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Extracellular Space in Chickens Estimated Using Sucrose, Thiosulfate, and Thiocyanate Injected Concurrently (40873)

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Abstract. The extracellular fluid (ECF) volume was estimated in chickens, a species for which remarkably high estimates (usually of thiocyanate space) have been reported. Chickens aged 8 weeks were given a single intravenous injection containing the three extracellular markers, [^{3}H]sucrose, [^{35}S]thiosulfate, and thiocyanate. After allowing 10 min for mixing to occur, plasma samples were obtained at intervals and marker concentrations were measured and used to estimate initial dilution of the markers in ECF. In contrast with most earlier studies, all three markers yielded estimates of ECF volumes that were in the range normally found in mammals. The mean sucrose space (17.8 ml/100 g body wt) was significantly lower than both the thiosulfate space (22.3 ml/100 g) and the thiocyanate space (23.9 ml/100 g).

Most estimates of extracellular fluid (ECF) volume in chickens have been made by measuring the dilution of the ECF marker, thiocyanate, in plasma samples taken at only a single time after injection of the marker. Usually no adjustment was made for renal loss of marker. Perhaps for these reasons some estimates (1, 2) have been remarkably high (more than 40% of body weight) compared with the values about 20% of body weight commonly quoted for mammals (3, 4). There are additional uncertainties associated with the use of thiocyanate since this marker is able to penetrate red blood cells and it is bound by serum albumin (5).

In experiments on glucose metabolism in chickens we encountered a need to estimate ECF volume reliably without the experimental complication of performing nephrectomy. The present paper describes experiments in which such estimates were obtained for the three ECF markers, thiocyanate, thiosulfate, and sucrose. To help assess the three markers, they were used simultaneously in each animal so that accurate comparisons could be made among them.

Materials and methods. Materials. Crossbred, white leghorn × australorp cocks, aged 5 to 6 weeks, were obtained from a commercial supplier. They were housed in groups of five and were given water and commercial poultry feed *ad libitum*. They were used in experiments when they were 8 weeks old, and weighed 892 ± 123 (SD) g.

The radioactive markers, sodium [outer S-^{35}S]thiosulfate and [6,6′(n)-^{3}H]sucrose, were obtained from the Radiochemical Centre, Amersham, U.K.

Experimental procedure. Chickens were anesthetized by injection of equithesin (6) into a leg vein using a cannula with a 26-gauge hypodermic needle attached for insertion into the vein. A dose of 1 ml/kg was given initially and additional doses were given at intervals during the experiment to maintain anesthesia. Another cannula was tied into the wing vein for collection of blood samples. A solution containing the three ECF markers ([^{3}H]sucrose, 370 kBq[3] in each animal; [^{35}S]thiosulfate, approximately 185 kBq in each animal; sodium thiocyanate (AR grade, Ajax Chemicals) in a dose adjusted for body weight, 1.233 mole/kg) was injected into a pectoral vein.

Blood samples (0.8 ml) were taken at 10,

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[3] The SI unit, becquerel, is one nuclear transformation per second. That is, one kilobecquerel (kBq) = 27.027 nCi.

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20, 30, 60, 90, 120, 150, and 180 min after the injection. In each case dead space fluid was discarded and the total volume was replaced with physiological saline (0.9 g/100 ml NaCl). Plasma was separated by centrifugation and the concentration of each marker was measured.

In one chicken, used to estimate the time taken for injected sucrose to equilibrate in the ECF, sucrose alone was injected and additional blood samples were taken at times before 10 min.

A double exponential function, reflecting loss of marker from the ECF, was fitted to the data for ECF marker concentrations at the times sampled. Concentrations of marker at zero time were obtained by extrapolation of these functions (Fig. 2) and the marker spaces were calculated using the dilution principle:

$$\text{Marker space} = \frac{\text{Amount injected}}{\text{Concentration at zero time}}.$$

Statistical Methods. A general computer program (7) for nonlinear regression, incorporating the Marquardt algorithm (8), was used to estimate the parameters of best fit (α_1, α_2, k_1, k_2) for the double exponential function:

$$Y = \alpha_1 e^{k_1 T} + \alpha_2 e^{k_2 T},$$

relating concentration of ECF marker (Y) to time after injection (T).

The experiment was of randomized completed block design (each chicken represented a block), and an analysis of variance appropriate to this design (9) was performed on the data for marker spaces.

Analytical procedures. Samples of plasma (100 μl each) were suspended in 10 ml Triton X-100–toluene (1:2 v/v) scintillator (10) and then counted for 10 min in a Nuclear Chicago Mark I liquid scintillation counter. Counts were determined, and corrections for quenching were made using the channels ratio method with external standardization (11). Concentrations of thiocyanate ion were measured in 200-μl samples of plasma using the method of Eder (5).

Estimation of equilibration time. The time taken for injected sucrose to equilibrate in the ECF was indirectly estimated for one chicken by repeatedly computing the apparent volume of distribution using subsets of data from which early samples were excluded from the calculation. In each case the calculation procedure was the same as described above for marker spaces. Figure 1 shows the pattern of increasing apparent volumes as progressively more of the early points are excluded. The half-time for this process was apparently 2.5 min; i.e., the equilibration was probably more than 90% complete by 7.5 min (three half-times) after the injection.

Results. Figure 2 shows the pattern of decline in plasma concentration of the three ECF markers, sucrose, thiosulfate, and thiocyanate, after simultaneously injecting them into 1 chicken. The data were well fitted by double exponential functions. For all three markers, these functions accounted for at least 99.8% of the total (within-animal) experimental variance in data from all 10 chickens used.

Mean marker spaces are shown in Table I, together with the analysis of variance. The mean space for the nonelectrolyte, sucrose (17.8 ml/100 g), was significantly smaller ($P < 0.05$) than the spaces for the

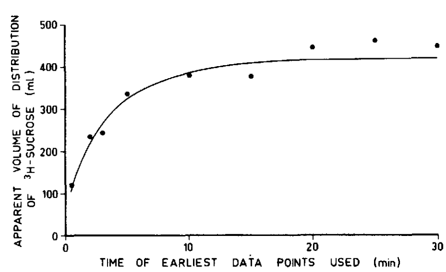

FIG. 1. Estimation of the equilibration time of intravenously injected [^{3}H]sucrose using repeated estimates of the apparent volume of distribution of [^{3}H]-sucrose. The estimates are based on subsets of data (plasma concentrations of [^{3}H]sucrose) obtained from a single experiment. Progressively more of the early data points were excluded from the extrapolation procedure (see text) in order to obtain successive estimates. For example, the 10-min apparent volume of distribution is based on only those [^{3}H]sucrose concentrations measured at 10 min and later. Estimates that include very early data points are low because mixing of the [^{3}H]sucrose into the ECF remains incomplete.

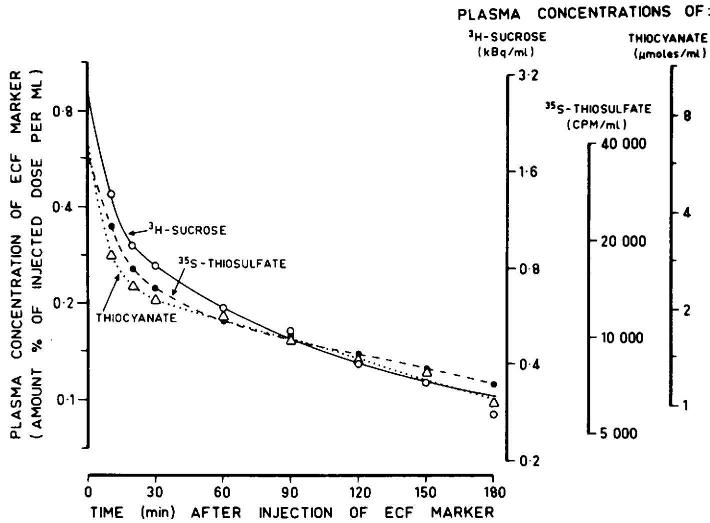

FIG. 2. Plasma concentrations of the extracellular markers, sucrose (○), thiosulfate (●), and thiocyanate (△), after their simultaneous injection into a chicken. The curved lines represent the double exponential functions fitted to the data; for sucrose, $Y = 0.301e^{-0.0067T} + 0.582e^{-0.1263T}$; for thiosulfate, $Y = 0.228e^{-0.0040T} + 0.346e^{-0.0975T}$; for thiocyanate, $Y = 0.237e^{-0.0047T} + 0.395e^{-0.1918T}$. These functions, reflecting loss of marker from the ECF, were extrapolated to zero time to obtain the concentrations used to estimate marker spaces.

two electrolytes, thiosulfate (22.3 ml/100 g) and thiocyanate (23.9 ml/100 g). Spaces for thiosulfate and thiocyanate did not differ significantly from each other.

The size of marker spaces tended to decrease with increasing body weight; for sucrose, the correlation coefficient (r) was -0.57 ($P < 0.1$); for thiosulfate it was -0.27 (not significant); and for thiocyanate it was -0.71 ($P < 0.05$).

Discussion. The sucrose, thiosulfate, and thiocyanate spaces reported here (Table I) are in good agreement with the mean inulin space (17.2 ± 0.25 ml/100 g) and mean iothalamate space (23.6 ± 0.61 ml/100 g) found for functionally nephrectomized (ureters tied) chickens of similar size by Harris and Koike (12). These appear to be the only two studies in which ECF volumes of chickens have been estimated using

TABLE I. SUCROSE, THIOSULFATE, AND THIOCYANATE SPACES (ml/100 g BODY wt) IN CHICKENS ($N = 10$)[a]

Sucrose space	Thiosulfate space	Thiocyanate space
Means ± SEM		
17.8 ± 1.4	22.3 ± 1.8	23.9 ± 2.9

Source of variation	Degrees of freedom	Mean square	Variance ratio
Analysis of variance			
Between chickens	9	66.98	1.84
Between marker substances	(2)	99.52	2.73
Electrolye vs nonelectrolyte	1	186.56	5.12[b]
Thiosulfate vs thiocyanate	1	12.48	0.34
Error	18	36.44	

[a] Group means and associated analysis of variance.
[b] $P < 0.05$.

methods that attempt to eliminate error arising through renal loss of marker. The good agreement indicates that the present method (not requiring functional nephrectomy) is not seriously affected by any systematic errors that might arise through incomplete mixing of the markers or by numerical errors associated with the extrapolation procedure (13).

The double exponential nature of the disappearance curves was as expected for compounds that meet a significant barrier to diffusion at the capillary wall (14). Initial decay is rapid while renal loss depletes the readily accessible vascular compartment, and then decay slows when renal loss is limited by the impeded diffusion from intravascular compartment to vascular compartment.

Most earlier estimates were made simply by measuring the concentration of thiocyanate in a single plasma sample collected 10 min after injection. Any renal loss of marker occurring in this time was disregarded. The earlier estimates were much higher than the present ones and those of Harris and Koike (12), e.g., 42.2 ml/100 g in chickens of the same age (8 weeks) as ours (2), 32.2 ml/100 g in slightly older (10 weeks) chickens (15), and more than 40 ml/100 g for younger chickens (1, 2). These studies had suggested that ECF volumes in chickens might be substantially higher than those of mammals for which numerous studies with the same markers have given estimates of the order of 20% (3, 4). The above-mentioned, early depletion of marker from the vascular compartment presumably led to some overestimation of spaces when only a single sample was used. Some indication of the magnitude of this error can be obtained from the present experiments in which disappearance curves for thiocyanate show a loss of about 20% in the earliest interval sampled—10 to 20 min after injection. Although substantial, this loss cannot account fully for the larger estimates of ECF volume obtained in earlier studies. During the first few minutes after injection, when marker is still equilibrating with ECF and remains at especially high concentration in the plasma, losses are presumably higher than 20% per 10 min. The curve fitting procedure used in the present study should account for the renal clearance of solute after solute mixing, but not for renal losses occurring during the mixing transients. Nevertheless, the procedure seems to strike a balance between overestimation of the distribution volume due to early tracer loss, and underestimation which would result if plasma solute concentrations taken before the solute equilibrates were used to calculate the distribution volume.

The present study was restricted to ECF markers of small molecular weight in order that equilibration should be rapid. Sucrose appeared to equilibrate within 10 min. This is in accord with an expected half-time of less than 2 min which we calculated for the process of mixing into the ECF, the calculation being based on a simple two-compartment model (14) and on Renkin's estimates of the permeability of capillaries to sucrose and the surface area of capillaries (16). It is likely that thiosulfate and thiocyanate also equilibrated within that time, since their molecular weights are lower than that of sucrose. Comparison of the estimates obtained with these three markers show clearly that estimates are biased to a small extent by choice of marker, sucrose giving about 20% lower values than thiosulfate and thiocyanate. Although it is not possible to state with certainty which is the most suitable marker, we have favored sucrose because it appears to be wholly confined to the ECF whereas thiosulfate and thiocyanate have been shown to penetrate red blood cells to some extent (5, 17, 18).

Chickens in the first few weeks of life appear to have substantially higher ECF volumes than do the 8-week-old ones used in the present experiments. Thiocyanate spaces, based on single 10-min blood samples (2) averaged 61% in 1-week-old chickens and then declined consistently with increasing age until they averaged 26% at 32 weeks. Although the accuracy of such estimates can be questioned, the evidence for a decline of ECF volume with age seems strong and is in accord with findings for mammals (19). This conclusion is supported by observations that isolated muscles of young chickens have ECF volumes exceeding 50% of body weight judged by estimates of chloride space and by direct histological examination (20). In young, growing animals the progressive reduction of ECF volume is probably partly determined by growth itself and partly by age, independently of growth (21). In our experiments, the negative association between body weight and ECF volume was not determined by age differences, since all chickens were of equal age.

The principal finding of the present study is that (by the time they are 8 weeks old) chickens have ECF volumes that are within the range usually found for adult mammals and are not especially high in the way suggested by earlier studies. This finding was obtained with all three ECF markers, sucrose, thiosulfate, and thiocyanate, but of these, sucrose gave consistently lower estimates than thiosulfate or thiocyanate.

Note added in proof. The present estimates of ECF volume are in good agreement with the value of 24.9 ml/100 g found for radiosulfate space in laying hens in which ureteral urine was collected in order to permit correction for renal loss of marker (Mongin, P., and Carter, N. W., Ann. Biol. Anim. Bioch. Biophys. 16, 649 (1976)).

1. Hegsted, D. M., Wilson, D., Milner, J. P., and Ginna, P. H., Proc. Soc. Exp. Biol. Med. **78**, 114 (1951).
2. Medway, W., and Kare, M. R., Amer. J. Physiol. **196**, 873 (1959).
3. Pitts, R. F., "Physiology of the Kidney and Body Fluids," 2nd ed., p. 22. Year Book Medical Publishers, Chicago (1968).
4. Spector, W. S., "Handbook of Biological Data," p. 340. Saunders, Philadelphia (1956).
5. Eder, H. A., Meth. Med. Res. **4**, 48 (1951).
6. Booth, N. H. *in* "Veterinary Pharmacology and Therapeutics" (L. M. Jones, ed.), 3rd ed. p. 165. Iowa State Univ. Press (1966).
7. Coulter, P. R., "A Computer Program for Nonlinear Regression Analysis and Function Minimisation," Report from the Chemical Engineering Department, School of Engineering, Monash University, CHER 70-2 (1970).
8. Marquardt, D. W., J. Soc. Indust. Appl. Math. **2**, 431 (1963).
9. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics," p. 134. McGraw–Hill, New York (1960).
10. Turner, J. C., Int. J. Appl. Radiat. Isotopes **20**, 499 (1969).
11. Kobayashi, Y., and Maudsley, D. V., "Biological Applications of Liquid Scintillation Counting," pp. 22–31. Academic Press, New York (1974).
12. Harris, K. M., and Koike, T. I., Proc. Soc. Exp. Biol. Med. **149**, 242 (1975).
13. Riggs, D. S., "The Mathematical Approach to Physiological Problems," p. 160. Williams & Wilkins, Baltimore (1963).
14. Landis, E. M., and Pappenheimer, J. R., *in* "Handbook of Physiology" Section 2: "Circulation," Vol. 2 (W. F. Hamilton, ed.), p. 1021. Amer. Physiol. Soc., Washington (1963).
15. Brown, K. I., Brown, D. J., and Meyer, R. K., Amer. J. Physiol. **192**, 43 (1958).
16. Renkin, E. M., Circ. Res. **41**, 735 (1977).
17. Cardozo, R. H., and Edelman, I. S., J. Clin. Invest. **31**, 280 (1952).
18. Elkington, J. R., and Danowski, T. S., "The Body Fluids," p. 78. Williams & Wilkins, Baltimore (1955).
19. Manery, J. F., Meth. Med. Res. **4**, 66 (1951).
20. Barlow, J. S., and Manery, J. F., J. Cell. Comp. Physiol. **43**, 165 (1954).
21. Widdowson, E. M., and McCance, R. A., Proc. Roy. Soc. Lond. B **152**, 188 (1960).

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