ROLE OF THE TIME FACTOR IN THE DISTRIBUTION OF INULIN IN THE EXTRACELLULAR SPACES OF VARIOUS TISSUES OF THE ORGANISM

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Together with other macromolecular compounds such as polyglucose and serum albumin, I^{131}-labeled inulin is a most suitable substance for use in determining the extracellular spaces of different tissues [1,3,8].

The accuracy of measurement of the extracellular spaces of individual tissues by the inulin method is dependent on the uniformity of distribution of this substance throughout the extracellular space. However, no agreement has been reached regarding the minimal time during which the distribution of inulin in the extracellular phase of the organism and of the individual tissues is sufficiently complete. This may be explained by the fact that some authors, when determining the extracellular space of the body, use a 2-h period of inulin distribution [5,6], whereas others use a 5-h period or longer [2,10,12,15]. To determine the extracellular spaces of the skeletal and smooth muscles, mainly in experiments in vitro, 2-h or 3-h periods of inulin distribution have been used [4,9,11].

Yet several investigations [2,10] have shown that complete inulin equilibrium is not achieved in the body in a period of 2 h, for the "inulin space" of the body continues to increase during subsequent hours.

The object of the present investigation accordingly was to study the role of the time factor in the distribution of inulin in the extracellular spaces of various body tissues.

EXPERIMENTAL METHOD

For inulin to be distributed uniformly throughout the extracellular phase, its concentration in the blood must be maintained for several hours at a strictly definite level. With a single injection of inulin, this can be achieved in experiments on nephrectomized animals, because removal of the kidneys prevents the excretion of inulin from the body. This method of distribution of inulin has therefore been used frequently in animal experiments [2,5-7,10, 11,15].

Experiments were carried out on 15 dogs weighing 8-15 kg. The animals were anesthetized with Nembutal (30 mg/kg), and the renal arteries and veins were then ligated through a lumbar incision. An intravenous injection of inulin (600 mg/kg body weight, as a 10% solution) was then given to the dogs. In 8 dogs, the inulin was distributed in 2 h, and in 5 dogs in 5 h. At the end of these periods of distribution of inulin, the animals were sacrificed by exsanguination, and the blood and the following tissues were taken for analysis for inulin: the skeletal muscle of the thigh, the heart (right and left ventricles), the muscular coat, and the mucous membrane of the stomach (the fundal portion), the aorta without the adventitia (thoracic and abdominal portions) and the liver. Small pieces of the tissues, from which all visible traces of blood and fat were first removed, were homogenized on ice. Samples of tissue homogenate weighing 200-400 mg and 0.2 ml of serum were taken for analysis of the inulin. The inulin in the blood serum was determined by Roe's method as modified by Roe and co-workers [13], and the inulin in the tissues was determined by the method of Ross and Mokotoff [14]. As controls, the blood serum and tissue of two dogs not receiving injection of inulin were used.

To calculate the volume of the intracellular space of the whole body and of the individual tissues, the following formulas were used:

$$\text{Total volume of extracellular fluid of the body (in ml)} = \frac{\text{Amount of inulin injected}}{\text{Amount of inulin in 1 ml serum}}$$

$$\text{Total extracellular space (in \% of body weight)} = \frac{\text{Volume of extracellular fluid (in liters)} \cdot 100}{\text{Body weight (in kg)}}$$

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Size of Inulin Space of the Tissues after 2-hour and 5-hour Distribution Periods of Inulin (M ± m)

Tissue	Extracellular space (in %)	
	2 h	5 h
Aorta		
Thoracic	31.3 ± 3.1	35.0 ± 3.2
	$P < 0.05$	
Abdominal	27.9 ± 2.4	32.1 ± 2.1
	$P < 0.01$	
Heart		
Right ventricle	13.8 ± 2.0	14.7 ± 1.0
	$P < 0.5$	
Left "	13.4 ± 1.4	14.2 ± 0.8
	$P < 0.5$	
Skeletal muscle	7.5 ± 1.5	8.0 ± 1.6
	$P < 0.5$	
Liver	15.3 ± 2.0	18.4 ± 1.1
	$P < 0.02$	
Stomach		
Muscular coat	27.8 ± 3.8	28.2 ± 4.9
	$P < 0.05$	
Mucous membrane	23.6 ± 3.1	31.6 ± 6.1
	$P < 0.05$	

$$\text{Extracellular space in tissues (in \% of 100 g fresh tissue)} = \frac{\text{Amount of inulin in 1 g fresh tissue} \cdot 100}{\text{Amount of inulin in 1 ml serum}}$$

EXPERIMENTAL RESULTS

The results obtained are given in the table. With a two-hour period of distribution of inulin, the total inulin space of the body was 14.7 ± 0.82%, and with a 5-hour period, 16.9 ± 0.83%. The difference between these two values was statistically significant ($P < 0.01$). Hence, with an increase in the distribution time of inulin by 3 h, the total inulin space of the body increased by 2.2%. An attempt was made to discover how the inulin space of the different tissues changed during this period. Analysis of the data for the individual tissues (see table) showed that the extracellular space in the smooth muscle of the stomach, the skeletal muscle, and the heart increased negligibly during the period of 3 h, by an amount which was not statistically significant ($P < 0.5$). For instance, in the smooth muscle of the stomach, this increase was 0.4%, in the skeletal muscle 0.5%, in the right ventricle 0.9%, and in the left ventricle 0.8%.

Meanwhile in tissues such as the mucous membrane of the stomach, the liver, and the aorta, the inulin space showed a significant increase during this period.

This difference was revealed more clearly by calculating the increase in the inulin space in the tissues over a period of 3 h by taking as 100 the inulin space of the tissues after a 2-hour period of distribution of inulin. The increase in the inulin space during a period of 3 h in the mucous membrane of the stomach was 25%, in the liver 17%, and in the aorta 12%. In the group of muscle tissues, the increase in the inulin space during the period of 3 h amounted to 6% (in the heart), 2.5% (in the skeletal muscle), and 1.25% (in the smooth muscle of the stomach). Consequently, the maximal increase in the inulin space during the period of 3 h was observed in the mucous membrane of the stomach, in the liver, and in the aorta.

Hence, in the intact organism, within a period of 2 h, inulin is uniformly distributed in the extracellular space only in the muscle tissues (the smooth muscle of the stomach, the skeletal muscle, the heart), for the increase in the inulin space in these tissues during the next 3 h is small and not statistically significant, especially in the smooth muscles of the stomach and the skeletal muscles. Consequently, a 2-hour period of inulin distribution can be used for determining the extracellular spaces in these tissues.

Meanwhile, in the mucous membrane of the stomach, the liver, and the aorta, inulin is not fully distributed in a 2-hour period, and the inulin space of these tissues continues to increase significantly during the next 3 h. Evidently, the extracellular space in the mucous membrane of the stomach, the liver, and the aorta can be determined more accurately only by using a 5-hour distribution period of inulin.

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