TABLE I. Contents of trypsin inhibitor and hyaluronidase inhibitor in preparation I to IV.

Trypsin inhibitor preparation	Amt. of preparation in reaction blend, mg	Units of trypsin inhibitor in reaction blend a	μg of hyaluronidase inhibited b	Amt of hyaluronidase inhibitor* in reaction blend c	c/a
I	.100	5.6	11.4	8	1.4
IV	.030	10.00	18.5	18	1.8
II	.100	18.8	23.3	32	1.7
III	.100	23.4	24.9	42	1.8

* expressed as μg of preparation V as estimated by interpolation on the curve in Fig. 1. (c/a for preparation V is 1.6).

esting that hylauronidase present in human urine also is influenced by an inhibitor in a dissociable manner(3).

Table 1 compares concentrations of trypsin inhibitor in Preparations I to IV with estimated amounts of hyaluronidase inhibitor. Column *a* shows amounts of trypsin inhibitor present in the reaction mixtures calculated from the activities of the trypsin inhibitor preparations. Column *b* shows amounts of hyaluronidase inhibited as estimated in the experiments. Column *c* shows amounts of hyaluronidase inhibitor present as calculated from the values in column *b* by interpolation on the curve in Fig. 1 and expressed as μg of preparation V. The last column shows the proportion between amounts of hyaluronidase inhibitor found and trypsin inhibitor present in the reaction mixtures. It is seen that approximately identical proportions are obtained.

It seems permissible to conclude that concentrations of trypsin inhibitor and hyaluronidase inhibitor parallel each other in the different preparations. Apparently the hyaluronidase inhibitor and trypsin inhibitor behave identically during the process of purification, suggesting that the two effects are caused by a single compound. This possibility is interesting in view of the relationship between urinary trypsin inhibitor excretion and pituitary-adrenal hormones as demonstrated previously (4,5).

Summary. Trypsin inhibitor preparations isolated from human pregnancy urine inhibit hyaluronidase. The activity curve indicates formation of a dissociable compound. The trypsin inhibitor concentration in preparations of different purity parallels the content of hylauronidase inhibitor suggesting identity of the 2 inhibitors.

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Received June 16. 1961 P.S.E.B.M., 1961, v108

Acute Effects of 9α-fluorohydrocortisone and 2-methyl-9α-fluorohydrocortisone on Renal Clearances of Sodium and Potassium in Man.* (27006)

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Before beginning a study of the modifying effects of sodium-retaining steroids upon mercurial diuresis, it was necessary to establish the usual pattern of response to the steroids in normal human subjects. The steroids used were 9 ∝-fluorohydrocortisone (FF) and 2-methyl-9 ∝-fluorohydrocortisone (2MeFF). The latter compound has been shown by Liddle and his associates(1) to exert a more potent sodium-retaining effect than aldosterone. The acute

* This study was supported by Grant from Nat. Heart Inst., N.I.H., U.S.P.H.S.

effects of these compounds upon renal hemodynamics and the renal clearances of sodium, potassium, and chloride in salt-loaded and salt-depleted humans are reported here.

Method. Standard acute renal clearance techniques were employed with 14 human male subjects without evidence of cardiovascular or renal disease. Seven of the subjects were salt-loaded for a period of 3 or more days prior to study by addition of 10 g of sodium chloride daily to the standard hospital diet. Seven subjects were salt-depleted by administration of a mercurial diuretic followed by 3 or more days of a 500 mg sodium diet prior to study. In all subjects, inulin and p-aminohippurate were infused intravenously in a 0.9% sodium chloride solution at a rate of 5 ml/min by a Sigma constant infusion pump. The subjects were well hydrated and in the morning post-absorptive state at time of study.

After 30 minutes of infusion, three 20 minute control clearance periods were obtained utilizing an indwelling bladder catheter and distilled water and air washouts. Following these control periods, the steroids were given intravenously to 12 of the subjects. 2MeFF was given to 6 subjects, 3 salt-loaded and 3 salt-depleted, as a single dose of 0.5 to 0.9 mg (8.1 to 10.6 μg/kg). FF was given to 6 subjects, 3 salt-loaded and 3 salt-depleted, in a priming dose of 5 mg, and in 4 subjects this was followed by a sustaining dose of 3 mg/hr. Total dosage of the latter compound varied from 5.0 to 19.5 mg (67 to 238 μg/kg). Two of the 14 subjects served as controls, 1 salt-depleted and 1 salt-loaded as described. The technique with these 2 subjects was identical with that utilized with the other 12, except that no steroid was given.

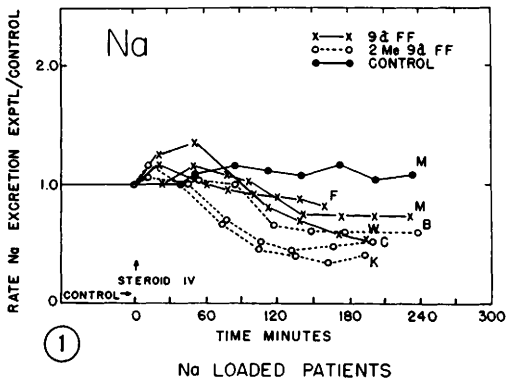
Following administration of the steroid, eight 30 minute urine collections were obtained. Blood was drawn at appropriate intervals for standard clearance calculations. Renal clearances of inulin, p-aminohippurate, sodium, potassium, and chloride were determined. The analytical methods employed were those of Schreiner(2) for inulin, Smith et al(3) for p-aminohippurate, Schales and Schales(4) for chloride, and flame photometry for sodium and potassium with the Baird internal standard flame photometer.

Results. 1. Renal hemodynamics. The data in all 14 experiments are summarized in Table I. Neither steroid significantly influenced the glomerular filtration rate (inulin clearance), with the exception of subject A.C., No. 11, who was salt-depleted and received 2MeFF. His C_{In} rose from 106 to 123 ml/min, an increase of 16% in glomerular filtration.

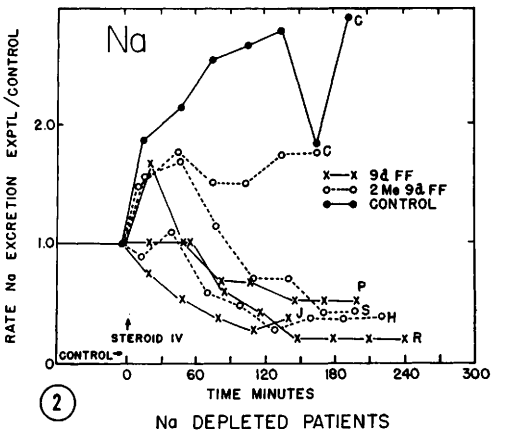
A slight but consistent depression of C_{PAH} was noted in all subjects during experimental periods, except subject A.C., in whom there was a rise of C_{PAH} from 526 to 726 ml/min, an increase of 38% in effective renal plasma flow. The magnitude of the C_{PAH} depressions (averaging 9%) and the fact that similar C_{PAH} depressions were noted in the 2 control subjects suggest that factors other than steroid administration were responsible for these small changes in effective renal plasma flow.

2. Sodium excretion. With the exception of subject J.F., No. 1, a significant decrease in rate of sodium excretion was produced in all subjects with both compounds. Within the 4 hour experimental period, minimum rate of excretion of sodium ranged from 0.76 to 0.20 of the rate excreted during the control period. The decrease in rate of sodium excretion was relatively less with FF during salt-loading as compared to the salt-depleted state. With 2MeFF, comparable reduction in sodium excretion occurred during both the salt-depleted and salt-loaded states. In the 3 salt-loaded subjects receiving FF, sodium excretion declined to 0.53, 0.76 and 0.95 of the rate excreted during the respective control periods, whereas in similarly loaded subjects receiving 2MeFF, sodium excretion was reduced to 0.39, 0.42, and 0.58 of the rate during control periods. In salt-depleted subjects receiving FF, sodium excretion dropped to 0.14, 0.25, and 0.57 of the rate excreted during control periods. Similar values with 2MeFF were 0.37, 0.40 and 0.44. By contrast, in both the salt-loaded and salt-depleted control subjects, sodium excretion increased during the experimental periods, reflecting the additive effect of administration of 0.9% NaCl intravenously throughout the experiment.

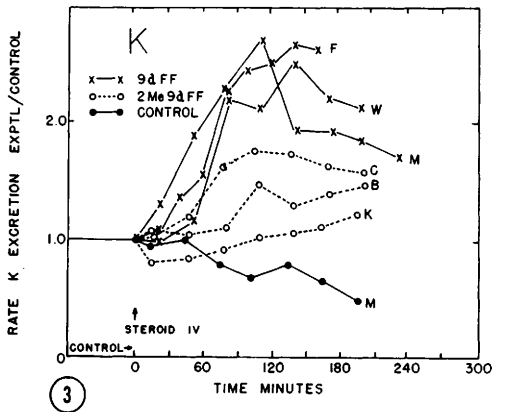
Fig. 1 gives the timed data in all 7 salt-loaded subjects indicating the relative effects of both compounds upon sodium excretion. The data on the ordinate are plotted as follows:



1 Na LOADED PATIENTS



2 Na DEPLETED PATIENTS



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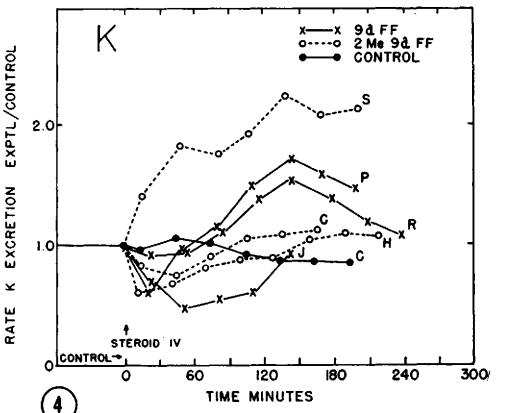
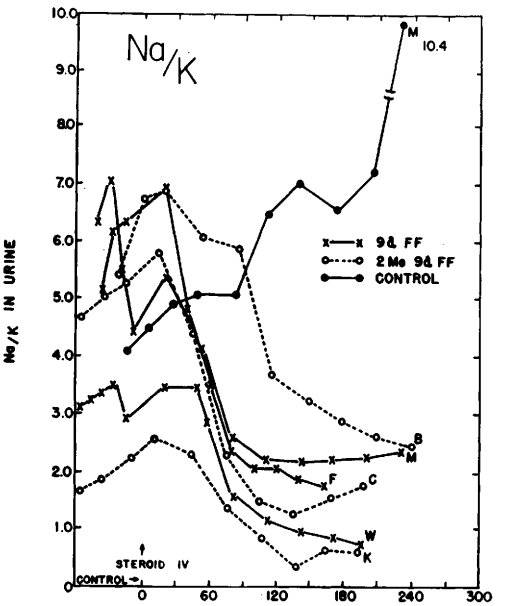
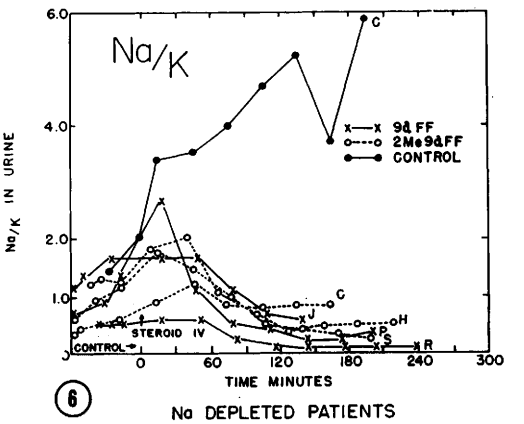


4 Na DEPLETED PATIENTS



5 Na LOADED PATIENTS



6 Na DEPLETED PATIENTS

$$\text{Exp.} \frac{\text{Sodium excreted}}{\text{Sodium filtered}} \text{ Cont.} \frac{\text{Sodium excreted}}{\text{Sodium filtered}},$$

which corrects for minor fluctuations of GFR and plasma concentrations. The control sodium excretion in these subjects ranged between 250 and 500 μeq/min, being fairly constant for each subject. Significant depression of sodium excretion began between one and 2 hours after administration of steroids. Rate of excretion of sodium reached the lowest point between 2 and 3 hours after administration, and effectiveness was still evident at the end of the 4 hour experimental period with both compounds. 2MeFF, given in a lower dosage than FF, produced an earlier and greater effect upon sodium excretion.

Fig. 2 presents similar studies in the 7 salt-depleted subjects. The control sodium excretion in these subjects ranged from 45 to 125 μeq/min, being fairly constant for each subject. Of interest is the increase in sodium excretion during the first hour in 2 of the subjects on 2MeFF and 1 on FF. Only in subject A.C., No. 11, with the sustained increase in sodium excretion, was there a significant rise in GFR and renal plasma flow. All patients receiving steroid excreted sodium at a rate much lower than the control patient.

3. Potassium excretion. The greatest increase in rate of potassium excretion occurred in salt-loaded subjects receiving FF, with peak values ranging from 2.12 to 3.08 times the rate of excretion during the control period (Table I). Salt depletion reduced this effect considerably, with actual potassium retention in one subject, H.J., No. 4. 2MeFF produced considerably less kaliuresis in both the salt-loaded and salt-depleted subjects with peak values ranging from 1.01 to 1.64 times control values.

Fig. 3 demonstrates the time relationship of the kaliuretic effects of both compounds in salt-loaded subjects. FF produced an earlier kaliuresis with considerably greater peak effects. The onset was almost immediate with this compound, and peak effects were attained in about 2 hours. With 2MeFF, the kaliuresis started in about one hour and increased gradually to much lower peak values. In contrast, the control subject who received no steroid, N.M., No. 13, revealed a progressive decline in rate of excretion of potassium as compared to the control period.

The modifying effects of salt depletion are seen in Fig. 4. The kaliuretic effects were variable with both compounds. Only one subject with 2MeFF showed an early sustained kaliuresis. Five other subjects revealed an early decrease in rate of potassium excretion, and, of these, only 2 showed later mild kaliuretic effects.

4. Chloride excretion. The effects upon chloride excretion were variable; in general, these reflected the summation of the effects of the steroids upon sodium retention and potassium excretion.

5. Urine Na/K ratios. The ratios of Na/K excretion in the urine are presented in Fig. 5 and 6. The most marked decreases in urinary Na/K are seen in the salt-loaded subjects. With FF, the lower ratios primarily represent marked kaliuresis, whereas similar ratios with 2MeFF reflect primarily sodium retention. By contrast, in both control subjects the urinary Na/K rose during experimental periods.

6. Plasma electrolyte concentrations. During the 4 hours after administration of the steroids, there were insignificant changes in plasma concentrations of sodium, chloride and potassium.

Discussion. It is apparent from these studies that, with the exception of one salt-depleted subject, intravenous administration of FF and 2MeFF in the dose range utilized exerted no discernible effects upon glomerular filtration rate. The changes in sodium and potassium excretion induced by these steroids appear to be primary tubular effects. A dissociation be-

FIG. 1. Rate of sodium excretion in salt-loaded patients; ratio of experimental period to control period.

FIG. 2. Rate of sodium excretion in salt-depleted patients; ratio of experimental period to control period.

FIG. 3. Rate of potassium excretion in salt-loaded patients; ratio of experimental period to control period.

FIG. 4. Rate of potassium excretion in salt-depleted patients; ratio of experimental period to control period.

FIG. 5. Ratio of sodium to potassium excretion in salt-loaded patients.

FIG. 6. Ratio of sodium to potassium excretion in salt-depleted patients.

TABLE I. Effects of 9α-fluorohydrocortisone and 2-methyl-9α-fluorohydrocortisone on renal hemodynamics and electrolyte excretion in salt-loaded and salt-depleted subjects.

			Subject	Total Dose (mg.)	C_{In} ml/min (mean)		C_{PAH} ml/min (mean)		Peak Effects Exp./Control						Urine Na/K		
									Sodium		Potassium		Chloride		Control (mean)	Exp. (Peak)	Exp./-Control
					Control	Exp.	Control	Exp.	Filtered	UV_{Na}	Filtered	UV_K	Filtered	UV_{Cl}			
FF	Salt Loaded	1	J.F.	5.0	101	107	877	721	1.16	.95	1.13	3.08	1.08	1.21	5.86	1.76	.30
		2	J.M.	5.0	115	117	593	551	1.06	.76	.99	3.00	1.07	.98	5.80	2.17	.37
		3	W.W.	16.7	138	138	739	615	1.11	.53	.86	2.12	1.13	1.04	3.25	.74	.23
	Salt Depleted	4	H.J.	12.5	78	77	448	420	.85	.25	.90	.83	.92	.40	1.46	.57	.39
		5	J.R.	19.5	108	113	676	648	1.13	.14	1.02	1.55	1.08	2.18	.53	.06	.11
		6	C.P.	11.1	118	131	777	706	1.14	.57	1.14	1.87	1.13	2.21	.99	.26	.26
2MeFF	Salt Loaded	7	R.C.	.5	134	134	1096	963	.95	.12	.90	1.59	1.01	.63	5.00	1.29	.26
		8	N.K.	.9	108	108	664	609	1.06	.39	.97	1.18	1.04	.71	1.89	.37	.20
		9	P.B.	.7	133	135	593	559	1.00	.58	.86	1.28	1.06	.67	6.36	2.41	.38
	Salt Depleted	10	U.H.	.7	89	94	497	484	1.02	.40	1.00	1.01	1.15	1.86	.95	.49	.52
		11	A.C.	.7	106	123	526	726	1.18	.37	1.12	1.23	1.28	2.67	.45	.85	1.89
		12	H.S.	.7	94	91	480	442	1.01	.44	1.03	1.64	1.08	1.52	1.27	.31	.24
Control	Salt Loaded	13	N.M.	—	124	119	545	492	.92	1.12	.95	.46	.92	1.13	4.47	10.37	2.32
	Salt Depleted	14	J.C.	—	98	114	318	306	1.20	2.88	1.26	1.16	1.23	4.00	1.75	5.92	3.38

In the C_{In} and C_{PAH} columns, mean value of three 20 min clearance periods is given in control column, and mean of six to eight 30 min clearance periods is expressed in experimental column. In the electrolyte columns, the experimental values and mean control values are used to calculate the ratios Exp./Control. C_{In} and C_{PAH} data are corrected to ideal surface area of 1.73m² with the exception of subject C.P.

tween the effects upon potassium and sodium excretion is present, 2MeFF having a relatively greater sodium-retaining effect and FF a greater potassium-losing effect. The urinary Na/K ratios may, therefore, be misleading when used as a sole guide of effectiveness. The kaliuretic effects of the steroids used appear to be blunted by prior salt depletion and augmented by salt loading. This might be explained by the variation of endogenous aldosterone excretion caused by salt loading and depletion.

In one subject in whom a paradoxical, sustained increase in sodium excretion occurred following intravenous 2MeFF, significant elevations in GFR and renal plasma flow were present. In this subject, the natriuresis associated with increased filtered loads of sodium predominated over the sodium-retaining effects of this potent electrocorticoid.

Following intravenous administration, the electrolyte effects of both steroids became manifest within one and 2 hours and were still present at the end of 4 hours.

Conclusion. 1. Intravenous administration of 9α-fluorohydrocortisone and 2-methyl-9α-fluorohydrocortisone in doses sufficient to produce profound electrolyte effects in 6 salt-depleted and 6 salt-loaded subjects produced no significant change in glomerular filtration rate, with the exception of one salt-depleted subject, in whom there was an increase in both glomerular filtration rate and effective renal plasma flow following 2MeFF. 2. 2MeFF had a more profound sodium-retaining effect than did FF. Even in salt-depleted subjects, the sodium-retaining effects of both compounds were demonstrable. 3. FF had a more profound potassium-losing effect than did 2 MeFF. Kaliuresis was greater in the salt-loaded than in the salt-depleted subjects. 4. Plasma concentrations of sodium, potassium, and chloride did not change significantly during the 4 hour study after intravenous administration of the steroids.

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Received June 19, 1961 P.S.E.B.M., 1961, v108

Possible Relations of Hypoglycaemia Induced by Mono-amine Oxidase Inhibition and Angina Pectoris.† (27007)

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The initially favorable results of the value of the mono-amine oxidase inhibitors in treatment of angina pectoris have latterly been tempered by further experiences of their use(1), and the response to therapy is probably, at best, unpredictable. However, it is of interest that Weiss et al.(2), using one of these drugs, iproniazid, in treatment of depressive patients, showed that those patients whose appetite, weight and sense of well being improved, demonstrated a definite hypoglycaemic response to the drug. It occurred to us that a similar correlation with hypoglycaemia may also exist in patients who showed improvement in the symptoms of angina pectoris as a result of such treatment. In considering the nature of this hypoglycaemia, the ganglion blocking action of iproniazid(3) may have

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† We would like to express our thanks to Dr. Rae Gilchrist, Edinburgh, Dr. George Discombe, London, and Dr. Paul Starr, Los Angeles, for their encouragement in this study.