. Hence after adrenalectomy we did not completely duplicate the response of intact animals to Su-15049. The basis of this failure is not understood but perhaps resulted from the kind or amounts of steroids used.

The effect of Su-15049 on potassium excretion, slight under any circumstances, varied in an unpredictable pattern unrelated to dose. Thus a kaliuresis, either statistically established or suggestive, was seen in the experiments recorded in Fig. 1, in Footnote †, and Table 1, Series 3. In other cases the drug was without effect or reduced potassium excretion.

Summary. 2-[2,6-Diphenyl-4(1-pyrrolidinyl) cyclohexyl]-pyridine (Su-15049) caused marked natriuresis and water diuresis in rats without consistent effects on potassium excretion. Its natriuretic actions were abolished by adrenalectomy. In adrenalectomized animals Su-15049 did not modify the response to aldosterone except when the latter was given in the massive doses (0.5 mg/rat) expected to have some glucocorticoid-like effects. Cortisol or corticosterone, however, made adrenalectomized animals responsive to Su-15049, and the presence of cortisol permitted the drug to antagonize the Na-retaining effects of aldosterone. No corticoid substitution therapy was found, however, that enabled adrenalectomized animals to respond to Su-15049 in regard to potassium excretion precisely as did normal ones. Su-15049 has some activity in conventional anti-inflammatory tests.

The authors are indebted to Mrs. H. Sylwestrowicz and her staff of the Biostatistics Section of this laboratory for the extensive statistical work involved in this study.

1. Robison, M. M., Renzi, A. A., Barrett, W. E., Experientia, 1967, in press.

2. Gaunt, R., Birnie, J. H., Eversole, W. J., Physiol. Rev., 1949, v29, 281.

3. Gaunt, R., Renzi, A. A., Chart, J. J., J. Clin. Endocrinol., 1955, v15, 621.

4. Formanek, K., Med. Pharmacol. exp., 1965, v13, 353.

5. Ingle, D. J., Acta Endocrinol., 1954, v17, 172.

6. Barrett, W. E., Rutledge, R. A., Plummer, A. J., The Pharmacologist, 1967, in press.

7. Cohen, M., Cafruny, E. J., The Pharmacologist, 1967, (in press).

Received April 13, 1967. P.S.E.B.M., 1967, v125.