span of time suitable for accurate quantitation. For example, data in Table I indicate at least 10 min. of stability in absorbance when CTC is being tested, and 30 min. stability for DMCTC and TC.

TABLE I—STABILITY OF TETRACYCLINES IN CHLOROFORM SOLUTION CONTAINING ALKALINE METHANOL

Time, min.	DMCTC, 372 mμ	TC, 366 mμ	CTC, 377 mμ
0	0.459	0.527	0.418
2	0.459	0.527	0.418
4	0.459	0.527	0.418
6	0.459	0.527	0.418
8	0.459	0.527	0.418
10	0.459	0.527	0.417
12	0.459	0.527	0.417
14	0.459	0.527	0.417
16	0.459	0.527	0.416
30	0.458	0.527	0.414

The concentrations of the three compounds tested were 0.80 mg./50 ml. for demethylchlortetracycline hydrochloride, tetracycline hydrochloride, and chlortetracycline hydrochloride, respectively.

The absorptivity for DMCTC, TC, and CTC was determined at the wavelength of maximum absorbance and found to be 2.89×10^{-2} for DMCTC, 3.071×10^{-2} for DMTC, 3.29×10^{-2} for TC, and 2.65×10^{-2} for CTC, as the hydrochlorides. The absorptivity is defined as the absorbance at the wavelength of each particular tetracycline for a solution containing 1 mcg. of tetracycline hydrochloride per milliliter, using a 1-cm. cell. Through examination of standards available in these laboratories, the absorptivity for each tetracycline and its epimer was found to be the same. The determination of absorbances of the column eluates after the addition of the auxochromic agent provided a quantitative spectrophotometric method for assaying tetracyclines in solvent solutions when compared with standards treated in like manner.

REFERENCES

(1) Woolford, M. H., Jr., and Chiccarelli, F. S., *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 400(1956).
(2) Chiccarelli, F. S., Woolford, M. H., Jr., and Avery, M. E., *ibid.*, **48**, 263(1959).
(3) Doskocil, J., and Vondracek, M., *Chem. Listy*, **46**, 564(1952).
(4) Selzer, G. B., and Wright, W. W., *Antibiot. Chemotherapy*, **7**, 292(1957).
(5) Kelly, R. G., and Buyske, D. A., *ibid.*, **10**, 604(1960).
(6) Addison, E., and Clark, R. G., *J. Pharm. Pharmacol.*, **15**, 268(1963).
(7) Hrdy, O., and Vesely, P., *Cesk. Farm.*, **10**, 126(1961).
(8) Kelly, R. G., *J. Pharm. Sci.*, **53**, 1551(1964).
(9) Griffiths, B. W., *ibid.*, **55**, 353(1966).
(10) Ascione, P. P., Zagar, J. B., and Chrekian, G. P., *ibid.*, **56**, 1393(1967).

In Vivo Method for the Simultaneous Determination of Potassium and Sodium Depletion

By GORDON S. BORN, STANLEY M. SHAW, and JOHN E. CHRISTIAN

An *in vivo* radiotracer technique utilizing whole body liquid scintillation counting for the simultaneous detection of ^{42}K and ^{24}Na retention within the intact animal was developed. The technique allowed direct comparison of the retention of potassium and sodium in treated and control animals. The differences in retention indicated the kaliuretic and natriuretic effects of proven diuretic agents. Experimentation with standards was used to develop the simultaneous technique for ^{42}K and ^{24}Na determination. The method was tested *in vivo* in rats and swine, using furosemide and hydrochlorothiazide to show the effects of these diuretic agents in altering potassium and sodium metabolism, to illustrate the adaptability of the method in studies with various animal species, and to confirm the versatility of the method with a whole body liquid scintillation detector of the "human type." Results indicated the technique to be a sensitive method for measuring alterations in potassium and sodium metabolism in rats and swine caused by the diuretic agents.

RADIOTRACER techniques utilizing *in vivo* whole body liquid scintillation counting have been used in studying the effects of diuretics on either potassium or sodium metabolism. Using ^{22}Na, Rupe, Bousquet, and Christian (1) evaluated compounds for their natriuretic and diuretic activity in rats. Born *et al.* (2) and Seno (3), using ^{42}K, studied the kaliuretic properties of diuretics in the rat. Shaw, Kessler, and Christian (4) investigated the kaliuretic and natriuretic properties of a diuretic by using ^{42}K and ^{24}Na in separate studies in swine. The advantages of the whole body liquid scintillation counting technique over photometric methods of determining potassium and sodium elimination have been stated in the above references. However, an undesirable aspect of the whole body liquid scintillation counting technique was the inability to study the kaliuretic and natriuretic properties of a diuretic in the same group of animals in a single experiment.

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The purpose of the following research was to develop an *in vivo* technique for the simultaneous detection of both potassium and sodium retention, thereby achieving simultaneous determination of the kaliuretic and natriuretic properties of a diuretic. The technique would limit biological error, reduce investigational time, and allow greater economy resulting from the utilization of fewer animals.

Following the administration of radioactive potassium and sodium, and their equilibration with the body stores of the electrolytes, whole body radioactivity was determined by dual channel counting, to establish the amount of each radionuclide present in the body. Subsequent measurements were made during drug or control dosage. Direct comparisons between the radionuclide retentions of treated and control animals allowed the evaluation of drugs for kaliuretic and natriuretic properties.

EXPERIMENTAL

The investigation was divided into three parts which were: development of the technique, study of the natriuretic and kaliuretic effects of furosemide[1] and hydrochlorothiazide[2] in the laboratory rat, and a study of the saluretic effects of furosemide in swine. The first study, which was conducted using the PUSAC (5), was carried out to develop a whole body liquid scintillation counting technique which would allow the resolution of the component parts from a ^{42}K and ^{24}Na mixture. The PUSAC was modified to allow dual channel counting by splitting the signal from the preamplifier with a tee connector and directing the split signal through separate amplifiers, pulse height analyzers, and scalers. One pulse height analyzer was calibrated to monitor the 1.26 Mev. Compton peak of ^{42}K with a 9% window (5.0 Mev. full scale). The other was calibrated to monitor the 2.5 Mev. Compton peak from ^{24}Na with a 20% window (3.2 Mev. full scale). The base line discriminators on both analyzers were set to maximize the sample-squared-to-background ratios at the stated window widths. Three mixtures of ^{42}K and ^{24}Na, with varying but known amounts of each radionuclide, were prepared. Additional standards of the single radionuclides at the same concentration were prepared. Mixture *1* was a standard containing both ^{24}Na and ^{42}K in the same concentration as the ^{24}Na standard 1 and the ^{42}K standard 1; other mixed samples were similarly prepared. Counts were taken on the standards and the mixtures of known concentration at 0, 6, 18, 30, and 42 hr. after preparation to simulate an *in vivo* study. At each time interval, the net count rate was obtained in each channel. The counting procedure yielded a counting error for the net sample counts of 5% or less at the 95% confidence level.

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

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

Observed net count rates for the mixtures (from both the potassium and sodium channels) were mathematically manipulated, resolving the relative amount of each radionuclide present. The simultaneous equation method was used to eliminate interference in each channel caused by spectral overlap of the other radionuclide. The ratio of any portion of the spectra to other portions was constant and independent of the activity.

The net count rate of a composite spectrum of the radionuclides ^{42}K and ^{24}Na, for the Compton peak channel of ^{42}K (N_K) may be written as:

$$N_K = K_K + f_{NaK}\, Na_{Na} \qquad \text{(Eq. 1)}$$

where K_K = count rate contribution by ^{42}K to the Compton-peak channel of ^{42}K, f_{NaK} = interference factor which expresses the fractional contribution of the Compton-peak channel of ^{24}Na to the Compton-peak channel of ^{42}K.

Similarly, the net count rate in the ^{24}Na channel (N_{Na}) may be written as:

$$N_{Na} = Na_{Na} + f_{KNa}\, K_K \qquad \text{(Eq. 2)}$$

where Na_{Na} = count rate contribution by ^{24}Na to the Compton-peak channel of ^{24}Na, f_{KNa} = interference factor which expresses the fractional contribution of the Compton-peak channel of ^{42}K to the Compton-peak channel of ^{24}Na.

Equations 1 and 2 are simultaneous linear independent equations containing two unknowns, K_K and Na_{Na}. The net count rates (N_K, N_{Na}) of the composite sample are obtained from the composite spectrum, and interference factors (f_{NaK}, f_{KNa}) obtained experimentally from standard spectra.

The solution to Eqs. 1 and 2 by substitution is:

$$K_K = N_K \cdot \frac{1}{1 - f_{NaK} f_{KNa}} - N_{Na} \cdot \frac{f_{NaK}}{1 - f_{NaK} f_{KNa}}$$

and

$$Na_{Na} = N_{Na} \cdot \frac{1}{1 - f_{KNa} f_{NaK}} - N_K \cdot \frac{f\mathrm{KNa}}{1 - f_{KNa} f_{NaK}}$$

Now let

$$C_1 = \frac{1}{1 - f_{NaK} f_{KNa}}$$

$$C_2 = \frac{f_{NaK}}{1 - f_{NaK} f_{KNa}}$$

$$C_3 = \frac{1}{1 - f_{KNa} f_{NaK}}$$

$$C_4 = \frac{f_{KNa}}{1 - f_{KNa} f_{NaK}}$$

The following new equations result:

$$K_K = C_3 N_K - C_4 N_{Na}$$
$$Na_{Na} = C_1 N_{Na} - C_2 N_K$$

where K_K = ^{42}K in the potassium channel, Na_{Na} = ^{24}Na in the sodium channel, N_K = observed count rate in the ^{42}K channel, N_{Na} = observed count rate in the ^{24}Na channel, and C_1, C_2, C_3, C_4 = constants whose value was derived from the ^{42}K and ^{24}Na standards.

The resolved potassium (K_K) and sodium (Na_{Na})

standard data at the first observation period were taken to represent 100% values. Both net count rates for later time intervals were corrected for counter efficiency changes, resolved, and then corrected for radioactive decay. Half-life values of 12.5 hr. for ^{42}K and 15.0 hr. for ^{24}Na were used. The per cent of potassium and sodium found in each of the three mixed samples was calculated and averaged for each observation period and the error (difference between observed value and 100%) calculated.

In using the simultaneous method to compare the effects of furosemide and hydrochlorothiazide upon potassium and sodium, two rat trials at different dosage levels were conducted. In addition to the simultaneous determination in trial *A*, an integral sodium study was conducted. In trial *B* an integral potassium determination was conducted as well as the simultaneous study, allowing comparison of the interference free (single radionuclide) integral and the simultaneous (dual radionuclide) detection methods. The PUSAC was utilized as previously described in this paper for the dual studies and as described by Born *et al.* (2) for the integral studies.

Female Sprague-Dawley albino rats (175–225 Gm.) were divided into groups consisting of 6 rats each. The animals were housed in individual metabolism cages. Twenty-four hours prior to the initiation of whole body counting, the animals were injected intraperitoneally with the isotope(s) in 0.5 ml. of neutral aqueous solution to allow equilibration of the radionuclides with body stores. For the simultaneous determinations, based upon supplier assay,[3] about 1 μc. of ^{42}K and 0.1 μc. of ^{24}Na were given each rat. In the case of the single radionuclide studies, approximately 3 μc. of ^{42}K or 0.3 μc. of ^{24}Na was administered. At the time of radionuclide administration, fasting was initiated and continued throughout the experiment. Distilled drinking water was allowed *ad libitum*. Immediately after initial determination of whole body radioactivity (time 0), the test drug (furosemide or hydrochlorothiazide) was administered. The animals were dosed again at each counting period (each animal receiving 220 mg./Kg./day in trial *A* and 250 mg./Kg./day in trial *B*). Whole body counts were made at 8, 16, 24, 32, and 40 hr. The net sample counts from each animal were manipulated as before to resolve the relative potassium and sodium counts. The resolved count rates for each radionuclide were treated separately, using a digital computer and Fortran programming for correcting data for radioactive decay, calculating the per cent ^{42}K and ^{24}Na retention in each animal, computing the average per cent retention of each electrolyte for the drug treated *versus* the control groups, and computing the Student *t* value for this difference in per cent retention.

To demonstrate the adaptability of the dual radionuclide detection method to other whole body counters, as well as to other test animals, domestic pigs were utilized in the Sinco-p (6) which was calibrated to allow dual channel counting of ^{24}Na and ^{42}K. A 20% window width (3.2 Mev. full scale) for each channel was used with this counter. The base line for each window was selected by maximizing the sample-squared-to-background ratio using ^{42}K and ^{24}Na standards.

Pigs of both sexes (25–30 lb.) were randomly divided into 2 groups of 3 animals. One group was designated the control group, and the other group selected for drug therapy. The animals were housed in metabolism cages throughout the experimental period. Forty-eight hours prior to the first drug administration, whole body ^{40}K was determined, the animals being immobilized in a steel container (4). Twenty-four hours prior to drug administration, ^{42}K (about 5 μc.) and ^{24}Na (about 1 μc.) were administered intraperitoneally to allow equilibration with body stores. Fasting, with water allowed *ad libitum*, was initiated and continued throughout the experiment. Following radionuclide equilibration, each animal was counted at 0, 6, 12, 24, 30, and 36 hr. Furosemide (100 mg./Kg./day) was administered in 2 equal aliquots at 12-hr. intervals starting at time zero.

The calculation of data was the same as previously described. However, natural ^{40}K radioactivity in each swine was subtracted from the net sample count of each channel before proceeding with further calculations.

RESULTS AND DISCUSSION

Table I shows the results of the study with standards which was undertaken to show the feasibility of a simultaneous method for detection of ^{42}K and ^{24}Na. The 100% values for ^{42}K and ^{24}Na at time zero were assigned, while the percentages at later time intervals were calculated. As can be seen, the greatest difference between known and observed standard activity was about 3% for ^{24}Na and less for ^{42}K. The sources used in this study had widely varied proportions of ^{42}K and ^{24}Na, representing extremes not often encountered in controlled experiments with animals. With observed count rates from ^{42}K and ^{24}Na approximating each other, as in the animal studies, the over-all error of simultaneous determinations should be reduced.

Table I—Per Cent of Isotope Found in Known Sources by the Simultaneous Method

Time[a] Elapsed	% [b] ^{42}K	% [b] ^{24}Na	% Error [c] ^{42}K	% Error [c] ^{24}Na
0	100.0	100.0	0.0	0.0
6	98.6	98.6	1.4	1.4
18	99.8	101.8	0.2	1.8
30	100.1	97.0	0.1	3.0
42	97.3	98.2	2.7	1.8

[a] Time elapsed in hours from first determination. [b] Average per cent for three samples. [c] One hundred per cent minus calculated per cent.

Table II shows the differences in per cent sodium retention between drug and control animals observed in trials *A* and *B*, and the calculated Student *t* values for these differences. As may be seen in Table II, both drug treatments caused a decrease in sodium retention in relation to control animals, and thereby, an increase in sodium excretion.

In trial *A*, in addition to the simultaneous study, an integral study of sodium retention was conducted allowing comparison of the data from both methods. In both studies in trial *A*, a significant difference

[3] New England Nuclear Corp., Boston, Mass.

TABLE II— EFFECTS OF FUROSEMIDE AND HYDROCHLOROTHIAZIDE UPON SODIUM RETENTION IN THE RAT

Time[a] Elapsed	Furosemide						Hydrochlorothiazide					
	Trial A[b]				Trial B[c]		Trial A				Trial B	
	Integral		Differential		Differential		Integral		Differential		Differential	
	D[d]	t[e]	D	t	D	t	D	t	D	t	D	t
8	13.6	9.72	9.4	4.19	13.0	9.26	9.7	7.02	6.5	4.01	9.9	11.07
16	13.2	12.10	14.7	9.33	15.3	8.15	6.8	6.33	8.8	5.66	10.8	6.38
24	14.5	8.76	16.9	12.60	18.5	9.39	7.0	4.18	8.4	5.36	10.7	6.27
32	14.5	13.15	12.6	12.03	17.2	8.82	6.4	6.75	5.1	2.80	7.9	5.16
40	11.2	7.82	12.7	7.51	15.5	8.60	4.6	3.55	3.7	2.36	7.2	4.83

[a] Time elapsed in hours from first drug administration. [b] Drug dosage 220 mg./Kg./day. [c] Drug dosage 250 mg./Kg./day. [d] Per cent retention controls minus per cent retention drug. [e] Student t value of 2.76 reflecting a confidence level of 99% for a "one tail" test.

(p = 0.01) in per cent retention was observed for sodium at all time intervals for both drugs. Figure 1 shows the average per cent retention of sodium,

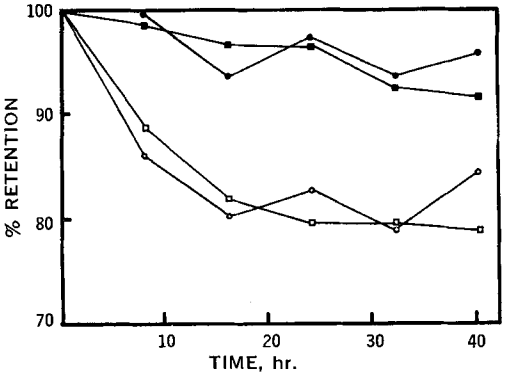

Fig. 1—Effect of furosemide on sodium retention. Key: ●, integral controls; ■, differential controls; ○, integral furosemide; □, differential furosemide.

TABLE III—DIFFERENCE IN EFFECT OF FUROSEMIDE AND HYDROCHLOROTHIAZIDE UPON SODIUM RETENTION (TRIAL A)

Time[a] Elapsed	Integral		Differential	
	D[b]	t[c]	D	t
8	3.8	3.29	3.0	1.51
16	6.4	8.23	5.9	3.72
24	7.5	8.27	8.6	5.49
32	8.0	8.30	7.5	4.05
40	6.6	5.77	9.1	5.65

[a] Time elapsed in hours from first drug administration. [b] Per cent retention hydrochlorothiazide treated minus furosemide treated. [c] Student t value of 2.76 reflecting a confidence level of 99% for a "one tail" test.

as affected by furosemide, for drug and control animals *versus* time for the simultaneous and integral studies. The observed retention values shown in Fig. 1 are representative of the average retention values used to calculate the differences in per cent retention. In Table III a comparison between furosemide and hydrochlorothiazide is made. In this case, the hydrochlorothiazide-treated group were considered as the controls, and activity of furosemide in relation to hydrochlorothiazide evaluated to show the differences in potassium and sodium elimination. In trial A of both integral and differential studies, furosemide was shown to cause significantly (p = 0.01) more sodium elimination than hydrochlorothiazide.

In Table IV the differences in per cent potassium retention between drug and control therapy are given for trials A and B, and their Student t values reported. In trial B, both integral and differential studies were conducted allowing comparison of the suitability of the two methods for potassium determination. From the data in Table IV, the integral method showed statistical differences (p = 0.01) in potassium retention between furosemide and control-treated animals at all time periods except the 16-hr. observation. In the differential study, the data were significantly different (p = 0.01) at the 8 and 24-hr. time period. With hydrochlorothiazide, only in the integral study was any difference in potassium retention between control and drug-treated animals observed, and then, only at two time intervals (8 and 32 hr.). The percentages of potassium eliminated in the integral and differential study are comparable. The statistical significance of potassium elimination in the integral study and nonsignificance in the differential study may indicate limitations to the reliability of a simultaneous method. The limitations are believed to be imposed by 3 combined factors: small number of animals utilized, counting error, and the inherently

TABLE IV—EFFECTS OF FUROSEMIDE AND HYDROCHLOROTHIAZIDE UPON POTASSIUM RETENTION IN THE RAT

Time[a] Elapsed	Furosemide						Hydrochlorothiazide					
	Trial A[b]		Trial B[c]				Trial A		Trial B			
	Differential		Integral		Differential		Differential		Integral		Differential	
	D[d]	t[e]	D	t	D	t	D	t	D	t	D	t
8	0.9	0.64	2.8	6.39	4.3	2.87	0.4	0.26	1.5	2.93	1.8	1.17
16	−0.0	−0.03	2.3	2.54	3.3	1.71	−2.2	−1.28	1.1	0.92	0.7	0.39
24	1.8	0.85	4.0	3.26	6.6	3.49	−0.3	−0.17	1.0	0.77	2.8	1.63
32	3.2	1.68	7.8	8.90	2.3	1.10	0.2	0.15	2.9	3.84	−1.9	−0.72
40	6.1	2.82	7.7	6.23	[f]	[f]	3.5	1.94	1.8	1.28	1.2	0.29

[a] Time elapsed in hours from first drug administration. [b] Drug dosage 220 mg./Kg./day. [c] Drug dosage 250 mg./Kg./day. [d] Per cent retention controls minus per cent retention drug. [e] Student t value of 2.76 reflecting a confidence level of 99% for a "one tail" test. [f] No observation made at this time interval.

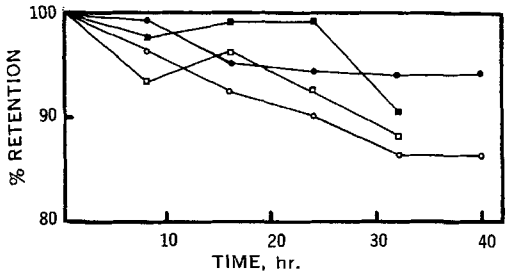

Fig. 2—Effect of furosemide on potassium retention. Key: ●, integral controls; ■, differential controls; ○, integral furosemide; □, differential furosemide.

TABLE V—DIFFERENCE IN EFFECT OF FUROSEMIDE AND HYDROCHLOROTHIAZIDE UPON POTASSIUM RETENTION (TRIAL *B*)

Time[a] Elapsed	Integral D[b]	Integral *t*[c]	Differential D	Differential *t*
8	1.3	2.21	2.5	2.13
16	1.3	1.56	2.6	1.28
24	3.0	3.89	3.8	1.94
32	4.9	6.14	4.2	1.77
40	6.0	6.04	...	...

[a] Time elapsed in hours from first day administration. [b] Per cent retention hydrochlorothiazide-treated minus furosemide-treated groups. [c] Student *t* value of 2.76 reflecting a confidence level of 99% for a "one tail" test.

small amounts of potassium actually excreted as a result of drug action. Figure 2 illustrates the average per cent potassium retention for furosemide and control therapy *versus* time for both the integral and differential study.

Table V shows differences in the effect of furosemide and hydrochlorothiazide on potassium metabolism, when the hydrochlorothiazide group was considered as control animals. The integral study shows that the two drugs affect potassium elimination to a different degree, furosemide causing a significant ($p = 0.01$) elimination of potassium as compared to hydrochlorothiazide. With the differential method, no statistically significant differences are observed, although the differences in per cent potassium elimination are comparable. Here again, any difference that may exist between the diuretics has been masked.

The effect of furosemide (100 mg./Kg./day) upon sodium and potassium retention in swine is shown in Table VI. The differences in per cent retention of ^{42}K and ^{24}Na between control and drug groups at different time intervals is indicated, as well as the Student *t* values for these differences. The furosemide treatment, when compared to control treatment, caused an insignificant excretion of potassium at all time periods. The results of the study show the natriuretic effect of furosemide upon sodium excretion, with significant differences being observed at 24, 30, and 36-hr. observation periods.

The results of the swine experiment are not in complete agreement with the integral study of the kaliuretic and natriuretic properties of furosemide by Shaw, Kessler, and Christian (4). While their investigation indicated a statistically significant

TABLE VI—EFFECTS OF FUROSEMIDE (100 mg./Kg./DAY) UPON POTASSIUM AND SODIUM RETENTION IN SWINE

Time[a] Elapsed	Potassium C−F[b]	Potassium *t*[c]	Sodium C−F	Sodium *t*
0[d]	0.0	0.000	0.0	0.000
6	3.0	2.229	13.3	3.237
12[d]	7.8	1.376	9.0	1.797
24[d]	0.2	0.087	17.9	7.732
30	2.0	0.376	18.9	12.903
36	11.7	1.907	23.7	4.358

[a] Time elapsed in hours from first drug administration. [b] Average per cent retention controls minus average per cent retention furosemide. [c] Student *t* value of 3.75 reflects a confidence level of 99% for a "one tail" test. [d] Drug or control dosage.

difference in potassium elimination between treated and control animals and this study indicates only a trend, as previously noted, the differential method lacks the sensitivity of the integral method in determining potassium retention. However, the sodium data are consistent with their study of furosemide.

SUMMARY AND CONCLUSIONS

Using standards, a suitable method was developed which would allow simultaneous detection of ^{42}K and ^{24}Na in a composite sample by large volume liquid scintillation counting. The standard data indicate that the determination of the amount of each radionuclide, in the presence of the other, can be accomplished with an error of about 3%. In rats, a comparison of the simultaneous and integral detection methods showed both methods capable of detecting potassium elimination as affected by furosemide or hydrochlorothiazide, though the integral was the more sensitive to small differences. The simultaneous method showed no statistical differences (between the 2 drugs) in potassium elimination. The simultaneous and integral methods were equally sensitive in the detection of sodium elimination. The effect of furosemide on the rat was shown, statistically, to be more pronounced than that of hydrochlorothiazide with respect to an increased elimination of sodium. Experimentation with swine illustrated the adaptability of the simultaneous method to other species and other whole body counters.

The results of the investigation indicate that the simultaneous method for determining the kaliuretic and natriuretic properties of diuretics would be applicable to animal studies and feasible for use in clinical evaluations. The high detection sensitivity of whole body counters, in combination with the short half-lived radionuclides ^{24}Na and ^{42}K, limits whole body exposure to ionizing radiation and allows clinical evaluation with a minimal health hazard.

REFERENCES

(1) Rupe, B. D., Bousquet, W. F., and Christian, J. E., *J. Pharm. Sci.*, **52**, 579(1963).
(2) Born, G. S., Shaw, S. M., Christian, J. E., and Kessler, W. V., *ibid.*, **54**, 1646(1965).
(3) Seno, S., unpublished data.
(4) Shaw, S. M., Kessler, W. V., and Christian, J. E., *J. Pharm. Sci.*, **54**, 1208(1965).
(5) Dunavant, B. G., and Christian, J. E., *Intern. J. Appl. Radiation Isotopes*, **8**, 223(1960).
(6) Christian, J. E., Kessler, W. V., and Ziemer, P. L., *ibid.*, **13**, 557(1962).