A close examination of Fig 3 shows that on two of the three tests of the theory with data the theoretical model appears to be in satisfactory agreement with the experiments. Firstly, the experimental $C^{\circ}{}_{H^+}$ dependence correctly fits the square root law[4] at essentially all times. Secondly, within the probable uncertainty of about a factor of two in theory, the coefficient, 1.5×10^{-4}, in Eq. 18, agrees[5] with data at large times for all $C^{\circ}{}_{H^+}$. Thus it appears that the disagreement between experiment and theory exists largely on the third point, the time dependence.

To amplify the idea that it is primarily the time dependence of the model which is at fault, let us consider two modifications of Eq. 18 which give better fit to the data. In Fig. 4 the data are compared with the following two equations

$$M = 1.4 \times 10^{-4} (C^{\circ}{}_{H^+})^{1/2} (t - \tau_1)^{1/2} \quad \text{(Eq. 20)}$$

and

$$M = 1.4 \times 10^{-4} (C^{\circ}{}_{H^+})^{1/2} (t^{1/2} - \tau_2^{1/2}) \quad \text{(Eq. 21)}$$

where we have taken τ_1 = six hours and τ_2 = one hour for the calculations. In these equations the coefficient and $C^{\circ}{}_{H^+}$ dependence have been retained from Eq. 18. Only changes in the time dependence, effective mainly at small t, have been incorporated. The meaning of τ_1, in Eq. 20, is that there is effectively a constant lag time, $\tau_1 \sim$ six hours, before the release process begins according to the theoretical model. The meaning of τ_2 in Eq. 21 is that there is not only a small lag time $\tau_2 \sim$ one hour, but there is effectively a small barrier in series with the pamoic acid barrier itself. This small effective barrier would, of course, be most important at small t values when the pamoic acid layer is thin. Actually, the data in Fig. 2 indicate a small lag time the order of one hour. It is more difficult to account for the barrier in series with the pamoic acid layer. It might be partially accounted for by the liquid diffusion layer and partially by a varying pamoic acid layer structure near the surface, *i.e.*, effectively smaller diffusion coefficients in the layer near the surface.

[4] If the coating phenomenon were absent, a linear law would be expected according to Eq. 11 with constant s.

[5] If the coating phenomenon were absent, rates ten to a hundred times greater would be expected.

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Investigation of *in Vivo* Tracer Techniques in Drug Screening Studies

By BILLY D. RUPE, WILLIAM F. BOUSQUET, and JOHN E. CHRISTIAN

An *in vivo* tracer method for screening drugs which act by altering the normal body metabolism of certain elements is described. These studies involved the detection of natruretic or antinatruretic action of six compounds. Further extensions of the present work are also discussed.

THE RELATIVELY RECENT development of large volume liquid scintillation counters ("Whole Body Counters") (1, 2) has made possible research on the development of new methodology for the qualitative and quantitative evaluation of pharmacologically active substances. *In vivo* assay of gamma ray emitting radioisotopes, as made possible with large volume liquid scintillators, is particularly attractive to the research pharmacologist in drug screening studies. The obvious advantages of this technique are three: (*a*) observations may be made on the intact animal; (*b*) serial observations may be made on the same animal over extended periods of time; and (*c*) it should be possible to reduce the size of experimental groups of animals. This technique has not been exploited to date.

One such counter is the Purdue University Small Animal Counter (PUSAC). The specifications and operating characteristics of this counter are fully described in the literature (2). The PUSAC and similar counters now being commercially produced are of such size that mice, rats, or guinea pigs may be used as experimental animals.

Large volume scintillation counters should

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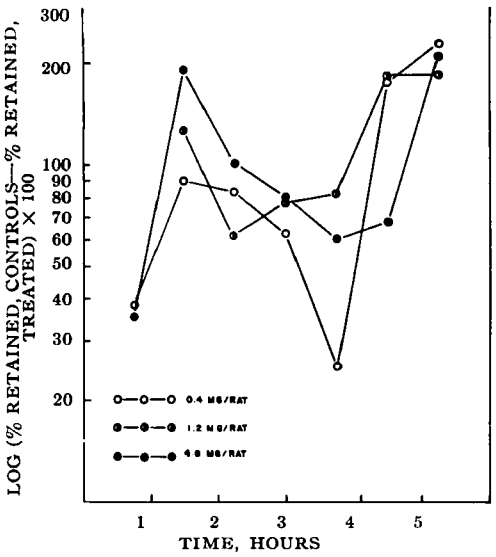

Fig. 1.—Effect of spironolactone on sodium retention in the rat.

provide a sensitive assay method for studying the effects of physiologically active substances on body pool, turnover time, and thus storage and excretion rates of several body ions of interest. However, the following criteria must be met: *A*, The ion must exist in the body in a measurable concentration. *B*, It must have been assigned a specific biochemical role, beneficial or otherwise. *C*, The concentration of the ion must be modified by drug administration or altered in disease conditions, so that a measurable change in concentration reflects the action of the drug or the development of a diseased state. *D*, The ion must have a suitable gamma emitting isotope.

Many ions existing in the body, such as sodium, potassium, calcium, and iodine meet all of the above criteria. Therefore, studies involving drugs or diseased states which alter the normal metabolism of these elements should be possible by the procedures discussed.

Classifications of the various possible studies concerning ions which meet the above criteria are: *A*, the role of the ion in normal physiology, with special reference to body pool, storage time, excretion patterns, and absorption studies; *B*, the effect of drugs in modifying normal body concentrations of the ion and interpretation of results in terms of drug-type and drug-dose, *i.e.*, screening methods; *C*, studies of normal ion concentrations as influenced by disease states. Relationship between body levels of specific metals and type and severity of disease, *i.e.*, diagnostic techniques; *D*, relating to items two and three above, studies of the effect of drug therapy on the diagnostic criteria and indications developed by whole body counting procedures, *i.e.*, evaluation of specific therapeutic procedures as opposed to screening techniques; and *E*, Industrial toxicology.

This study is an example of one of the many possible applications of large volume liquid scintillation counters to pharmacological studies.

The effects of various pharmacologic compounds, mainly diuretic agents, on sodium metabolism in the rat using sodium-22 were studied. This isotope emits a gamma ray of 1.30 Mev., thus making it possible to detect and quantitatively measure the amount of this isotope using external liquid scintillation detectors.

The most common method of studying the diuretic or natruretic activity of pharmacologically active compounds is to collect urine specimens and determine the volumes and/or electrolyte content. The electrolyte content is determined in most cases by flame photometry (3–5); however, this value is not always precise due to interference of trace ions present in the urine.

Since all diuretic agents affect sodium reabsorption in the kidney in some manner, a measure of the amount of sodium retained and by difference the amount excreted should give an indication of the pharmacological activity of the

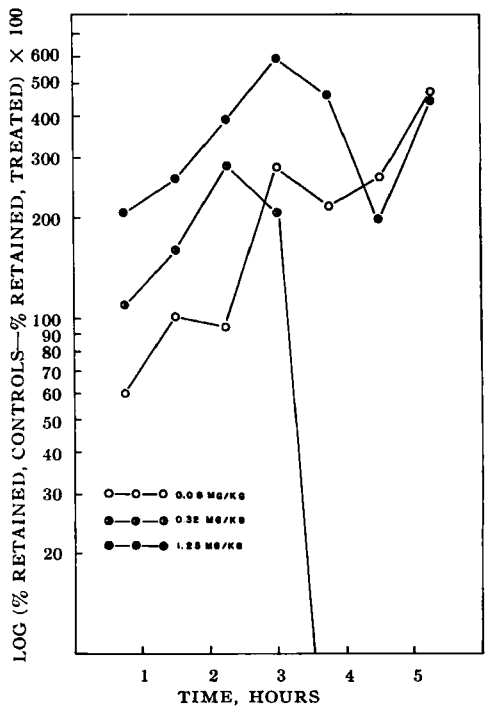

Fig. 2.—Effect of hydrochlorothiazide on sodium retention in the rat.

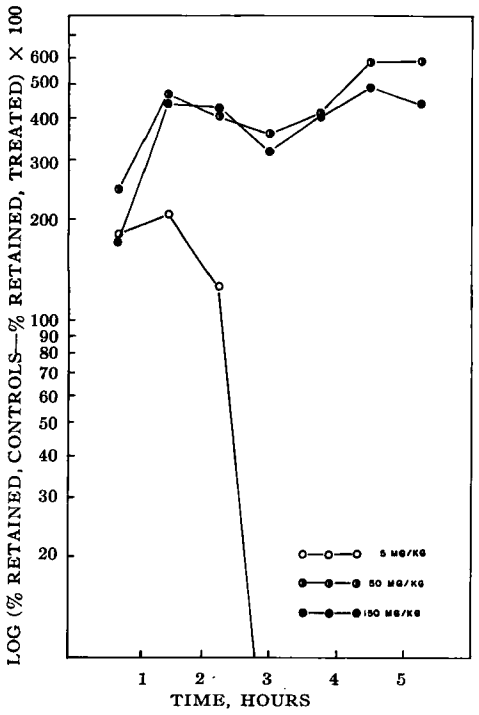

Fig. 3.—Effect of acetazolamide on sodium retention in the rat.

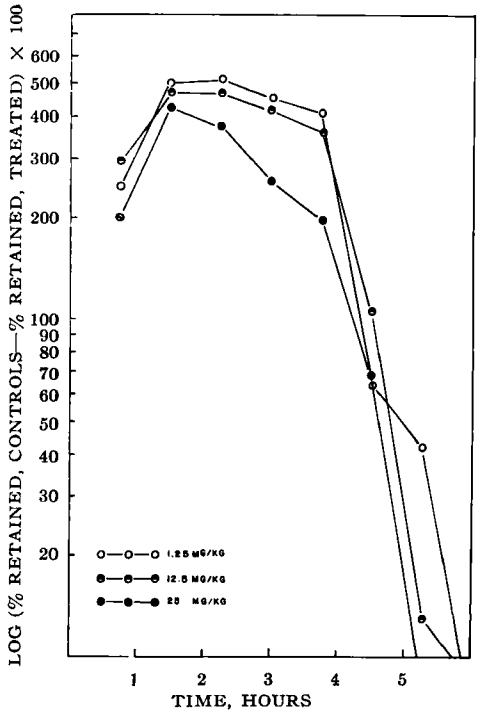

Fig. 4.—Effect of chlorothiazide on sodium retention in the rat.

drug. Wiebelhaus, *et al.* (6), found that if animals used in testing diuretics were first hydrated with 0.9% sodium chloride solution, the resulting values obtained for the natruretic activity of various diuretic compounds also were a good indication of their diuretic activity. This finding has also been reported elsewhere in the literature (7, 8).

In this study rats were hydrated with isotonic sodium chloride solution containing sodium-22. Known diuretic agents were administered and the whole body retention of sodium-22 measured, at several time intervals, as an indicator of natruretic efficacy. In other experiments urine was collected in addition to sodium-22 measurements on the whole animal to provide a correlation between natruretic and diuretic effect.

METHODS

Female Sprague-Dawley rats weighing between 180 and 220 Gm. were used throughout this study unless otherwise noted. The rats received treatment prior to test as indicated.

Desoxycorticosterone Blocking Effect of Spironolactone.[1]—This study dealt with the detection of the ability of spironolactone to relieve desoxycorticosterone acetate (DCA) induced sodium retention in the adrenalectomized rat. Four groups of six animals were adrenalectomized and maintained as described by Kagawa and Brown (3).

At the 24th hour after adrenalectomy, DCA in corn oil (12 mcg. in 0.05 ml.) was injected subcutaneously in the shoulder region of the animals. All animals were then hydrated by injecting subcutaneously 2.5 ml. of 0.9% sodium chloride solution, containing approx. 0.05 μc. of sodium-22, at divided sites in the shoulder region.

Three hours later, at the 27th hour after adrenalectomy, the rats were again injected with DCA as described above. At this time the control group received 0.5 ml. of corn oil subcutaneously, and group I received 0.4 mg.; group II, 1.2 mg.; and group III, 4.8 mg. of spironolactone in 0.5 ml. of corn oil.

The animals were then studied for a period of 5¼ hours by periodically determining the percentage of the original whole body sodium-22 activity remaining by *in vivo* radioassay.

Natruretic Activity of Four Diuretic Agents.—The natruretic activity of four diuretic compounds at three dose levels, chlorothiazide[2] (1.25, 12.5, and 25 mg./Kg.), hydrochlorothiazide[3] (0.08, 0.32, and 1.25 mg./Kg.), acetazolamide[4] (5, 50, and 150 mg./Kg.), and meralluride[5] (0.7, 3.5, and 7.0 mg. Hg/Kg.), was studied. Four groups of six

[1] 3-(3-oxo-7α-Acetylthio-17β-hydroxy-4-androsten-17α-yl) propionic acid γ-lactone. Marketed as Aldactone by G. D. Searle and Co.

[2] 6-Chloro-7-sulfamoyl-2H-2,3,4-benzothiadiazine 1,1-dioxide. Marketed as Diuril by Merck Sharpe and Dohme Co.

[3] 6-Chloro-3,4-dihydro-7-sulfamoyl-2H-1,2,4-benzothiadiazine 1,1-dioxide. Marketed as Hydrodiuril by Merck Sharpe and Dohme Co.

[4] 5-Acetamido-1,3,4-thiadiazole-2-sulfonamide. Marketed as Diamox by Lederle Laboratories.

[5] N[[3-(1,2,3,4,5,6-Hexahydro-1,3-dimethyl-2,6-dioxopurin-7-ylmercuri)-2-methoxypropyl]carbamoyl] succinamic acid. Marketed as Mercuhydrin by Lakeside Laboratories.

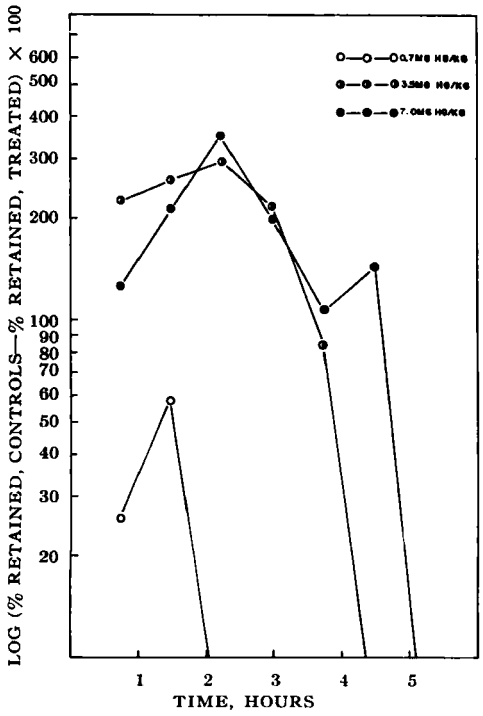

Fig. 5.—Effect of meralluride on sodium retention in the rat.

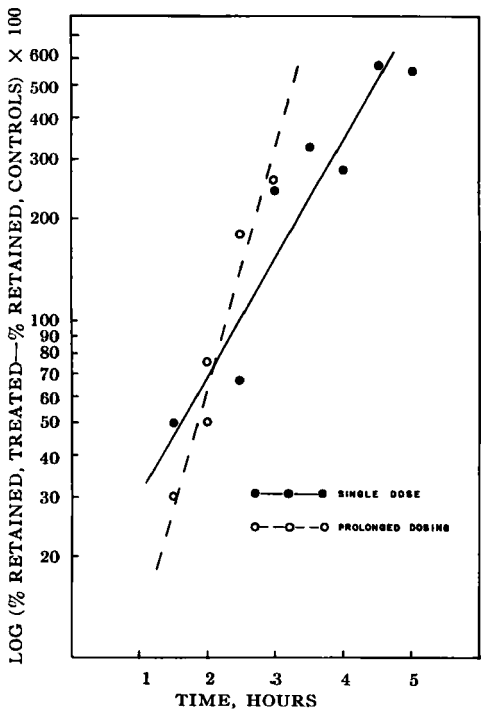

Fig. 6.—Effect of amphenone-B on sodium retention in the rat.

animals were used for each compound. One group was kept as a control in each case.

Hydration consisted of intraperitoneal injection of 25 ml./Kg. of 0.9% sodium chloride solution containing 0.05 μc. of sodium-22. The animals then received an intraperitoneal injection of each compound and control immediately after hydration. The amount of radioactivity retained was then determined by periodically determining the total body radioactivity at 45-minute intervals over 5¼ hours.

All meralluride injections were intramuscular.

Effect of Amphenone B[6] on Sodium Metabolism.—Vogt (9) noted that amphenone B would either increase or decrease sodium excretion in rats dependent upon whether the animal received a single dose of the compound or prolonged doses over a period of 2–17 days. Since the activity of this compound is evidenced by a change in sodium metabolism brought about by a change in aldosterone biosynthesis, it was subjected to examination by this technique.

In the single dose study, rats were injected intraperitoneally with 200 mg./Kg. of amphenone B in distilled water following hydration; the controls received an equal volume of distilled water. In the case of prolonged doses, the animals received 200 mg./Kg. of the compound in distilled water daily for 5 days. Controls again received an equal volume of distilled water at the same time. Hydration was undertaken just prior to the injection of the compound on the fifth day of dosing. Sodium retention was determined as previously described.

[6] 3,3-Bis(*p*-aminophenyl) butane; Ciba Pharmaceutical Products, Inc.

Ability of Diuretic Agents to Concentrate Sodium in the Urine.—Since there is reason to believe that diuretic activity and natruretic activity are related, all conditions in the previous experiments (with the exception of the amphenone B experiment) were duplicated; the animals were placed in metabolism cages and urine samples collected. The radioactivity contained in the samples was determined by placing the urine in a 20-ml. glass bottle and then placing in the PUSAC. The percentage of the original whole body radioactivity that was contained in the sample was determined.

RESULTS AND DISCUSSION

It was possible to detect effects on sodium metabolism elicited by each of the drugs used. Both natruretic responses and inhibition of sodium excretion were readily observed.

The results obtained in the experiments involving the DCA-blocking effect of spironolactone indicate a positive response at each dose administered (Fig. 1.)[7] These results suggest that the drug has a rapid onset and a duration of action of over 5 hours as well under the conditions of this experiment. The changes in slope are possibly explained by the mode of administration of DCA, since two oily subcutaneous injections were made 3 hours apart.

[7] In Fig. 1 and in all figures showing natruretic activity, a semilogarithmic plot was obtained by plotting the difference of the per cent of sodium retained by the controls, minus the per cent retained by the treated animals, multiplied by 100, along the *y*-axis and time along the *x*-axis. Where sodium retention was indicated, the difference of the per cent retained by the treated animals, minus the per cent retained by the controls, multiplied by 100, was logarithmically plotted on the *y*-axis.

TABLE I.—STUDIES OF SODIUM CONCENTRATION IN THE URINE[a]

Drug	Av. Urine Vol.	% of Orig. Act.[b]	DI, ml. %[c]
Acetazolamide			
Control	7.2	4.7	33.7
Treated	8.5	8.8	75.1
Chlorothiazide			
Control	8.9	7.2	64.4
Treated	6.4	12.2	77.8
Hydrochlorothiazide			
Control	6.3	6.6	41.4
Treated	10.3	8.8	91.8
Meralluride (10–20 hours)			
[d]Control	5.7	4.1	23.3
[d]Treated	12.3	2.7	37.1
Spironolactone			
Control	5.1	1.2	6.0
Treated	7.3	2.0	14.5

[a] Five-hour samples unless otherwise stated. Doses: acetazolamide (50 mg./Kg. BW), chlorothiazide (12.5 mg./Kg. BW), hydrochlorothiazide (2.5 mg./Kg. BW), meralluride (3.5 mg. Hg/Kg. BW), spironolactone (1.2 mg./rat). [b] $\frac{\text{Activity in urine specimen}}{\text{Activity in whole animal at time } 0} \times 100$. [c] Urine volume × av. per cent of original activity. [d] Data from six animals, all others from three animals.

This could allow for a fluctuation in the blood level of DCA.

The results obtained from the studies involving the four known diuretic agents are shown in Figs. 2–5. In each case it was possible to detect the natruretic activity of the drug under study in the chosen test period. These compounds represent three physiologically different natruretic actions, suggesting the feasibility of this procedure for screening diuretic agents. The results also indicate a possibility that more elaborate experimental setups, primarily a more varied dose schedule, might give quantitative as well as qualitative indications regarding the efficacy of a particular drug. This was evidenced by the fact that the results obtained here closely correlate with the known action of the drugs in respect to onset and duration of action.

Amphenone B was shown to inhibit sodium excretion as a single dose or after prolonged doses. These results were reproducible under the conditions of these experiments, although they are not in complete agreement with earlier experiments reported in the literature (10).

Ability of Diuretic Agents to Concentrate Sodium in the Urine.—Results in Table I indicate that neither urine volume measurements alone nor sodium-22 excretion alone gave consistent indications of the activity of the known diuretic compounds in every case. The explanation for this may be that the experimental groups in this particular study were small and that the periods of observation were short. However, the DI (Diuretic Index) did give definite indications of drug action in all cases studied here. (See Table I.) (DI = Urine Volume × Average % of Original Whole Body Radioactivity in Urine.) The percentage of original activity was calculated by dividing the net amount of activity contained in the urine sample by the net amount of activity contained in the corresponding animal at time *0*, and then converting to per cent. This value was calculated for each animal, and the average of the values was determined for each test compound.

It appears from these results that an enhancing or inhibiting effect on sodium excretion by compounds known to affect sodium metabolism could be determined in a simple 5-hour experiment.

SUMMARY

Procedures have been described for detecting drug effects on sodium metabolism in rats over a period of 5 hours by whole body radioassay techniques in an effort to evaluate the feasibility of *in vivo* tracer techniques in drug screening studies. These procedures have the following advantages: short experiment times are possible; it is not necessary to collect urine samples; fewer test animals are required than in diuretic screening procedures presently in use; and it is not necessary to sacrifice the animal, thereby allowing for serial study of drug action on the same animal.

The procedures described here involve studies of sodium metabolism only; however, whole body liquid scintillation counting should be an effective tool in investigations of pharmacologically active substances, especially in cases where the drug in question exerts its major effect on a body constituent which is available in a labeled form. Compartmental analyses of the excretion curves of many metabolically active ions (Na, K, Ca, Fe, I) may be expected to serve qualitatively and quantitatively as sensitive indicators of drug action.

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