A METHOD OF SIMULTANEOUS MEASUREMENT OF EXTRA - AND INTRACELLULAR FLUID IN RATS

(UDC 612.014.461.3-083)

Yu. I. Ivanov and B. A. Pakhmurnyi

Department of Pharmacology (Head—Prof. E. B. Berkhin),
Altai Medical Institute, Barnaul
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 4,
pp. 123-125, Arpil, 1965
Original article submitted October 9, 1963

Water in an organism is usually divided into intracellular and extracellular. The determination of the volumes of intracellular and extracellular fluid is of considerable practical importance since in many pathological processes, in particular those accompanying edema, changes of the water balance of an organism are observed. Several methods have been proposed which were based on injecting a substance into the organism which is uniformly distributed in the investigated space, after which the degree of its dilution is calculated.

The extracellular fluid was determined by means of substances which do not penetrate into the cell: sodium thiocyanate, mannitol, sodium thiosulfate, inulin, etc. The most widespread method (by virtue of simplicity) is the use of sodium thiocyanate, but it yields somewhat overestimated figures as a consequence of its partial binding with the cells of the liver and erythrocytes [2]. The high molecular weight of inulin impedes its penetration inside the cell, but it does not combine with plasma proteins, which permits it to be used for accurately determining the intracellular fluid.

The total quantity of fluid was investigated by means of antipyrine, which nicely diffuses and is uniformly distributed in all tissues [1].

The most accurate, but not always accessible, is the method with the use of heavy water. The intracellular space is calculated by the difference between the total volume of fluid and the extracellular water.

Of great interest is the one-stage determination of the volume of the intra- and extracellular fluid, which makes it possible to study the interrelationship between these values when investigating one animal. Determination of the extracellular space by means of sodium thiocyanate and thiosulfate is the simplest method but both these substances stain with the same reagents as antipyrine. Furthermore, the determination of antipyrine, as was suggested in the literature [1], is cumbersome and requires spectrophotometry in view of the weak intensity of the stain.

The purpose of our investigations was to develop a convenient method for a one-stage determination of the volume of intra- and extracellular fluid in rats with the use of inulin, which does not impede the investigations of antipyrine, and also a modification of the method of determining inulin and the development of a new method of determining antipyrine.

The method of determination is as follows. Before the experiment, into a 5% solution of antipyrine we add inulin based on 20 mg per 1 ml and dissolve it with slight heating. The weighed samples of the substances must be taken very accurately. Under light ether anesthesia the abdominal cavity of the rats is opened and bilateral nephrectomy is performed (in order to avoid excretion of the investigated substances). Then 1 ml of the prepared solution is injected by an accurately graduated syringe into the inferior vena cava. The abdominal cavity is sutured, the rat weighed, and after 3 h the rat is again anesthesized, the abdominal cavity opened, and not less than 3 ml of blood is taken from the abdominal aorta by a syringe washed with heparin. The blood is centrifuged at 2500 rpm for 15 min. Inulin and antipyrine is determined in the plasma.

Water Spaces in Rats Weighing 90-150 g (in %)

Test substances	Number of animal	Total water	Extracellular space	Intracellular space
Antipyrine	23	64,6±1,59		
Inulin	24		20,3±0,38	
Inulin and antipyrine	36	64,1±1',17	20,0±0,28	44,0±1,19

To determine inulin we used our modification of Schreiner's method [5]. The proteins were precipitated with zinc sulfate and caustic soda. We dissolved 12.5 g of zinc sulfate in 125 ml of 0.25 N solution of sulfuric acid and brought the obtained volume up to 1 liter with distilled water. To 8.5 ml of this solution we added 0.5 ml of plasma and 1 ml of 0.75 N solution of caustic soda. After 30 min the fluid was filtered through a dense paper filter. To 1 ml of the filtrate we added 1 ml of 0.1% freshly prepared resorcinol solution in 96% ethanol and 3 ml of 30% hydrochloric acid. The test tube with the solution was placed in a waterbath for 30 min at a rigorously maintained temperature of 80° and was shaken periodically. Then the test tube was cooled for 5 min in running water and colorimetrically analyzed on a photoelectrocolorimeter with a green filter (wavelength 508 mμ) in a 10-mm wide cuvette. The investigated fluid was compared with distilled water.

The calibration curve was constructed using solutions with concentrations from 10 to 150 mg%. The extracellular space is expressed in percent of body weight. The calculation is performed by the formula:

$$\text{Volume (in ml)} = \frac{\text{quantity of injected inulin (in mg)} \times 100}{\text{concentration of inulin in plasma (in mg \%)}}$$

$$\text{Volume (in \%)} = \frac{\text{volume (in ml)} \times 100}{\text{body weight (in g)}}$$

Consequently,

$$\text{Volume of fluid (in \%)} = \frac{\text{quantity of injected inulin (in mg)} \times 10{,}000}{\text{concentration of inulin in plasma (in mg \%)} \times \text{body weight (g)}}$$

The antipyrine is determined by the method proposed by us: to 1 ml of plasma add 1 ml of 10% solution of trichloroacetic acid, after 10 min centrifuge (10-15 min at 2500 rpm), after which filter through a small cotton filter. To 1 ml of filtrate add 1 ml of the reagent with trivalent iron.*

After 10 min the filtrate is analyzed colorimetrically on a photoelectrocolorimeter with a blue filter (wavelength 453 mμ) in a 5-mm wide cuvette. A comparison is made with a solution containing 1 ml of distilled water, 1 ml of 10% solution of trichloroacetic acid, and 2 ml of the reagent with iron. The antipyrine space is calculated by the formula given for inulin.

Determination of the spaces was carried out by the described method on 36 rats weighing from 90 to 150 g. In addition, we made a separate determination of the antipyrine and inulin spaces in order to elicit whether the presence of inulin affects the determination of antipyrine and vice versa. As is apparent from the table, the data obtained coincide.

The inulin space of rats weighing more than 200 g varied within the limits of 14.8-18.8%, whereas the extracellular space increased with decrease of body weight [4].

To check these data we set up an additional series of experiments on five rats weighing from 250 to 300 g. The inulin space of these rats was 17.8 ± 0.81%. The antipyrine space, which was 47.8 ± 3.00% also differed. Consequently, in older rats the water content decreased, especially at the expense of the intracellular space, which was 30.0 ± 2.98%.

*Ferric chloride 33.45 mg, concentrated nitric acid 25 ml, and distilled water up to 1 liter; filter the solution.

It was of interest to compare the magnitudes of the extracellular space obtained by various methods. For this purpose we determined the thiocyanate space of 7 rats by the method proposed by Bowler [3]. The data we obtained corresponded to the data in the literature, being equal to 29.4 ± 1.07% of body weight. Consequently, the thiocyanate space is somewhat overestimated in comparison with the inulin space, which is in accord with the data of Morrison [4].

Thus, the proposed method makes it possible to simultaneously determine the main water spaces of an organism in experiments on small animals.

LITERATURE CITED

1. K. Blazha and S. Krivda, Theory and Practice of Revivification in Surgery. Bucharest (1962).
2. I. Bulbuka, et al. Methods of Investigating Hydro-Electrolytic Equilibrium. Bucharest (1961).
3. R. G. Bowler, Biochem. J., 38, (1944), p. 385.
4. A. B. Morrison, Am. J. Physiol., 201, (1961), p. 329.
5. G. E. Schreiner, Proc. Soc. exp. Biol. (N. Y.), 74, (1950), p. 117.