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# Investigation of the Saluretic and Kaliuretic Properties of a Diuretic Agent in Swine by *In Vivo* Whole Body Counting

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**A simple and concise analytical technique was developed for the determination of the kaliuretic and saluretic properties of diuretic agents in large animals. Whole body liquid scintillation counting, in conjunction with the radioactive isotopes $^{24}Na$ and $^{42}K$, was utilized to determine directly the retention of the isotopes in swine during control and diuretic treatment. A radioactive tracer was orally administered and allowed to equilibrate in the animals, and control or drug treatment was initiated. Animals were counted at selected time intervals. The per cent retention of the radioactive isotope was calculated for each whole body tracer determination. Control and treated data were compared for the loss of sodium and potassium due to diuretic action. The diuretic agent, furosemide, was found to exhibit a marked saluretic activity in swine, while causing a small loss in potassium.**

MANY BIOLOGICAL testing methods have been devised to evaluate diuretics. The measurement of urine volume, employing rats and dogs as test animals, is commonly used to indicate diuretic activity (1–5). Sodium excretion in the urine is used as an index of diuretic activity (6–8). In the biological comparison of the effectiveness of various diuretic compounds, as well as the determination of their effect on electrolyte balance, the Van Arman method or modifications (9–12) may be employed for dogs, while in rats the Lipschitz method or modifications may be used (1, 10, 13–16). Generally, the animals are fasted, hydrated, and dosed; the urine is collected, volume measured, and the urine analyzed by flame photometry for ionic content.

The development of large volume liquid scintillation counters has made possible whole body measurement of minute amounts of $\gamma$-emitting radioisotopes. The pharmacological action of diuretic agents upon the excretion of sodium and potassium by the rat has been investigated by small animal whole body liquid scintillation counting (17, 18). Microcurie amounts of the electrolyte studied were administered to the experimental animals. Following equilibration of the isotope with normal body electrolyte, whole body radioactivity was determined, and subsequent measurements were made during drug or control treatment. Direct comparison of radioisotope retention in treated and in control animals allowed the evaluation of drug action upon the excretion of the ion of interest.

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Although small animals, such as rats, are important in the study and evaluation of diuretic agents, larger animals, such as the dog, are commonly employed. This investigation was undertaken to study the utilization of a large animal whole body liquid scintillation counter for the determination of the saluretic and kaliuretic properties of a diuretic compound in large animals. Small swine were chosen as the experimental animal because of the biological similarity of swine to man.

## EXPERIMENTAL

The investigation was divided into two parts. Initially, radioactive potassium, $^{42}K$, was utilized to study the effect of the diuretic agent furosemide[1] upon whole body potassium retention. This procedure was followed by the study of the effect of the diuretic upon whole body sodium retention with radioactive sodium, $^{24}Na$. A total of nine swine, weighing from 9.1–17.3 Kg., was employed in the investigation. Five animals were utilized in the $^{42}K$ study and four in the $^{24}Na$ study. The animals served as their own controls, with drug treatment following the control study immediately after the decay of the radioactive isotope (5–10 days).

Swine were housed individually in dog metabolism cages. Food was removed 12 hr. before radioactive isotope determinations were initiated. Fasting was continued for the duration of the experiment. Distilled water was given *ad libitum*. Urine was collected, and the volume was measured throughout each experimental period. Animals were weighed at various time intervals during each study.

Radioactive isotopes were obtained as the chloride in an aqueous stock solution.[2] An accurately meas-

[1] Furosemide is 4-chloro-*N*-(2-furylmethyl)-5-sulfamoylanthranilic acid. This compound was supplied by Lloyd Brothers, Inc., Cincinnati, Ohio, and has the trade name of Lasix.
[2] Oak Ridge National Laboratory, Oak Ridge, Ten

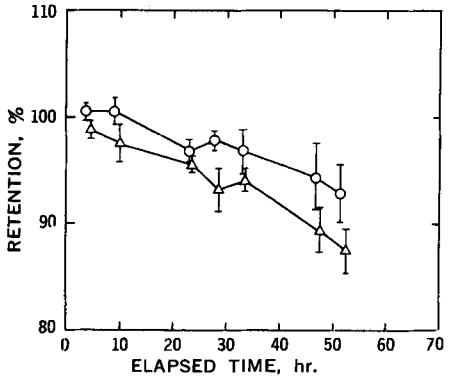


Fig. 1.—The effect of furosemide upon potassium retention in the swine. Key: O, control treatment; △, furosemide treatment.

ured aliquot of the stock solution was tranferred to a lactose filled veterinary capsule. The radioactive isotope was administered *per os* to each animal by placing the capsule into the throat with a boling gun. Swallowing was aided with small amounts of water. Radioactivity was given 24 hr. previous to the initiation of whole body counting to allow uniform distribution and interchange of the isotope with the naturally occurring element in the animals.

Radioactive isotope was measured in animals by large animal whole body liquid scintillation counting. The basic mechanisms and characteristics of an earlier version of the liquid scintillation detector have been previously published (19). The detector has been modified and improved by the addition of an upper detecting tank, which allows $4\pi$ sample geometry. Individual swine was immobilized for counting in a specially designed and constructed container. The semicircular container (made of steel with a 0.06-in. thick stainless steel, water-tight liner in the bottom) was constructed 4 ft. long and 18 in. in diameter. End plates were made with a series of variously spaced holes to receive a double rod which could then be positioned at various distances from the bottom of the container. An animal was placed in the container without being tranquilized or anesthetized. The swine was positioned on its back, legs tied down to clamps along the sides of the metal supports of the container, and a double metal rod was placed in an appropriate position in the end plates and between the legs and over the head to immobilize the animal. A protective semicircular metal plate was placed over the animal. The container with the swine was moved above the detector conveyor and lowered into position by an electric lift supported by a monorail. The electrically operated conveyor moved the animal into a reproducible position in the detector.

Following 24 hr. of radioisotope equilibration ($^{42}K$ or $^{24}Na$), whole body radioisotope content was determined in each animal by liquid scintillation counting immediately preceding the initiation of control or drug treatment. Each animal was counted three times daily during the 53 hr. of experimentation. The diuretic agent was given at a dosage level of 100 mg./Kg. of body weight per day in two equal aliquots at 12-hr. intervals. Lactose was administered during control studies in a similar manner. The oral administration of drug or control capsules was accomplished utilizing the same procedures employed in isotope administration.

Each whole body count was corrected for coincidence loss (as necessary), background, and variation in counter efficiency. The natural $^{40}K$ radioactivity contained in the individual swine was subtracted and the net count ($^{24}Na$ or $^{42}K$) was corrected for radioactive decay to time zero. Time zero was specified for each animal as that time at which the first whole body count of the animal was taken following the equilibration of the radioactive isotope. The per cent retention of the isotope at each counting interval was calculated based on 100% retention at time zero. Data were averaged and statistically treated to determine significant differences in potassium or sodium retention.

## RESULTS AND DISCUSSION

Figure 1 presents the average per cent retention of $^{42}K$ in the five experimental swine at various time intervals during control and drug studies. The furosemide treatment may be seen to cause a small decrease in the retention of potassium, and thereby an increase in excretion, in the swine as compared to control conditions. Although intervals of counting following the initiation of drug or control treatment were not exactly equal, data were statistically treated by the $t$ test to determine the occurrence of significant differences. Significant differences ($p = 0.05$) were found after a single dose of the diuretic agent and throughout the entire period of study, with the exception of the 23-hr. counting interval. The kaliuretic properties of furosemide have also been shown in other investigations (18, 20, 21). An extensive increase in urine volume was observed during the collection period following the initiation of diuretic administration (54%) and throughout subsequent intervals of urine measurement. These data, presented in Table I, indicate a definite diuretic effect due to the pharmacological activity of furosemide. Furosemide has also been shown to produce an extensive increase in urine excretion in dogs at various dosage levels (21), including 100 mg./Kg. (22), and in other experimental subjects (20, 23).

The results of the investigation of the effect of

TABLE I.—EFFECT OF FUROSEMIDE UPON URINE VOLUME IN SWINE

| Collection[b] Interval | Urine Vol.[a] (ml./hr.) Control | Diuretic |
|---|---|---|
| | $^{42}$Potassium Study | |
| 1 | 70.7 | 47.5 |
| 2 | 31.3 | 23.8 |
| 3 | 39.6 | 86.7[c] |
| 4 | 22.9 | 44.1 |
| 5 | 41.1 | 51.2 |
| 6 | 19.9 | 37.6 |
| | $^{24}$Sodium Study | |
| 1 | 29.0 | 30.8 |
| 2 | 32.1 | 125.6[c] |
| 3 | 22.7 | 44.5 |
| 4 | 22.5 | 72.3 |
| 5 | 11.7 | 62.5 |

[a] Average of five animals for the $^{42}K$ study and average of four animals for the $^{24}Na$ experimentation. [b] Average collection interval of 12 hr. [c] Diuretic administration initiated at the beginning of this collection interval.

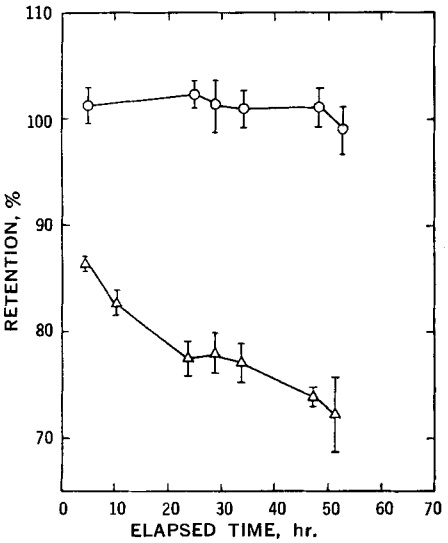


Fig. 2.—The effect of furosemide upon sodium retention in the swine. Key: O, control treatment; △, furosemide treatment.

furosemide upon the retention of sodium in swine are graphically presented in Fig. 2. Following a single dosage of the diuretic agent (one-half the daily dose), the retention of sodium decreased significantly (13.7%) within a few hours and continued to decrease during the course of drug treatment, indicating a marked increase in urinary sodium loss. The saluretic properties of furosemide have been noted in previous investigations (20–23). Urine volume was greatly increased due to furosemide treatment (Table I). The greatest increase in volume occurred during the collection period following the initiation of diuretic therapy.

The results of the investigation indicate that neither the initial administration of the diuretic agent nor subsequent dosing over a period of 53 hr. produced a marked loss in potassium. In contrast, sodium was found to be excreted extensively. A marked decrease was detected within 4 hr. following the initiation of drug treatment. Sodium was noted to be continually lost during the entire period of diuretic therapy, but to a lesser extent than produced by the initial drug administration. The determination of the saluretic properties of furosemide by whole body counting techniques could have been accomplished in a lesser interval of time with the realization of the same information as found in the longer study. Also, the kaliuretic properties of the diuretic agent were indicated during the initial period of experimentation. The ability to determine the saluretic and kaliuretic properties of a compound during a short time interval adds to the desirability of whole body liquid scintillation counting as an analytical procedure. It is desirable to be able to study the effect of a compound on sodium and potassium excretion during a period of continued drug administration.

The investigation has shown that large animal whole body liquid scintillation counting techniques provide a sensitive analytical method for determining the saluretic and kaliuretic properties of a diuretic agent. The short half-lives of $^{24}Na$ (15.05 hr.) and $^{42}K$ (12.47 hr.) allow the utilization of the same animals for control and drug studies. Utilizing this assay procedure, the difficulties found in flame photometric analysis are eliminated. There is no need for tedious laboratory procedures, such as catheterization of dogs, collection of urine, and multiple flame photometric sodium and potassium determinations which are subject to error from trace element interference.

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