Measurement of extracellular fluid compartment volume using inulin:

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Summary:
Background:
There is a need to find methods to assess the size of the extracellular fluid (ECF) volume without involving radioactive tracers. For this purpose, the simple delusion method was used to measure the ECF in rabbits and the inulin which is a polysaccharide was used as a marker of ECF measurement.
Methods:
18 male rabbits were used in this study. 8 of these animals were bilaterally nephroctomized to calculate the exact time to get diffusion equilibrium time after a bolus dose of inulin at a dose of 25mg/kg of a solution of inulin 10 mg/ml. intravenously. The blood samples were taken after 1, 15, 45, 60, 90, 120, and 180 min.
Results:
ECF volume was about 144.5 to 149.7 ml/kg depending on the inulin concentration changing with time because the equilibrium time of inulin and the ECF could not be obtained to the end of the experiment.
Conclusion:
The inulin takes a space in the body fluids differ than the true ECF volume. It is better to use the term "inulin space" to reflect such measurements rather than the ECF volume.
Key words: inulin, extracellular volume.

Introduction :

There is a need for reasonably simple methods to measure the extracellular fluid (ECF) volume in experimental animal and also in critically ill patients. Although the ECF volume is maintained remarkably constant in healthy humans [1], marked changes occur in severe trauma, major surgery, and critical illness [2,3,4]. For example, this fluid space is greatly expanded in burn injuries after proper fluid therapy [5]. In septic patients, there is often interstitial edema, which may increase the body weight by more than 10% [6].

Methods for measuring the ECF volume that can be easily applied in the clinical environment would offer the possibility of maintaining patients within a predetermined volume range through fluid administration and diuretics. This is not done today because of the shortcomings of the current methods. Several of them use radioactive tracers, which are not readily accepted in clinical medicine. Furthermore, laboratory analyses must be reasonably simple and daily measurements possible. The time needed for each assessment should also be relatively short [7].

An accurate pharmacokinetic description of the interstitial space is essential for the development of a physiologically based pharmacokinetic model (PBPK) for extracellular solutes.

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Although PBPK models have been used extensively to describe human pharmacokinetics, nearly all of these studies have involved solutes that have intracellular distributions, and, thus, do not require detailed modeling of the interstitial space. One exception is the extracellular solutes inulin and the beta-lactam antibiotics [8].

The inulin which is a polysaccharide, white, odorless, and low sweet powder. Its molecular weight is about 5000A which a large enough to stay out side the cells [9]. It is the gold standard exogenous marker used in glomerular filtration rate (GFR) estimation because it is uncharged, freely filtered at the glomerulus, not bound to plasma proteins, non toxic, not metabolized within the body, and neither reabsorbed nor secreted by renal tubule properties [10].

In the present study, a method using single bolus weight dependent intravenous inulin injection was tested. A correcting factor of normal renal elimination of inulin was calculated as a reference in future studied.

Animal and methods:
18 male rabbits with body weight rang from 630 to 2150 gm were anaesthetized with ketamine (panpharma) 40mg/kg intra-muscular injection and marginal veins in both ears were canulated for intra-venous giving. The blood collecting was though the jugular vein in the neck.

In 8 rabbits labrotomy was performed and bilateral nephrectomy was done. Then the animal allowed recovering from anesthesia until the righting reflex was regained (about 1 h.).

A single injection of inulin solution (10 mg/ml; Fluka AG, Chem. Fabril Ch-9470 Buchs) was given in a dose of 25mg/kg as slow intravenous injection in at least 90 seconds.

Then venous blood samples (1 ml each) were taken at 1, 15, 45 and 60 min. after the injection.

The plasma inulin was tested by color reaction. A method called "Heyrovesky method" [11] which is a method of determination of inulin by color reaction that is a purple-violet color with indole 3 acetic acid in conc. HCL and testing spectrophotometer at 520nm.absorbance.

The results:

Table 1. The level of inulin in plasma according to the time in non nephroctomized rabbits.

Time (Min)	Plasma inulin level Mean ± SD (in mg/ml)	Inulin index
1	0.378±0.0197	-------
15	0.140±0.0075	-------
45	0.044±0.0033	3284.27
60	0.031±0.003	4688.64
90	0.016±0.0006	9191.125
120	0.008±0.0005	18712.5
180*	0.007-0.0063	--------

*only 2 animal survived to this time.

Table 2. The plasma inulin levels according to time in nephroctomized rabbits.

Time (Min)	Plasma inulin level Mean ± SD (in mg/ml)	Extracellular ml/ kg
1	0.418±0.0010	---
15	0.197±0.0047	---
45	0.173±0.0032	144.508
60	0.172±0.0035	145.348
90	0.170±0.0027	147.058
120	0.167±0.0029	149.701

The calculations:

The extracellular volume= volume of the marker given (inulin) x concentration of the marker given (inulin)/ the concentration in the plasma after equilibrium

The time taken to get an accepted equilibrium between the inulin and the extracellular fluid is between 60 to 90 min. At these times, the ECF could be calculated according to the equation above [12].

Discussion

The molecular weight of inulin is ~ 5000, so it is too large to pass through cell membrane channels, but it is small enough to easily pass through porous capillaries. There are no other significant transport pathways for inulin [13]. This only pathway was blocked in nephroctomized animal. Therefore, to a good approximation, we can assume inulin is uniformly dissolved in the extracellular fluid that makes up about 20% of the body weight [14]. This is true after equilibrium time, which is the time taken for inulin to equally diffuse throughout the whole ECF.

In this study a true equilibrium time could not be obtained since even after 120 min there was a continuous decrease in the plasma inulin.

Our data shows a rapid decreased in the inulin levels in plasma. This was followed by a decrease in the level at a slower rate. This was explained that there was some sort of trap of inulin molecules in some tissues or organs in the interstitial fluid compartment [15]. Handelsman and Sass (1956) [16] found that there was a difference in the inulin level between plasma (and serum) and the whole blood on the same subject blood because approximately 8% to 10% of the inulin given was "trapped" by the erythrocytes. This is important clinically because hemolyized samples may give higher inulin level than the true inulin level in the plasma (false positive).

According to Levitt, (2003) [13], bilateral nephrectomy is the method for studying the distribution properties of a marker with characters such as inulin in defacement tissues in the animal studies to determine the equilibrium concentration after a bolus intravenous injection. Although this is not applicable for humans, a good approximation to this situation can be obtained in subjects with sever renal failure and very low rates of clearance. This is important in calculating the tissue binding, extravascular collection, and extrarenal clearance of the marker studied.

Van-Westen and coworkers (2002) [17] studied the extrarenal clearance of GFR markers (inulin and iohexol) in healthy and nephroctomized pigs and found that the extrarenal clearance of inulin was about 0.7 ml min^{-1} 10kg^{-1} in nephroctomized animal. This indicates that this is a source of error decrease the calculated renal function.

The extrarenal clearance and the inulin trapped in some tissues may be the cause that the values of systemic clearance were higher than the renal clearance (up to 20%) [18, 19].

As The ECF volume calculated in this study, the index of inulin which is a virtual number for predicting the inulin distribution at that time and could be used according to the equation:

Extracellular fluid (inulin distribution) =index of inulin at the time of sample taking x plasma inulin concentration at the same time

These numbers listed in table 1 under the name "inulin index" could be used in future studies for predicted e ECF volume in rabbits using the above equation.

However, the new textbooks use the term "inulin space" rather than the extracellular volume [12, 20].

As a conclusion, when inulin is injected into the blood of an animal, it usually disperses almost completely throughout the ECF within 45 to 90 min. Some of inulin will take further time to diffuse into and out of the dense tissues or even diffuse into some cells and this will affect the measurements of ECF volume or the GFR.

References:

Leth, A., and Binder, C., (1970): The distribution volume of $^{82}Br^-$ as a measurement of the extracellular fluid volume in normal persons. Scand J Clin Lab Invest, 25:291–7.

Kim, J., Wang, Z., and Gallagher, D., (1999): Extracellular water: sodium bromide dilution estimates compared with other markers in patients with acquired immunodeficiency syndrome. J Parent Ent Nutr, 23:61–6.

McCullough, A.J., Mullen, K.D., and Kalhan, S.C., (1991): Measurements of total body and extracellular water in cirrhotic patients with and without ascites. Hepatology, 14:1102–11.

Shaffer, S.G., Ekblad, H., and Brans, Y.W., (1991): Estimation of extracellular fluid volume by bromide dilution in infants of less than 1000 grams birth weight. Early Human Develop, 27:19–24.

Zdolsek, H.J., Lisander, B., Jones, A.W., and Sjöberg, F., (2001): Albumin supplementation during the first week after a burn does not mobilise tissue oedema in humans. Intensive Care Med, 27:844–52.

Lucas, C.E., Ledgerwood, A.M., Rachwal, W.J., et al. (1991): Colloid oncotic pressure and body water dynamics in septic and injured patients. J Trauma, 31:927–33.

Zdolsek, J.H., Lisander, B., and Hahn, R.G., (2005): Measuring the Size of the Extracellular Fluid Space Using Bromide, Iohexol, and Sodium Dilution. Anesth Analg, 101:1770-1777.

Levitt, D.G., (2002): PKQuest: capillary permeability limitation and plasma protein binding - application to human inulin, dicloxacillin and

ceftriaxone pharmacokinetics. BMC Clin Pharmacol, 2:7.

Fischer, P.A., Bogoliuk, C.B., Ramirez, A.J., Sanchez, R.A., and Masnatta, L., (2000): A new procedure for evaluation of renal function without urine collection in rat Kidney Int, 58: 1336-41.

Levey, A., (1990): measurement of renal function in chronic renal disease. Kidney Int, 38:167-184.

Heyrovesky, A., (1956): Determination of inulin. Clin. Chim. Acta. 1: 470.

Gyton, J.E., and Hall, J., (2000): Extracellular fluid volume measurements. Textbook of medical physiology. 10th ed. Edited by Gyton A and Hall J. Sauders Company, Philadelphia, London, p 268-269.

Sturgeon, C., Sam II, A.D., and Law, W.R., (1998): Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. J Appl Physiol 84: 2154-2162.

Levitt, D.G., (2003): the pharmacokinetics of the interstitial space in humans. B.M.C. Clin. Pharmacol. 3:3.

Takada, K., (1995): Pharmacokinetic two compartment model. pp. 51-77. In: Basic and clinical Pharmacokinetics. Yakugyojigyosha, Tokyo.

Handelsman, M.B., and Sass, M., (1956): Serum vs. plasma vs. whole blood J. Lab. Clin. Med. 48:759.

Van-Westen, D., Almen, T., Chai, C.M., Frennby, B., Mansson, S., and Sterner, G., (2002): Biliary and total etrarenal clearance of inulin and iohexol in pigs. A source of error when determining GFR as body clearance, Nephron, 91: 300-307.

Hellerstein, S., Berembom, M., Alon, U., and Warady, B.A., (1993): The renal clearance and infusion clearances of inulin are similar but not identical. Kidney Int, 44: 1058-1061.

Van-Acker, B.A., Koomen, G.C., and Arisz, L., (1995): Drawbacks of the constant infusion technique for measurement of renal function. Am. J. Physiol., 268: 543-552.

Cieslar, J., Huang, M.T., and Dobson, G.P., (1998): Tissue spaces in rat heart, liver, and skeletal muscle in vivo. Am J Physiol, 275:1530-36.