SIMULTANEOUS DETERMINATION OF VOLUMES OF EXTRACELLULAR FLUID

A. G. Rummel' and G. S. Chudnovskii

UDC 612.014.461.3-019 : 599.742.1

A method of simultaneous determination of the distribution volumes of the dye T-1284, sodium thiosulfate, and antipyrin and also of the circulating blood volume is described. The accuracy of the method is ±7.7%.

* * * *

For an objective assessment of water-salt homeostasis information regarding all water spaces of the body is essential. However , most methods so far devised require the successive determination of the body fluids. Methods of simultaneous determination of the water spaces of the body so far described [6, 11, 12] require the use of radioactive substances and are very laborious. Methods of simultaneous determination of the fluid volumes of the body without the use of isotopes [9, 10, 13] have not been widely used in experimental and clinical practice because they do not reflect the true values of the water spaces of the body or they involve administration of reagents and collection of urine over many hours.

In this paper we describe a comparatively simple method of simultaneous determination of the water spaces of the body.

EXPERIMENTAL METHOD

Reagents. 1) Mixture of the dye Evans T-1824 (200 mg), sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, 200 g), antipyrin (30 g), and sodium carbonate (Na_2CO_3*; 100 mg/liter water†). This mixture will subsequently be called SETA. 2) Tungsten reagent, prepared on the day of the experiment from 100 ml 0.1 N H_2SO_4 and 100 ml 1% Na_2WO_4, made up to one liter with water. 3) 0.004 N iodine solutions. 4) 0.2 N NaOH, prepared on the day of the experiment. 5) Zinc reagent. On the day of the experiment 26.7 ml 10% $ZnSO_4 \cdot 7H_2O$ solution in 0.25 N H_2SO_4 was made up to 100 ml with water. 6) 4 N H_2SO_4. 7) 0.2% sodium nitrite solution ($NaNO_2$).

Apparatus. 1) SF-4 or SFD-2 spectrophotometer with special cuvette-holder capable of measuring optical density in 1.2 ml of solution in rectangular quartz cuvettes with an absorption layer thickness of 10 mm. LP-58 potentiometer with calomel (comparing) and platinum (indicator) electrodes. 3) Abbe refractometer. 4) Centrifuge giving 4000 rpm with 10- and 25- ml tubes. 5) Hematocrit. 6) Calibrated syringes.

Technique of Experiment. Under chloralose anesthesia (80-100 mg α-chloralose /kg body weight) cannulas were inserted into the femoral artery and jugular vein. The hematocrit index was determined‡. The blood obtained was at once centrifuged at 3000 rpm for 7-10 min and the plasma separated**. This plasma was subsequently used as control sample. SETA was then injected into the jugular vein by the calibrated syringe at a speed of about 4 ml/min in a dose of 1 ml/kg body weight, after which the cannula was washed out with 5-10 ml physiological saline. The time when injection of SETA began was taken as

* The sodium carbonate is used to preserve the sodium thiosulfate.

† Mixture made up bidistilled, boiled (free from carbon dioxide) water, poured into 25-ml ampoules, and sterilized.

‡ For determination of blood volume.

** Subsequently the blood was immediately centrifuged after being taken, and the plasma was separated.

Laboratory of Physiology and Pathology of Endocrine Regulation, Department of Experimental Biology, Institute of Cytology and Genetics, Siberian Division, Academy of Sciences of the USSR, Novosibirsk (Presented by Academician V. V. Parin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 2, pp. 117-120, February, 1968. Original article submitted June 14, 1966.

zero, from which counting began. Blood was then taken in volumes of 3-5 ml from the femoral artery after intervals of 15, 30, 45, 60, 90, 120, and 180 min. The removed blood was replaced by physiological saline through the jugular vein. The control sample and all seven samples of experimental plasma, together with 5-6 ml samples of SETA in water in dilutions of 1 : 50, 1 : 200, and 1 : 750 were kept in a refrigerator (4°).

Quantitative Determination of T-1824, Sodium Thiosulfate, and Antipyrin was carried out next day. All samples of plasma were recentrifuged at 1500-3000 rpm for 10-15 min and the supernatant plasma was used in the experiment. The total protein content in the control plasma was determined (in g%) refractometrically.

Determination of T-1284. The control and the first four plasma samples were analyzed. Water, SETA solution, (1 : 50), and control and experimental plasma, in volumes of 1.2 ml each, were added to the cuvettes and their optical density measured at a wavelength of 618 mμ. The water was used to establish zero, and the optical density of the control plasma was subtracted from the optical density readings of the experimental plasma samples. If hemolysis or lipemia were present, a correction was introduced [5].

Determination of Sodium Thiosulfate. The control and the first four experimental samples of plasma, after spectrophotometry, were used for determination of sodium thiosulfate. Water, SETA solution (1 : 200), and the control and experimental plasma samples were added in volumes of 0.2 ml each into 25-ml centrifuged tubes. Next, 20 ml of tungsten reagent was added to each tube. After centrifugation (1000-1500 rpm, 10-15 min), supernatant fluid was transferred in volumes of 15 ml into 50-ml beakers for titration. Titration was carried out with 0.0004 N iodine solution on the LP-58 potentiometer to a predetermined equivalent point. The volume of iodine solution used up in titrating each sample was recorded.

Determination of Antipyrin. Antipyrin was determined by the method of Brodie and co-workers [4], slightly modified by ourselves. Water, SETA solution (1 : 750) control plasma, and the 4th, 5th, 6th, and 7th samples of blood plasma were added in volumes of 0.5 ml each to centrifuge tubes with a capacity of 5-10 ml. To each tube were added 0.75 ml of 0.2 N NaOH solution and 0.75 ml of zinc reagent, drop by drop; the contents of the tubes were thoroughly mixed and centrifuged at 3500-4000 rpm for 20 min. Supernatant fluid was transferred in volumes of 1.2 ml into quartz cuvettes, and after addition of one drop 4 N H_2SO_4 the optical density of the samples was measured at 345 mμ. Next, one drop of 0.2% sodium nitrite solution was added to each cuvette, and the optical density of all the samples measured again 30 min later under identical conditions. Water samples were used to establish zero and the control plasma samples for determining the optical density of the nitroso-antipyrin in the samples was given by the difference between the results of the 2nd and 1st measurements.

CALCULATIONS

The distribution spaces of T-1824, sodium thiosulfate, and antipyrin were calculated from the following formula:

$$\text{D.S.} = \frac{A \cdot C \cdot V}{B \cdot P}\left[1 - \frac{\text{Plasma protein (in g \%)}}{100}\right],$$

where D. S. represents the distribution space of T-1824, sodium thiosulfate, or antipyrin (in ml/kg body weight); A the optical density of a standard solution of SETA (for T-1824 and antipyrin) or the volume (in ml) of 0.0004 N iodine used up in the titration of sodium thiosulfate in a standard SETA solution; B the optical density of the samples (extrapolated to zero time) or the volume (in ml) of 0.0004 N iodine solution used up in the titration of sodium thiosulfate in the samples (extrapolated to zero time); C the degree of dilution of the standard SETA solution; V the volume (in ml) of added SETA; P the weight of the animal (in kg); while the term $1 - \frac{\text{plasma protein (in g \%)}}{100}$ represents the content of water in the plasma in percent.

To determine theoretical concentration at the moment of addition (time zero; C_0) the method of extrapolation was used [3]. On semilogarithmic paper the time was plotted along the simple scale and the concentration of substances in samples of blood plasma along the logarithmic scale [in this case, the optical densities of the plasma samples for T-1824 and antipyrin or the volume (in ml) of iodine solution used up in titration of sodium thiosulfate in the plasma samples].

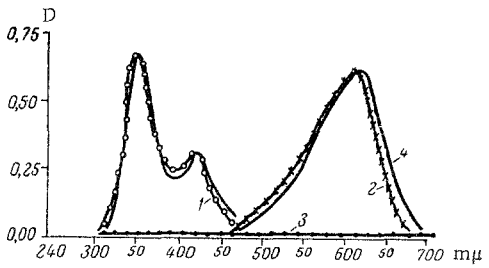

Fig. 1. Optical density (D) of T-1824, sodium thiosulfate, antipyrin, and SETA as a function of wavelength (in mμ). 1) Antipyrin; 2) T-1824; 3) sodium thiosulfate; 4) SETA.

EXPERIMENTAL RESULTS

The plasma volume of normal male dogs varied from 38.2 to 71.2 ml/kg body weight, the blood volume from 66.1 to 102.8 ml/kg body weight, the extracellular fluid from 171.1 to 343.5 ml/kg body weight, and the total body water from 502.7 to 794.3 ml/kg body weight. Calculations showed that the mean plasma volume was 47.8 (44.4-51.2)* ml/kg, the extracellular fluid 261.4 (243.1-279.7) ml/kg, and the total body water 638.6 (579.5-697.7) ml/kg.

According to data summarized by V. S. Asatiani [1], the mean total water content in dogs is 628 ml/kg with variations from 503 to 756 ml/kg, while the volume of extracellular fluid varies, depending on the method of determination from 166 to 408 ml/kg. The plasma volume, calculated from the distribution space of T-1824, according to different authors averages 42.2 [2], 55.7 [7], and 57.3 [8] ml/kg. Our results are in agreement with those just mentioned.

Analysis of the light absorption curves (see Fig. 1) showed that the components of our SETA mixture did not influence each other when determined quantitatively. Sodium thiosulfate, T-1824, and antipyrin did not interfere with each other during their quantitative estimation.

As the result of five repeated quantitative estimations of the components of the SETA added to heparinized dog's blood in the concentrations usually used experimentally, a mean value of 99.7% T-1824, 99.2% sodium thiosulfate, and 99.5% antipyrin was found.

Appropriate dilutions of SETA showed that the minimal detectable concentrations of T-1824, sodium thiosulfate, and antipyrin were 0.02, 7.2, and 0.12 μg/ml respectively, so that the sensitivity of the method of determination of these substances was many times greater than their concentration in the blood plasma during the experiment.

To assess the reproducibility of the method and the accuracy of investigation of the distribution spaces of T-1824, sodium thiosulfate, and antipyrin, the experiment was repeated in 7 dogs. The results showed that during repeated determinations, the difference between the volumes of plasma, extracellular fluid, and total body water did not exceed ±10, ±59, and ±50 ml/kg respectively. Determination of the significance of the differences between the results of two successive determinations of the plasma, extracellular fluid, and total body water volumes showed that the differences between repeated determinations of all these volumes were not significant (in every case $P > 0.05$). From the results of successive determinations of the plasma, extracellular fluid and total body fluid volumes the error of the method was ±7.6 (or ±16%), ±54 (or ±21%), and ±49 ml/kg (or ±7.7%).

Hence, our suggested method of simultaneous determination of the body water spaces of the dog reflects with satisfactory accuracy the distribution spaces of the dye T-1284, sodium thiosulfate, and antipyrin.

LITERATURE CITED

1. V. S. Asatiani, Biological Tables [in Russian], Part 1, Tbilisi (1960).
2. S. S. Polushkina, Pat. Fiziol., No. 4, 69 (1962).
3. I. Bulbuka, et al., Methods of Investigation of Hydro-Electrolytic Equilibrium [in Russian], Bucharest (1962).
4. B. Brodie, et al., J. Biol. Chem., 179, 25 (1949).
5. H. Davis and L. Isenberg, J. Lab. Clin. Med., 41, 789 (1953).
6. I. S. Edelman, et al., Science, 115, 447 (1952).
7. F. H. Epstein and T. B. Ferguson, J. Clin. Invest., 34, 434 (1955).
8. J. B. Flanagan, et al., Am. J. Physiol., 160, 89 (1950).
9. C. H. Hendricks, et al., Am. J. Med. Sci., 230, 648 (1955).

*Arithmetic means are given with confidence limits for $P = 0.05$.

10. K. Masaoka, J. Jap. Obstet. Gynec. Soc., 5, 400 (1958).
11. J. D. McMurrey, et al., Metabolism, 7, 651 (1958).
12. F. D. Moore, Science, 104, 157 (1946).
13. D. Torelli, Recenti Prog. Med., 16, 240 (1954).