PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 242–247 (1975)

Apparent Volumes of Distribution of ^{125}I-Iothalamate and Inulin in Chickens[1] (38781)

KAREN M. HARRIS[2] AND T. I. KOIKE

Department of Physiology, University of Arkansas Medical Center, Little Rock, Arkansas 72201

A precise definition and accurate measurement of extracellular fluid volume (ECFV) in mammals has not been possible due, in part, to the heterogeneity of this fluid phase (1, 2). There is little information regarding this problem in chickens although similar difficulties in estimating this fluid phase undoubtedly exist in this species as well. In searching for a convenient substance to determine the volume of the extracellular compartment in intact chickens by the dilution method we have examined the possibility of using labeled iothalamate as an indicator. The labeled compound is a tri-iodinated benzoic acid derivative that is easily measured, is apparently excluded from most intracellular compartments (3, 4), and does not appear to bind to plasma protein (3, 5, 6). The present study was undertaken to establish the equilibration time following injection of labeled iothalamate, to observe the effects of saline infusion on the apparent volume of distribution of ^{125}I-iothalamate, and to compare, simultaneously, the volume distributions of ^{125}I-iothalamate and inulin.

Materials and Methods. Twenty White Leghorn pullets weighing between 0.69 and 1.25 kg (mean 1.01 kg) were used in these studies. The birds were housed individually in screen-bottomed cages and were maintained on a commercial growing ration (Starter-Grower, Ralston Purina Co.) and water *ad libitum* for at least a week before they were used. Animals, fasted overnight but allowed water *ad libitum*, were anesthetized with pentobarbital sodium (30 mg/kg iv) and a brachial vein and artery cannulated with PE 50 tubing. Both ureters were located by blunt dissection (7), isolated with ties, and occluded 10–15 min prior to injection of the indicator(s). The indicators used in this study were ^{125}I-sodium iothalamate and inulin. One ml of 0.15 *M* NaCl containing 1–2 μCi ^{125}I-iothalamate was administered intravenously at zero time followed by 1.5 ml of 0.15 *M* NaCl to clear the tubing. In one series of experiments 50 or 100 mg inulin were simultaneously injected with the labeled iothalamate. Subsequent to injection of the indicator(s) blood sample or samples (approximately 1.2 ml each) were obtained by displacing the saline in the tubing with arterial blood and drawing the sample into heparinized syringes. The blood samples were centrifuged immediately and the plasma drawn off for analysis of inulin and/or ^{125}I-iothalamate.

Plasma disappearance curve of ^{125}I-iothalamate. Five pullets were used in an initial series of experiments to characterize the plasma disappearance curve of ^{125}I-iothalamate following injection. Blood samples were obtained at 3, 10, 20, 40 and 60 min subsequent to injection of the indicator. The amount of radioactivity in one ml of plasma, expressed as a percent of the injected dosage, was plotted as a function of time to construct the plasma disappearance curve and to establish the equilibration time for the indicator.

Effect of saline infusion on apparent volume of distribution of ^{125}I-iothalamate. A second series of experiments on five animals was performed in which the apparent volume of distribution of labeled iothalamate was determined serially prior to and following the intravenous administration of 0.15 *M* NaCl. The volume of saline administered was adjusted to the body weight and equalled about 15% of the estimated ECFV (assumed to be 20% of the body wt). Blood samples were obtained at 50 and 65 min following ^{125}I-iothalamate injection (presaline samples). The calculated volume of saline was then

[1] Supported in part by P.H.S. Research Grant No. AM-10393 and the Arkansas Heart Association.

[2] Recipient of P.H.S. Predoctoral Fellowship 5 FOI GM49672. Present address: 12520 S. W. Faircrest, Portland, Oregon 97225.

infused intravenously over a period of about 3 min (66–69 min following the injection of the indicator) and five additional blood samples were taken over a period of an hour at 130, 145, 160, 175 and 190 min (postsaline blood samples). The initial postsaline sample (130 min) was taken an hour following completion of saline infusion and about 2 hr following ^{125}I-iothalamate injection.

In this series of experiments a sample of heart muscle (ventricle) and liver were obtained in addition to the two kidneys immediately following the final blood sample. The apparent ^{125}I-iothalamate space in the tissues was calculated by dividing the amount of radioactivity in the tissues (cpm/g wet wt) by the amount found in the final plasma sample (cpm/ml).

Comparison of ^{125}I-iothalamate and inulin volumes of distribution. ^{125}I-iothalamate and inulin were simultaneously injected into five birds and postinjection blood samples obtained at 20, 40, and 60 min. The 60-min sample was used to calculate the ^{125}I-iothalamate and inulin spaces. The 20- and 40-min samples were analyzed for radioactivity only. In these experiments a plasma sample was obtained before injection of the indicators in order to obtain a plasma inulin blank. The plasma ^{125}I-iothalamate data from this series were also used in constructing the disappearance curve (above).

The apparent volume of distribution of the indicators was calculated by dividing the quantity of indicator injected (corrected for the total amount found in the two kidneys) by the concentration in plasma. The amount of indicator(s) administered in any given experiment was determined by measuring radioactivity and inulin (where used) on duplicate aliquots of the injection solution. Correction for kidney content of the indicator was made since it was anticipated that glomerular filtration, although attenuated by ureteral obstruction, would probably continue to some extent (8).

Radioactivity of plasma and tissues was measured in a Nuclear Chicago Autogamma counter. The inulin concentration of TCA filtrates of plasma or kidney homogenates was determined by the anthrone method (9). In order to correct for noninulin chromogen in renal tissue inulin blanks of kidney tissue were estimated on five additional birds not injected with inulin. The average value of the noninulin chromogen (96 $\pm$ 10.3 μg/g wet wt) was used to calculate the total amount of inulin present in the two kidneys at the end of the experiments where inulin was used as an indicator.

^{125}I-sodium iothalamate (Glofil-125) was obtained from Abbott Laboratories, Radiopharmaceutical Products Division. Radiopurity was checked by ascending paper chromatography using tertiary amyl alcohol saturated with 5.7% NH_4OH as the solvent system and was found to exceed 99%. Inulin was obtained from Nutritional Biochemicals. Student's *t* test was used to evaluate differences between means unless otherwise noted.

Results. The plasma disappearance curve for ^{125}I-iothalamate is shown on Fig. 1. The amount of ^{125}I-iothalamate in one ml of plasma, expressed as a percent of the total amount injected, is plotted on the vertical, logarithmic axis as a function of time. Under the conditions of these experiments, the movement of labeled iothalamate out of the plasma compartment apparently follows closed two compartment kinetics and the data can be fitted to a curve described by the equation, $P_t = 0.68e^{-0.18t} + 0.35$ (Where t = time and P_t = plasma concentration of iothalamate at time, t).

The apparent volume of distribution of ^{125}I-iothalamate before and following the intravenous infusion of saline is summarized on Fig. 2. The presaline iothalamate space at 50 and 65 min averaged 23.6 $\pm$ 0.61 (SE) % of the body weight and did not differ significantly from each other. The postsaline volumes of distribution averaged 28.4 $\pm$ 0.22 (SE) % of the body weight (Range 27.8 $\pm$ 1.0 to 29.1 $\pm$ 1.1) and also did not vary significantly over the sampling period of 1 hr. However, the five postsaline volumes of distribution of ^{125}I-iothalamate were elevated significantly from presaline values (Fig. 2). The 65- and 130-min iothalamate spaces (i.e. pre- and postsaline volumes of distribution are tabulated on Table I together with a comparison of the observed and expected post-saline values. The expected postsaline space was computed by adding the volume of saline infused to the 65-min volume of distribution. The ratios of the observed and

expected values for the 130-min ^{125}I-iothalamate space averaged 104 ± 2.3 (SE) % and did not differ significantly from each other ($P > 0.2$, t test for paired differences).

The simultaneous volumes of distribution of ^{125}I-iothalamate and inulin 60 min subsequent to the injection of the two indicators are summarized in Table II. The apparent volume into which inulin penetrates after an hour is 75% of the labeled iothalamate space and the difference between the two is statistically significant ($P < 0.005$).

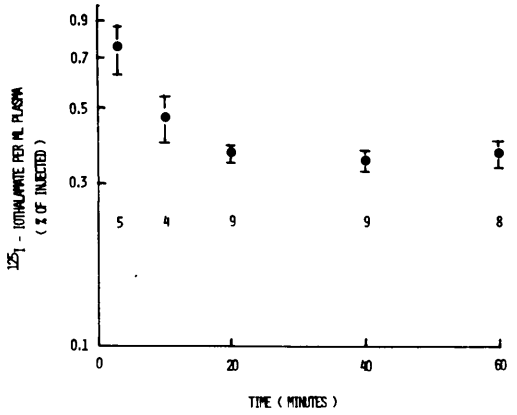

FIG. 1. Plasma disappearance curve for ^{125}I-Iothalamate following its injection at zero time. The number under each point within the figure indicates the number of animals used to calculate the mean ±SEM.

The ^{125}I-iothalamate space of heart muscle, liver, and kidney in the saline infused birds are summarized in Table III. Labeled iothalamate spaces of the three tissues differed significantly from each other ($P < 0.001$) with kidney tissue having the highest content of the indicator.

Discussion. The only other substance that has been used to estimate the volume of the extracellular phase in intact chickens by the dilution method has been thiocyanate (11, 12). The 10-min thiocyanate space in White Leghorn chickens of comparable age and weight to those used in the present study averaged 30.0 ± 2.7 (SD) % of the body weight (12). This value is greater than ^{125}I-iothalamate or inulin spaces at 60 min (Table II). Data that would provide reasons for these differences are not available. It appears, however, that the volume into which the indicator penetrates is inversely related to the molecular dimension of the substance (the molecular weights of thiocyanate, iothalamate, and inulin being 58, 607, and

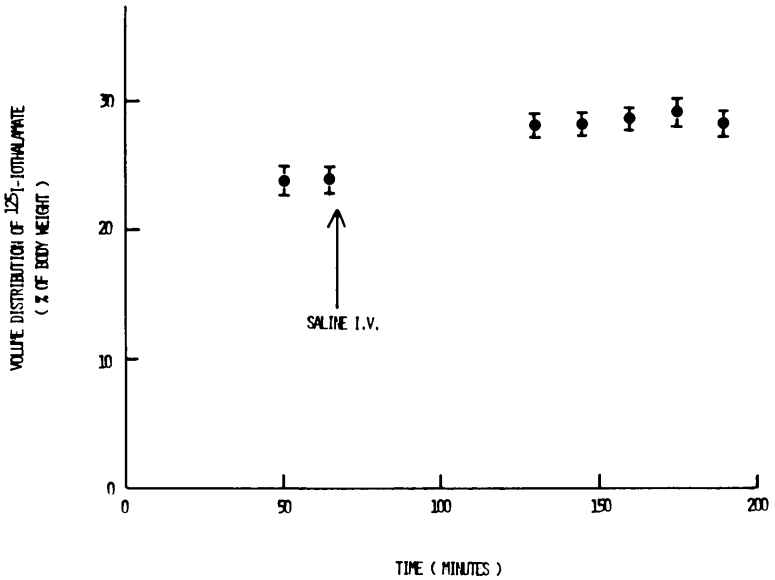

FIG. 2. Apparent volume distribution of ^{125}I-iothalamate prior to and following the intravenous infusion of 0.15 *M* NaCl. The data were evaluated by a single factor analysis of variance on repeated measurements followed by Newman-Keuls' test on the ranked means at the 0.05 level of significance (10).

TABLE I. ^{125}I-IOTHALAMATE VOLUME DISTRIBUTION (% OF BODY WEIGHT) PRIOR TO AND AFTER SALINE ADMINISTRATION. SALINE WAS INJECTED IV FROM 66–69 min. DIFFERENCE BETWEEN OBSERVED AND EXPECTED 130 min ^{125}I-IOTHALAMATE SPACES WAS ANALYZED BY STUDENT'S PAIRED *t* test.

Animal	Body weight (kg)	^{125}I-iothalamate space 65 min	130 min	Saline injected (ml)	Postsaline space[a] $\frac{\text{Observed}}{\text{Expected}} \times 100$
3	1.18	21.5	25.8	35	105
4	1.17	25.3	28.1	35	99
6	1.08	22.6	28.4	35	110
8	0.85	26.1	31.2	25	107
11	1.18	22.9	25.8	35	98
Mean		23.7	27.9		104
±SEM		0.87	1.00		2.3

[a] The "expected" postsaline space was computed by adding the volume of saline infused to the 65-min volume of distribution.

about 5000, respectively). As would be expected from the relative molecular weights thiocyanate, in addition to its greater volume of distribution, equilibrates more rapidly than does labeled iothalamate; i.e., within 10 min (11, 12) compared to 20 min or more for iothalamate (Fig. 1).

Biliary excretion of ^{131}I-iothalamate in aglomerular fish (3) and the continued formation of glomerular filtrate following occlusion of the ureters in dogs (8) suggest that the liver and kidneys are possible sites where removal of the indicator from plasma might occur in the present studies. The relatively high amounts of ^{125}I-iothalamate found in these two organs (Table III) are in agreement with such a suggestion.

Since renal clearance of ^{125}I-iothalamate is equal to the glomerular filtration rate in mammals (5, 6, 13), it is probable that the high levels of labeled iothalamate observed in kidney tissue in the present study (Table III) were due to filtration of the indicator. The total weight of the two kidneys averaged 0.79 ± 0.10 (SD) % of the body weight in these studies and contained 6.0 ± 0.37 (SE) and 3.7 ± 0.36 (SE) % of the total amount of ^{125}I-iothalamate injected after 60 and 190 min, respectively. The difference between the two values (2.3 ± 0.51) is significant ($P < 0.005$). Whether the difference is related to temporal events and/or to the saline infused in the longer experiments (190 minutes) is not clear. If the ^{125}I-iothalamate contained in the kidneys decreases with time it would obviously introduce errors into the volumes of distribution computed from plasma samples obtained at various intervals prior to the final sample (e.g., as in Fig. 2). However, the difference in renal content of labeled iothalamate between

TABLE II. SIMULTANEOUS VOLUMES OF DISTRIBUTION OF ^{125}I-IOTHALAMATE AND INULIN (% OF BODY WEIGHT) 60 MIN SUBSEQUENT TO INJECTION OF THE INDICATORS.

Animal	Body weight (kg)	^{125}I-iothalamate 60 min	Inulin 60 min	$\frac{^{125}\text{I-iothalamate}}{\text{Inulin}}$
15	1.10	22.9	16.3	1.40
16	1.19	28.3	17.1	1.65
21	1.07	19.7	17.5	1.13
22	1.06	20.6	17.1	1.20
24	1.25	24.2	17.8	1.36
Mean		23.1	17.2	1.35
±SEM		1.52	0.25	0.09

TABLE III. ^{125}I-IOTHALAMATE SPACE IN TISSUES (ml/100 g WET WEIGHT).

Animal	Heart	Liver	Kidney
3	28.9	76.1	126
4	29.6	74.5	154
6	26.5	79.5	134
8	34.0	85.5	149
11	27.4	77.5	98
Mean	29.3	78.8	132
±SEM	1.3	1.8	9.9

experiments terminated 60 or 190 min after injection is not large and the 65-min volume of distribution in the latter series (Table I) did not differ significantly from the former (Table II) ($P > 0.5$).

We also noted that the renal content of inulin was significantly greater ($P < 0.05$) than that of ^{125}I-iothalamate [8.6 ± 0.92 (SE) vs. 6.0 ± 0.37% of the injected dosages]. Micropuncture studies have shown that tubular permeability to ^{125}I-iothalamate, but not inulin, increases when intratubular pressures are elevated (4). Thus, the difference in fractional amounts of the two indicators found in the kidneys here may relate to differential rates of reabsorption since the plasma to filtrate concentration ratios of inulin and iothalamate would most likely be equal (5, 6, 13).

The 60-min inulin space in these studies was significantly less ($P < 0.005$) than the simultaneously determined ^{125}I-iothalamate space (Table II). The value of 17.2% of the body weight for inulin space in chickens is similar to the 30- and 60-min inulin space in the rat (14) and dog (15), respectively. The difference between iothalamate and inulin spaces may relate to slower rate of equilibration of the latter and/or more rapid entry of iothalamate into cellular compartment(s). Long term studies in mammals have shown, however, that inulin space increases progressively with time and that the removal of inulin from the extracellular fluid can involve binding (15), biliary excretion (16), or entry into cellular compartment(s) (14). Additional studies are needed in chickens in order to obtain information on the relationship between the ECFV and the temporal changes in apparent volume of distribution of inulin.

The constancy of the plasma concentration of ^{125}I-iothalamate from 20 to 60 min (Fig. 1) and 130–190 min (Fig. 2) after injection of the indicator indicates that the movement of labeled iothalamate from the equilibration volume of distribution is not perceptible within these time frames. Furthermore, the agreement between the expected and observed postsaline volumes of distribution for ^{125}I-iothalamate suggests that the infused saline and ^{125}I-iothalamate penetrate at parallel rates into an equal, although not necessarily an identical, fluid space. Plasma volume, estimated by determining T-1824 space in 5 birds of similar age, averaged 4.8 ± 0.3 (SE) % of the body weight (Harris and Koike, unpublished observations). Thus, the extravascular phase with which plasma iothalamate equilibrates, is four times the plasma volume and equals about 19% of the body weight.

The results of the present studies would suggest that ^{125}I-iothalamate could be used to measure the fluid phase represented by the plasma compartment and the extravascular fluid with which plasma is in rapid diffusion equilibrium. The magnitude of the renal and extrarenal clearances from the extracellular fluid phase would have to be assessed under any given experimental condition, however, in order to determine whether correction for such losses might be necessary.

Summary. The suitability of utilizing ^{125}I-iothalamate to estimate the volume of extracellular fluid was assessed in ureterally ligated chickens. Subsequent to intravenous administration the movement of labeled iothalamate from the plasma compartment follows closed two-compartment kinetics and equilibration between vascular and extravascular phases is attained in about 20 minutes. The volume of distribution of ^{125}I-iothalamate prior to and following the infusion of 0.15 *M* NaCl (equal to 15% of the estimated ECFV) averaged 23.6 ± 0.61 and 28.4 ± 0.22% of the body weight, respectively. The observed postsaline labeled iothalamate space did not differ statistically from the expected value. When administered simultaneously inulin penetrates into an apparent volume that is 75% of the labeled iothalamate space after 60 minutes. The content of ^{125}I-iothalamate is relatively high in liver and kidney tissue and suggests that these are major sites where removal of the indicator from plasma occur. It is suggested that ^{125}I-iothalamate, under appropriate conditions, could be used to measure the plasma volume and the extravascular fluid with which plasma is in rapid diffusion equilibrium.

^{125}I-Sodium Iothalamate for parts of this study was kindly provided by Abbott Laboratories of North Chicago, IL.

We thank Loyce Neldon for excellent technical

assistance and L. Pryor for computer analysis of parts of the data.

We thank Drs. J. N. Pasley and G. C. Bond for comments.

1. Edelman, I. S., and Liebman, J., Amer. J. Med. **27,** 256 (1959).
2. Walser, M., *in* "Compartments, Pools, and Spaces in Medical Physiology" (P. E. E. Bergner and C. C. Lushbaugh, eds.), p. 241 U. S. Atomic Energy Commission, Oak Ridge (1967).
3. Griep, R. J., and Nelp, W. B., Radiology **93,** 807 (1969).
4. Lorentz, W. B., Jr., Lassiter, W. E., and Gottschalk, C. W., J. Clin. Invest. **51,** 484 (1972).
5. Anderson, C. F., Sawyer, T. K., and Cutler, R. E., J. Amer. Med. Ass. **204,** 653 (1968).
6. Sigman, E. M., Elwood, C. M., and Knox, F., J. Nucl. Med., **7,** 60 (1966).
7. Dantzler, W. H., Amer. J. Physiol. **210,** 640 (1966).
8. Ullman, E., and Wilde, W. S., Amer. J. Physiol. **207,** 1273 (1964).
9. Davidson, W. D., and Sackner, M. A., J. Lab. Clin. Med. **62,** 351, (1963).
10. Winer, B. F., "Statistical Principles in Experimental Design," 2nd ed., McGraw-Hill Book Co., New York (1971).
11. Hegsted, D. M., Wilson, D., Milner, J. P., and Ginna, P. H., Proc. Soc. Exp. Biol. Med. **78,** 114 (1951).
12. Medway, W., and Kare, M. R., Amer. J. Physiol. **196,** 873 (1959).
13. Israelit, A. H., Long, D. L., White, M. G., and Hull, A. R., Kidney Int. **4,** 346 (1973).
14. White, H. L., and Rolf, D., Amer. J. Physiol. **188,** 151 (1957).
15. Knoefel, P. K., Kraft, R. P., Jr., Knight, R. D., and Moore, S. K. Life Sci. **13,** 1591 (1973).
16. Schanker, L. S., and Hogben, C. A. M., Amer. J. Physiol. **200,** 1087 (1961).

Received December 9, 1974. P.S.E.B.M. 1975, vol. 149.