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Kaliuretic Properties of Furosemide and Hydrochlorothiazide by *In Vivo* Liquid Scintillation Counting

By GORDON S. BORN, STANLEY M. SHAW, JOHN F CHRISTIAN, and WAYNE V. KESSLER

A simple and concise analytical technique was developed for the determination of the kaliuretic properties of diuretic agents. The kaliuretic properties of furosemide and hydrochlorothiazide were compared in electrolyte and nonelectrolyte supplemented rats. ^{42}Potassium was employed as a tracer, and whole body liquid scintillation counting was utilized. The per cent of ^{42}K retention was determined for drug and control groups at various time intervals and comparisons were made for differences in potassium depletion due to drug action. Whole-body liquid scintillation counting was found to be applicable for the study of the kaliuretic properties of diuretic agents.

In the search for new and improved diuretics, the excretion and depletion of body electrolytes by these agents must be evaluated. Many new diuretic agents increase urinary potassium excretion and may cause hypokalemia. The technique of determining electrolyte content in urine samples by flame photometry is subject to complications and errors, such as the interference of trace ions present in the urine. There exists a need for the development of new and improved analytical techniques for the study of potassium excretion as affected by diuretic and allied medicinal agents.

The development of large-volume liquid scintillation counters such as the Purdue University Small Animal Counter (PUSAC) has made possible the detection and measurement of minute amounts of γ-emitting radioactivity administered to an intact test subject (1–3). Following equilibration of an isotope with normal body electrolyte, whole body radioactivity may be determined and subsequent measurements made over a period of time. Direct comparison of radioisotope retention in treated and in control animals allows simple evaluation of drug effect on the ion of interest. An alteration in the excretion of the tracer isotope, and thereby normal body electrolyte, may be noted easily as a change in the whole body radioactivity contained within the animal. Data obtained are indicative of the direct effect of a medicinal agent upon the excretion of the ion from the entire body of the animal.

This study dealt with the development of an *in vivo* tracer method for the investigation of potassium metabolism using ^{42}K in conjunction with whole body liquid scintillation counting. The effects of furosemide[1] and hydrochlorothiazide,[2] diuretic compounds, on potassium metabolism in the fasted rat were studied. In the first phase of study (*Experiment A*), the effects of the diuretic agents on potassium excretion in fasted animals not receiving electrolyte supplement were investigated. In the second phase of investigation (*Experiment B*), replacement electrolyte supplement (sodium and potassium) was administered to the fasted rats while studying potassium excretion as affected by the diuretic agents. The supplement was given to simulate the ingestion of electrolyte through food, as occurs in the human, and perhaps allow greater kaliuretic drug action.

EXPERIMENTAL

Experiment A.—The effects of furosemide and hydrochlorothiazide were investigated at dosage

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[1] Furosemide is 4-chloro-*N*-(2-furyl-methyl)-5-sulfamoylanthranilic acid. This compound was supplied by Lloyd Brothers, Inc., Cincinnati, Ohio, and is marketed as Lasix.

[2] 6 - Chloro - 3,4 - dihydro - 7 - sulfamoyl - 2H - 1,2,4-benzothiadiazine-1,1-dioxide. The compound was supplied by Merck Sharp & Dohme, West Point, Pa., as Hydrodiuril

TABLE I.—EFFECTS OF FUROSEMIDE UPON POTASSIUM RETENTION IN THE RAT

Time[a] Elapsed	400 mg./Kg./Day				200 mg./Kg./Day				50 Gm./Kg./Day			
	Expt. A		Expt. B		Expt. A		Expt. B		Expt. A		Expt. B	
	D[b]	t[c]	D	t	D	t	D	t	D	t	D	t
4	1.62	1.440	1.36	2.961	−0.23	−0.289	1.32	1.639	−1.09	−1.564	0.04	0.063
8	2.16	2.664	4.05	3.372	0.44	0.660	3.18	6.198	−0.43	−0.482	0.86	1.207
12	3.03	3.100	4.45	7.294	0.83	0.920	4.06	5.395	0.10	0.114	1.83	2.338
16	8.48	6.698	4.92	2.870	3.03	4.709	4.51	5.309	−0.49	−0.782	2.55	2.552
20	7.06	9.474	5.13	5.103	3.45	4.141	5.32	4.430	0.56	0.884	4.51	3.122
24	7.10	10.620	6.18	7.852	2.48	2.076	6.92	4.974	0.21	0.203	3.65	2.815
28	9.03	6.843	7.00	7.811	5.27	5.052	6.34	6.397	1.87	2.407	2.31	2.094
32	9.09	17.456	8.64	9.273	5.27	4.388	7.35	8.835	2.31	3.215	3.67	3.136
36	8.41	7.101	8.15	11.403	5.48	3.833	8.11	9.360	4.12	3.300	4.87	4.897
40	9.87	11.473	10.15	6.954	6.76	4.791	9.05	7.166	3.52	3.224	3.78	3.475
44	10.93	9.100	11.60	8.569	8.06	5.496	9.33	9.898	5.03	4.839	6.58	7.586
48	10.10	5.430	8.14	5.829	7.09	6.776	8.63	5.290	1.56	1.767	4.76	2.448

[a] Time elapsed in hours from first drug administration. [b] Per cent retention controls minus per cent retention drug. [c] Student t value of 3.169, reflecting a confidence level of 99%.

levels of 400, 200, and 50 mg./Kg. of body weight per day. Each experiment was repeated for verification of results.

Female Sprague-Dawley albino rats (146–227 Gm.) were divided into groups consisting of six rats in each group. The animals were housed two per wire-bottomed cage throughout the experimental period. A 24-hr. equilibration period was utilized to insure the exchange of radioactive ^{42}K with the body stores of potassium (4); therefore, 24 hr. prior to the initiation of whole body counting, the animals were injected intraperitoneally with approximately 2.7 μc. of ^{42}K in 0.5 ml. of aqueous solution.

Twelve hours after the injection of ^{42}K, the food was removed from the cages. Distilled water was allowed *ad libitum*. Whole body radioactivity was determined in the whole body counter (PUSAC) at the end of the 24-hr. equilibration period.

The test drug was administered immediately following the initial whole body measurement. The drug under investigation was administered by intubation in divided doses every 8 hr. in 0.5 ml. of distilled water as a suspension during the remaining 48-hr. period of investigation. The controls were given a blank dosage of 0.5 ml. of distilled water. Total body radioactivity measurements were made every 4 hr. in each animal with a counting error of less than 1%. The net sample count observed at the first counting determination was taken to represent 100% retention of potassium in the rat. The per cent of potassium remaining at later time intervals was calculated for each animal based upon the first sample count obtained for the animal at the initiation of whole body counting. Computations of data were accomplished through the utilization of an analog computer with FORTRAN-type programming.

Experiment B.—Two dosage levels (200 and 50 mg./Kg./day) of furosemide and hydrochlorothiazide were studied in rats which were fasted, but which were given a replacement supplement of sodium and potassium. The experimental procedures as explained in the preceding discussion were employed with the following exceptions.

In each experiment, there was a control group which received no replacement electrolyte supplement and a control group which received electrolyte supplement. Two additional groups of animals received supplement and either furosemide or hydrochlorothiazide. Animals were housed in individual metabolism cages which allowed for urine collection. The urine was collected at 12-hr. intervals during the 48-hr. period of whole body ^{42}K determinations. Total potassium content was determined in each sample by flame photometric analysis.[3] Fasting of the rats was initiated 24 hr. prior to the administration of diuretic and control therapy. Total body ^{42}K was determined every 6 hr. in each animal using the PUSAC.

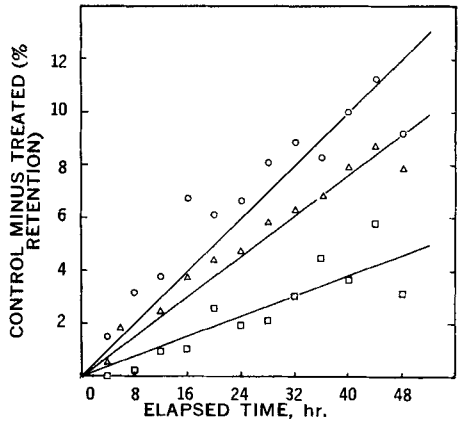

Fig. 1.—Effects of furosemide at three dosage levels upon potassium retention in the rat. Key: ○, 400 mg.; △, 200 mg.; □, 50 mg.

Immediately following the first ^{42}K determination, drug or control therapy was initiated. Drug or blank dosage of distilled water was administered by intubation every 12 hr. The electrolyte supplement was also given in 3.0 ml. of distilled water in divided doses every 12 hr. by intubation. The daily electrolyte supplement consisted of 125 mg. of sodium as sodium chloride and 25 mg. of potassium as potassium chloride. Previous work (5) had indicated that rats treated with furosemide (50 mg./Kg./day) excreted 20–30 mg. of potassium and about five times more sodium daily.

RESULTS AND DISCUSSION

Nonsupplement Experiment.—The effects of furosemide at the three dosage levels studied upon potassium retention in the rat are shown in Table I

[3] The analysis was conducted by Lloyd Brothers, Inc. Cincinatti, Ohio.

TABLE II.—EFFECTS OF HYDROCHLOROTHIAZIDE UPON POTASSIUM RETENTION IN THE RAT

Time[a] Elapsed	400 mg./Kg./Day				200 mg./Kg./Day				50 mg./Kg./Day			
	Expt. A		Expt. B		Expt. A		Expt. B		Expt. A		Expt. B	
	D[b]	t[c]	D	t	D	t	D	t	D	t	D	t
4	0.13	0.115	−0.51	−0.605	−0.53	−0.679	−0.54	−0.819	−0.54	−0.415	0.76	0.815
8	1.29	1.475	0.58	0.633	0.28	0.474	−0.06	−0.116	−0.88	−0.619	1.59	2.264
12	3.19	6.436	0.03	0.044	0.62	0.962	0.33	0.518	−0.96	−0.649	0.65	0.856
16	1.30	1.005	−0.35	−0.417	0.22	0.162	0.88	1.078	−1.17	−0.829	1.41	1.468
20	1.67	2.527	1.06	1.130	1.40	1.377	1.21	1.146	1.19	0.681	1.16	0.945
24	3.48	4.405	0.25	0.222	0.53	0.493	1.02	0.829	−1.25	−0.762	0.80	0.569
28	1.88	1.539	0.96	0.726	2.15	2.115	0.02	0.017	−0.18	−0.167	−0.11	−0.108
32	2.34	2.327	0.53	0.583	1.91	1.612	0.91	1.132	0.34	0.242	2.04	2.135
36	3.28	2.438	1.10	1.020	2.01	1.799	0.91	1.371	1.43	1.023	0.79	0.895
40	2.60	1.866	0.66	0.444	3.63	3.048	−1.31	−1.429	2.61	1.690	−1.54	−1.721
44	3.06	2.525	0.17	0.113	3.71	1.566	0.34	0.399	0.71	0.608	2.04	2.399
48	0.67	0.314	−2.47	−1.366	3.37	2.255	−0.64	−0.372	1.20	0.897	0.36	0.193

[a] Time elapsed in hours from first drug administration. [b] Per cent retention controls minus per cent retention drug. [c] Student *t* value of 3.169, reflecting a confidence level of 99%.

and are summarized and compared in Fig. 1. Linear relationships were obtained by the method of least squares. The data indicate that all dosage levels of furosemide increase the excretion rate of potassium. Also, the kaliuretic activity is increased with increased dosages of furosemide. Successive administrations of the drug increase the

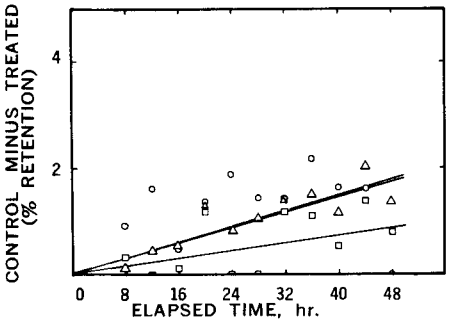

Fig. 2.—Effects of hydrochlorothiazide at three dosage levels upon potassium retention in the rat. Key: ○, 400 mg.; △, 200 mg.; □, 50 mg.

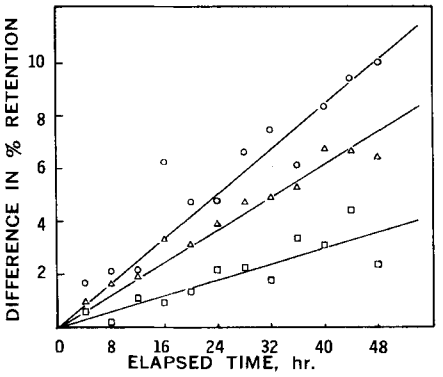

Fig. 3.—Effects of furosemide *vs.* hydrochlorothiazide upon potassium retention in the rat. Key: ○, 400 mg.; △, 200 mg.; □, 50 mg.

TABLE III.—EFFECT OF DIURETIC AGENTS UPON POTASSIUM RETENTION IN THE ELECTROLYTE SUPPLEMENT-TREATED[a] RAT

Time[b] Elapsed	Furosemide				Hydrochlorothiazide			
	Control Minus Drug		Supplement Control Minus Drug		Control Minus Drug		Supplement Control Minus Drug	
	D[c]	t[d]	D[e]	t[d]	D[c]	t[d]	D[e]	t[d]
	200 mg./Kg./Day				200 mg./Kg./Day			
6	1.50	2.104	0.67	1.670	0.30	0.492	−0.52	−1.041
12	4.16	5.345	2.23	2.772	2.25	4.324	0.32	0.578
18	6.44	8.157	2.02	2.294	3.23	4.025	−1.19	−1.366
24	9.31	9.322	4.77	4.466	5.20	5.518	0.66	0.658
30	12.43	17.455	4.59	4.839	8.21	10.344	0.36	0.361
36	f	...	...	...	f	...	...	...
42	17.29	14.969	8.19	11.066	13.73	10.859	4.63	5.141
48	18.09	14.422	6.26	6.167	12.03	9.368	0.20	0.189
	50 mg./Kg./Day				50 mg./Kg./Day			
6	5.09	8.064	0.97	1.808	4.36	5.185	0.24	0.311
12	5.76	6.557	1.70	2.165	5.69	5.744	1.63	1.794
18	10.06	9.818	2.71	2.703	6.85	7.331	−0.50	−0.550
24	9.10	8.786	2.94	2.969	8.33	10.317	2.17	2.899
30	11.15	12.245	1.73	2.448	10.40	11.201	0.98	1.347
36	13.87	12.079	3.51	4.170	11.95	10.402	1.59	1.892
42	11.36	9.715	4.02	4.818	11.46	11.528	4.12	7.308
48	13.82	12.328	4.17	8.558	13.35	10.078	3.70	4.319

[a] Electrolyte supplement containing 25 mg. of potassium and 125 mg. of sodium administered daily. [b] Time elapsed in hours from first drug administration. [c] Per cent retention control without supplement minus per cent retention drug. [d] Student *t* value of 3.169, reflecting a confidence level of 99%. [e] Per cent retention control with supplement minus per cent retention drug. [f] No observation made at this time interval.

difference in potassium retention in drug animals as compared to controls. The results indicate that the maximal dose of furosemide may be above 400 mg./Kg./day in the rat since there was a significant increase in potassium excretion with increasing dose levels of the compound. Timmerman *et al.* (6) found a large and increasing excretion of sodium in rats treated with furosemide. Sodium excretion was noted to increase extensively with an increase in the dose of the diuretic agent.

The effects of hydrochlorothiazide at the three dosage levels studied upon potassium retention are shown in Table II. Figure 2 graphically illustrates the effects of hydrochlorothiazide at the three dosage levels studied. Hydrochlorothiazide slightly increases the excretion of potassium at all three of the dosage levels. The increased potassium excretion, however, is less for all levels than the excretion caused by the lowest dose level of furosemide treatment. The alteration in potassium excretion by hydrochlorothiazide at the 400 and 200 mg./Kg./day dose levels is very similar. This could indicate that the kaliuretic effect of the drug is maximal at the 200 mg./Kg./day level or even lower, since the difference between the 50- and 200-mg. dosage levels is small. It has been shown that the maximal natriuretic response of hydrochlorothiazide in the rat is below 100 mg./Kg./day (6).

The effects of furosemide *versus* hydrochlorothiazide upon potassium retention at the three dosage levels studied were determined by subtracting the average values of the differences in potassium retention between treated and control groups for hydrochlorothiazide from the corresponding values for furosemide. The resulting values were taken to represent the difference in the effect between the two agents upon potassium metabolism. Figure 3 illustrates that the magnitude of potassium excretion increases with additional furosemide administration in comparison to hydrochlorothiazide at all dose levels.

Supplement Experiment.—Furosemide and hydrochlorothiazide at the two dosage levels cause an increase in the excretion of potassium as compared to that exhibited by both control and supplement control animals (Table III). The supplement treatment also had an increasing effect upon potassium excretion in relation to the unsupplemented controls. This effect may be observed by noting the differences in the values of drug *versus* control and drug *versus* supplement control data presented in Table III.

Figure 4 graphically illustrates the effects of furosemide (200 and 50 mg./Kg./day) upon potassium excretion as compared to supplement-treated control animals. The 200 mg./Kg./day dose level of the drug caused increased potassium excretion in relation to the 50-mg. treatment. This altered potassium retention is in close agreement with the results of the nonsupplement study (Fig. 1), as is the statistical significance of the results.

Figure 5 shows the effects of the two dosage levels of hydrochlorothiazide upon potassium excretion in relation to supplement-treated control animals. The 50 mg./Kg./day dose level may be observed to cause a greater increase in potassium excretion than that produced by the higher level of hydrochlorothiazide. This is not in agreement with the results of the nonsupplement investigation, in which the opposite effect was found. Also, differences in potassium retention between drug and electrolyte control animals at the 50-mg. dose level were statistically significant at the later period of the study. The presence of electrolyte supplement in the drug and control animals may have allowed for the differing drug actions. Hydrochlorothiazide (200 and 50 mg./Kg./day) exhibited a lesser kaliuretic action than seen in the lower dosage level of furosemide (Fig. 4). This is in agreement with the results found in the nonsupplement investigation.

The effects of furosemide *versus* hydrochlorothiazide upon potassium retention at the two dosage levels studied were determined in the same manner as in the nonsupplement investigation. These calculations were conducted only for the relation of treated animals to supplement control rats. Figure 6 graphically portrays that the magnitude

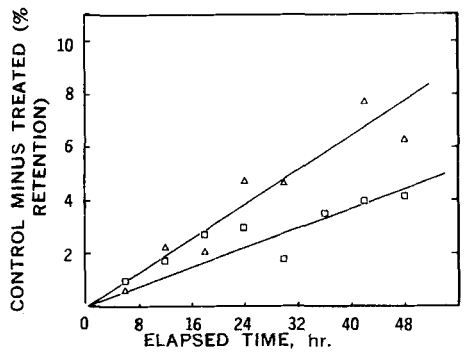

Fig. 4.—Effects of furosemide at two dosage levels upon potassium retention in the supplement-treated rat. Key: △, 200 mg.; □, 50 mg.

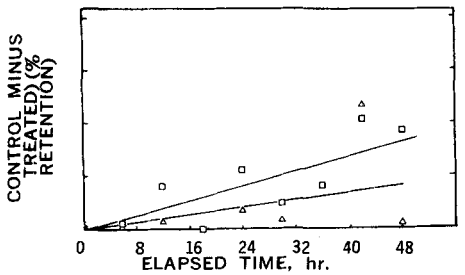

Fig. 5.—Effects of hydrochlorothiazide at two dosage levels upon potassium retention in the supplement-treated rat. Key: △, 200 mg.; □, 50 mg.

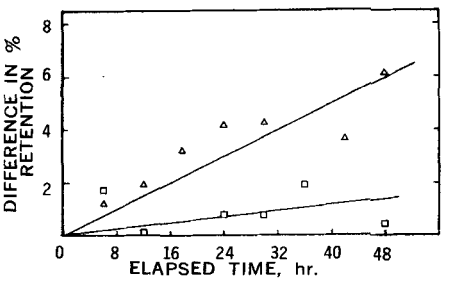

Fig. 6.—Effects of furosemide *vs.* hydrochlorothiazide upon potassium retention in the supplement-treated rat. Key: △, 200 mg.; □, 50 mg.

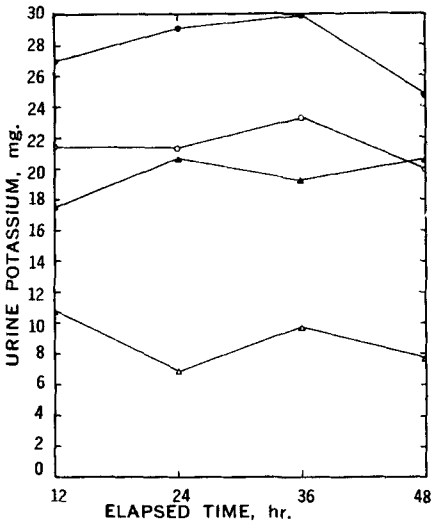

Fig. 7.—Effects of furosemide and hydrochlorothiazide at the 200-mg. level upon urinary potassium in the rat. Key: ●, furosemide (200 mg.) + Na and K; O, hydrochlorothiazide (200 mg.) + Na and K; ▲, control + Na and K; △, control.

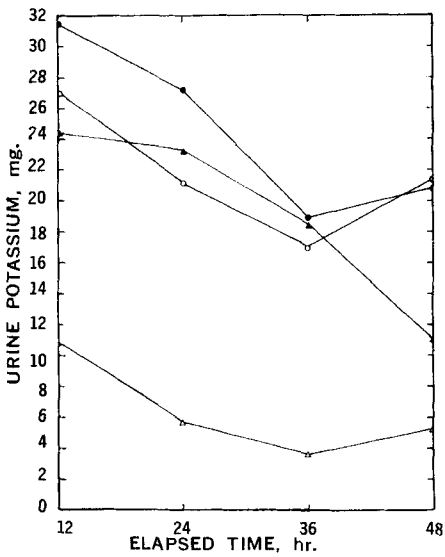

Fig. 8.—Effects of furosemide and hydrochlorothiazide at the 50-mg. level upon urinary potassium in the rat. Key: ●, furosemide (50 mg.) + Na and K; O, hydrochlorothiazide (50 mg.) + Na and K; ▲, control + Na and K; △, control.

of potassium excretion increases with additional furosemide administration in comparison to hydrochlorothiazide. However, the difference in kaliuretic activity between the two compounds at the 50 mg./Kg./day dose level is much smaller than was observed in the nonsupplement study (Fig. 3), while the difference in kaliuretic action was only slightly reduced at the 200 mg./Kg./day level.

Furosemide produced a large increase in urine volume as compared to electrolyte controls and nonelectrolyte control animals. Hydrochlorothiazide caused a small increase in urine volume in relation to both groups of controls. Timmerman *et al.* (6) observed that furosemide exhibits an extensive natriuretic action (mg. to mg. basis) in relation to hydrochlorothiazide. Figures 7 and 8 graphically illustrate the excretion of total potassium, as determined by flame photometric analysis, in the urine of the experimental animals. Urinary potassium excretion generally agrees with the whole-body retention data.

SUMMARY AND CONCLUSIONS

The effects of furosemide and hydrochlorothiazide upon whole body potassium retention in the rat without electrolyte supplement have been investigated at three dosage levels. Furosemide was found to cause an extensive loss of potassium at all dosage levels, which was about proportional with the increase in dosage. Hydrochlorothiazide produced only a slight increase in potassium excretion which remained essentially the same at all dosage levels. In animals receiving electrolyte replacement therapy, the extent of potassium excretion caused by the diuretic agents was found to be in agreement with the results of the nonsupplement study. The effect of diuretic treatment was determined by flame photometric analysis of total urinary potassium in the electrolyte supplement experimentation. The results obtained were found to be in agreement with the results observed by whole body liquid scintillation counting.

In conclusion, an analytical technique utilizing *in vivo* whole body liquid scintillation counting has been developed to follow potassium metabolism. The radioassay procedure has excellent potential for the investigation of therapeutic agents which affect potassium retention and excretion.

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