EXTRACELLULAR SPACE MEASUREMENT IN CANINE FEMORAL ARTERY UNDER TENSION

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ABSTRACT

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By

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The extracellular space (ECS) of canine femoral artery under tension was estimated using the tracer uptake technique. The vessels were placed under tension via a cannulated stainless steel rod. When sucrose ^{14}C was used as the tracer, the tissue was so sensitive to tension that this tracer is believed to enter the smooth muscle cells. The ECS was 44.6 ml/100 gm tissue for a "relaxed" artery, 54.4 ml/100 gm tissue for an artery in "moderate" tension and 66.0 ml/100 gm tissue for one in "tight" tension. However, when inulin ^{14}C was used as a tracer, no statistical difference could be detected between vessels under different degrees of tension. The inulin space was 41.3 ml/100 gm tissue. The total water volume (measured by the weight change after evaporation) was 76.0 ml/100 gm tissue. This leaves an intracellular volume of 34.7 ml/100 gm tissue.

When the potassium concentration in the surrounding fluid was lowered to 25% of the normal concentration, the

sucrose space did not change. The inulin space, however, dropped from 40.5 ml/100 gm tissue to 36.0 ml/100 gm tissue when the potassium concentration was lowered. The water volume remained unchanged which indicates that the intracellular volume increased from 35.5 ml/100 gm tissue to 40.0 ml/100 gm tissue with a decrease in the potassium concentration.

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By

Carl R. Beck

A THESIS

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To my parents

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Electrical potential differences are found across the membranes of all living cells. Changes in these potential differences are responsible for the electrochemical impulses conducted by nerve cells and the contractile characteristics of muscle cells. These potential differences are in part established by an energy consuming active transport mechanism which preferentially pumps certain ions across the membranes, and is influenced by the diffusion of ions through the membranes because of concentration differences. In studies aimed at attempting to better understand these processes, there are several parameters for which it would be useful to have numerical values. Two such parameters are the intracellular and extracellular volumes of the tissue, since the distribution of total volume is important in the interpretation of data relative to both active and passive transport across cell membranes.

There are several methods for measuring the extracellular space (ECS) of a particular type of tissue. The "anatomical" ECS can be calculated by measuring the

appropriate areas on electronmicrographs of sections procured from the tissue. However, electronmicrographs are typically taken of an area which is densely populated with cells, excluding a relatively "cell free" fraction of the tissue. This cell free tissue fraction contains a variety of materials, such as connective tissue, fibroblasts, glandular tissue and interstitial cells, of which an electronmicrograph would be of little value since the degree of ion permeability would be unknown. Since an ionic distribution study requires knowledge of the ECS which includes all the space accessible to the ions, this method is undesirable (7).

The method most often used for this type of study is the tracer uptake technique. With this method a tracer molecule which cannot enter the cells, is allowed to diffuse into the ECS. The amount of this tracer taken up by the tissue is then measured to determine the ECS. This was the method chosen for this investigation.

The ECS of different types of tissue vary considerably because of the different structures and the varying complexity of these structures. Even among different kinds of blood vessels there is considerable variation in structure. Therefore, the ECS must be determined for each particular tissue of interest. In this work the canine femoral artery was investigated.

In actual physiological conditions, arteries are under tension resulting from blood pressure in the lumen.

In metabolically active tissue, the arteries constrict in response to an increase in blood pressure and dilate in response to a decrease in blood pressure. This phenomenon is called the "Bayliss response" or myogenic response. It tends to maintain blood flow relatively constant despite changes in blood pressure. The arteries used in this work were cannulated with a stainless steel rod to mimic the tension produced by blood pressure.

Traditionally, the ECS of blood vessels has been measured using helically cut strips, see Figure 1. By cutting the vessels in this way, excess moisture on the surface of the tissue can be removed by blotting, and muscle cell damage will be reduced, since the cells are arranged in a circular fashion around the vessel. Reduction of cell damage is critical since a damaged cell may allow the tracer to enter, increasing the ECS (24). The helical cuts were not necessary in this study because the arteries were cannulated with a stainless steel rod which prevented moisture from being trapped in the lumen. Consequently, only the outer surface of the vessel required blotting and the vessel sustained less cell damage.

Another, more recent observation in vascular smooth muscle, is vasoconstriction in a reduced potassium environment (14). If constriction significantly changes the dimensions of smooth muscle cells, these changes should be reflected in measurements of the ECS and the total water space. In this study the ECS and the total water space

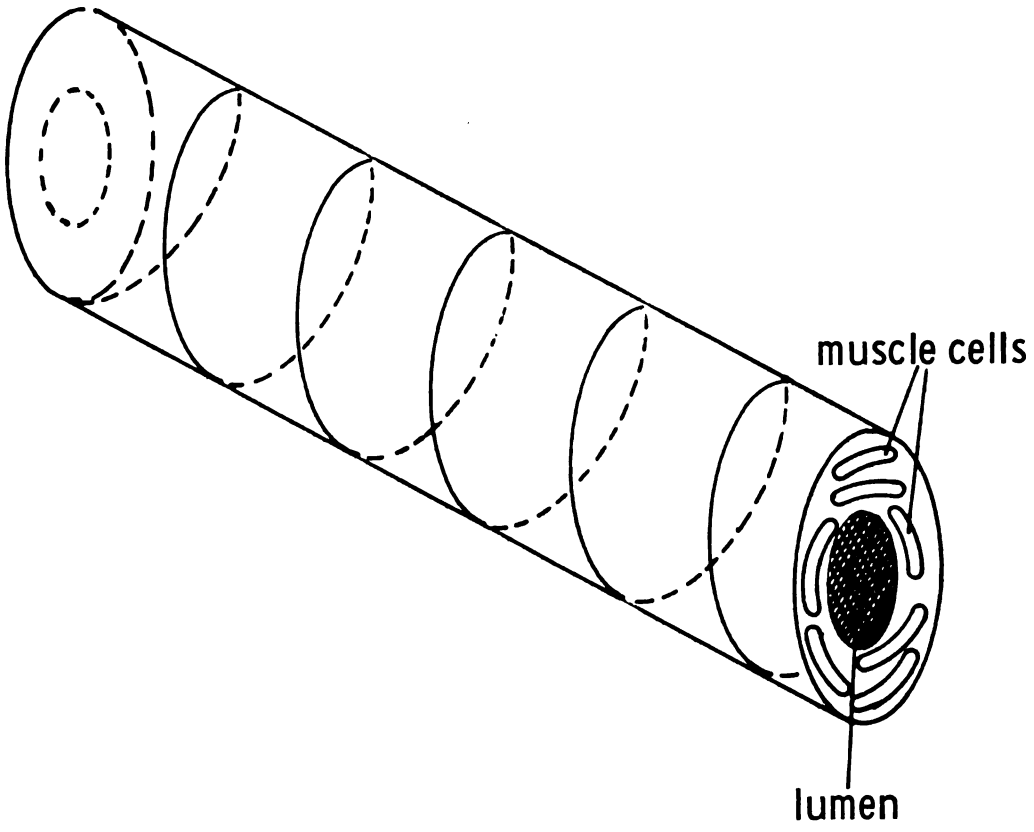

Figure 1.--Sketch of a helically cut artery segment, showing the circularly arranged cells.

were measured in sections of artery subjected to a potassium concentration which was 25% of the normal physiological potassium environment. These volumes were then compared to those that were measured in the "normal" potassium solution.

BACKGROUND AND THEORY

Two Methods of Measurement of ECS

There are two methods with which the accessible, or free extracellular volume may be calculated. One method is to use the Donnan equilibrium principle (9). This method requires data on the amounts of circulatory space, connective tissue, and nerve tissue in the ECS, the water and chloride content of these tissues, and the total electrolyte content. These data can be determined and this method works well for skeletal muscle, however in smooth muscle there is evidence of cation and anion binding which would invalidate the calculations (8, 25).

The other technique is based on tracer uptake, where a tracer is chosen which will presumably not enter the muscle cells, or will do so at a very slow rate. This presents the problem of choosing the proper tracer. Table 1 lists some tracers which have been used or tested for ECS measurements in smooth muscle tissue. From this large array of choices one must determine which is mot effective for measurement in a specific type of tissue.

TABLE 1.--Tracers used or tested for FCS measurement.

Tracer	Reference
Arabinose	2
Mannitol	2
Sucrose	1, 2, 24, 25
Raffinose	2
Inulin	2, 15, 19, 24, 25
Fructose	6
Sorbitol	12
Albumin	2, 25
Sulfate	3, 25
Ferrocyanide	25
Polyglucose	12
Ethanesulfonate	12, 13
EDTA	17
Thiosulfate	18, 19
Thiocyanate	15
Calcium	12
Lithium	12
Chloride	12
Sodium	12
Bromide	3

Complications of Measurement of ECS

The extracellular volume of smooth muscle tissue is filled with a complex heterogeneous mixture of materials, including collagen, blood vessels, nerve and Schwann cells, macrophages, fibroblasts, elastic tissue and a gel-like matrix of mucopolysaccharides. It is in this twisted maze that the tracer must diffuse. This labrinth is further complicated by "micropinocytic" or "plasmalemmal" vesicles which appear to be connected with an intracellular endoplasmic reticulum and could quite possibly increase the effective ECS (7).

Thus the problem becomes one of accessibility of the tracer to the desired space. The ideal tracer would enter only those spaces which are available to the free (unbound) extracellular water and solutes. The differences in the tracers are reflected in the different values for ECS which have been reported. Some representative values of ECS for vascular smooth muscle are given in Table 2. Many investigators have used chloride as a tracer, since it is known to be excluded from most cells and is very small sterically. However, recent observations indicate a large fraction of chloride may be present inside the cells of smooth muscle and the possibility exists that chloride may be "bound" either extracellularly or intracellularly (25, 10).

TABLE 2.--ECS and Water Space measurements in vascular smooth muscle.

Muscle Type	ECS	Method	Water	Reference
mouse femoral artery	30	electronmicro.	--	23
pig carotid artery	39	electronmicro.	--	22
cow carotid artery	39	thiosulfate	--	18
dog carotid artery	44	EDTA	72.2	17
dog carotid artery	25	inulin	--	16
dog carotid artery	36	inulin	73.6	11
dog carotid artery	37-39	inulin	--	20
dog carotid artery	40	sucrose	70.1	25
dog lingual artery	47	EDTA	75.5	17
rat aorta	35	inulin	68.3	15
rabbit aorta	62	inulin	72.7	4
dog aorta	44	inulin	--	20
rat portal vein	45	sucrose	77.9	1

ECS, ml/100 gm tissue

Water Space, gm/100 gm tissue

A Comparison of Tracers

A few investigators have used the uptake technique in smooth muscle to compare different tracers. Barr and Malvin (2) examined seven tracers in canine intestine and found sucrose, inulin and raffinose to measure approximately the same space and not to approach the total tissue water with long incubation times. They suggested that these tracers may be a suitable means of estimating the ECS in this tissue. An analysis performed by Goodford and Leach (12) indicated that the inulin volume increased in the guinea-pig taenia coli when the tissue was treated with the enzyme hyaluronidase which metabolizes hyaluronic acid. Since hyaluronic acid has been found in the interspaces of many tissues, this data suggests that inulin is sterically inhibited by hyaluronic acid from entering portions of the ECS, an observation previously predicted in a partition study by Ogston and Phelps (21). As a result of this observation and a comparison of data from other papers, monosaccharides or disaccharides were recommended for ECS measurement. Villamil et al. (25) tested five tracers on canine carotid arteries. In that investigation hyaluronidase had no effect on the inulin space. However, they found that inulin could only partially penetrate isolated dense adventitia suggesting that an obstruction to inulin diffusion occurs in the adventitia, rather than in the mucopolysaccharide matrix of the ECS. Since

the sucrose space approached but never exceeded the total tissue water when the cellular barriers were metabolically abolished and because the values were near those calculated by combined light and electron microscopy, sucrose was recommended as the best ECS indicator of the five tested. Although these studies all seem to ratify sucrose as the most reliable extracellular marker, other investigators advocate the use of larger molecules (8). In fact, most authors have chosen inulin for this function (5). Some evidence has been shown that sucrose enters smooth muscle cells (6, 9) and therefore a larger molecule should be used. The strongest indication of this is given by Bozler and Lavine (6) who noticed that the smooth muscle cells of frog stomach swell in isosmotic sucrose solution and the sucrose space was larger than inulin space.

In this investigation inulin and sucrose were used as ECS markers because they appeared to be the best representatives of both large and small molecules.

The Intracellular and Water Space

The intracellular volume is determined by subtracting the ECS from the total volume available to the solutes. One way total volume is determined is through the use of a tracer, such as urea, which is assumed to enter all the available space, both intracellular and extracellular (1). It has been shown however, that urea space exceeds the

total water content of certain smooth muscle tissue (2) and therefore binding of urea may be occurring.

Another popular method for determining total available space is to weigh the tissue to obtain a "wet weight." Then the tissue is heated in an oven to remove all the moisture, and a water-free "dry-weight" is obtained. The difference is taken to be the total water volume available to the tissue. This method requires the removal of extraneous solution from the outside of the tissue. The most reproducible method of achieving this according to Hagemeijer et al. (15) is with absorbant paper. A method very similar to this was adopted here. Table 2 lists some values of water space.

EXPERIMENTAL PROCEDURES AND APPARATUS

Artery Procurement

Mongrel dogs ranging in weight from 20 to 40 kg were anesthetized by intravenous injection of sodium pentobarbitol (30 mg/kg) and ventilated with a mechanical positive pressure respirator via an intratracheal tube. The femoral artery was surgically exposed, ligated, and cannulated with a stainless steel rod. The artery and rod were then removed from the hindlimb and placed in a vial containing oxygenated Ringers solution at 37°C for transportation to another laboratory. The arteries were approximately 1.5 cm in length, 1.5 mm in diameter, and weighed about 18 mg.

Tissue Preparation

The artery, attached to the stainless steel rod on which it was originally cannulated, was placed in a petri dish (filled with oxygenated Ringers solution) while the loose adventitia was removed. At this time the artery was moved along the rod with a pair of forceps to establish the degree of tension.

Three criteria of tension were distinguishable: (1) a relaxed tissue was defined as one which could be easily slipped off the rod, i.e., held on the rod only by the surface tension of the solution. A tissue sample of this size was very difficult to obtain, as a perfect compromise had to be made between "too loose" and some degree of tension. A loose artery presumably, would trap water between the steel rod and the inner wall of the vessel, which would add to the wet weight of the vessel. (2) An artery was defined as being under moderate tension if it exhibited some degree of resistance to being displaced on the rod. (3) A tight artery was one which was difficult to move along the rod.

After the mounted tissue had been examined in the petri dish, it was taken from the Ringers solution and the residual moisture was removed by blotting the tissue and rod on Kimwipe disposable wipers. The artery and rod combination were then weighed on a Torbal balance and the "wet weight" obtained from the difference of this weight and the weight of the rod. The time lapse between artery procurement and the wet weight determination was approximately 30 minutes.

Incubation and Washout

Immediately after weighing, the mounted artery was placed in a centrifuge tube containing the incubation medium, in which a mixture of 95% O_2 and 5% CO_2 was

continuously bubbling, see Figure 2. The incubation medium consisted of 3 ml of Ringers solution, plus 0.05 ml of labelled sucrose or 0.10 ml of labelled inulin solution and was kept near 37°C by a water bath.

After the tissue was incubated in the labelled Ringers solution for 30 minutes it was blotted as described previously. The artery was slipped off the rod and placed in another centrifuge tube containing distilled water, 5 ml for a sucrose experiment or 3 ml for an inulin experiment. After one hour the tracer was assumed to have equilibrated throughout the tissue and the solution.

Measurements and Calculations

Calculation of the Water Volume. After the washout step, the tissue was removed from the washing medium and placed on a watchglass in a Cenco constant temperature oven at 105°F to evaporate the water. A 24 hour period in the oven was sufficient for the tissue to achieve a constant weight. This dry weight was then subtracted from the wet weight previously calculated to give the total water weight (or water volume if a density of 1.0 is assumed).

The Number of Counts in the Tissue. A 1.0 ml aliquot of the washing medium was placed in a scintillation vial containing 10 ml of Insta-Gel liquid scintillation fluid, and counted on a Packard Tri-Carb liquid scintillation spectrometer. Since the volume of the washing medium is

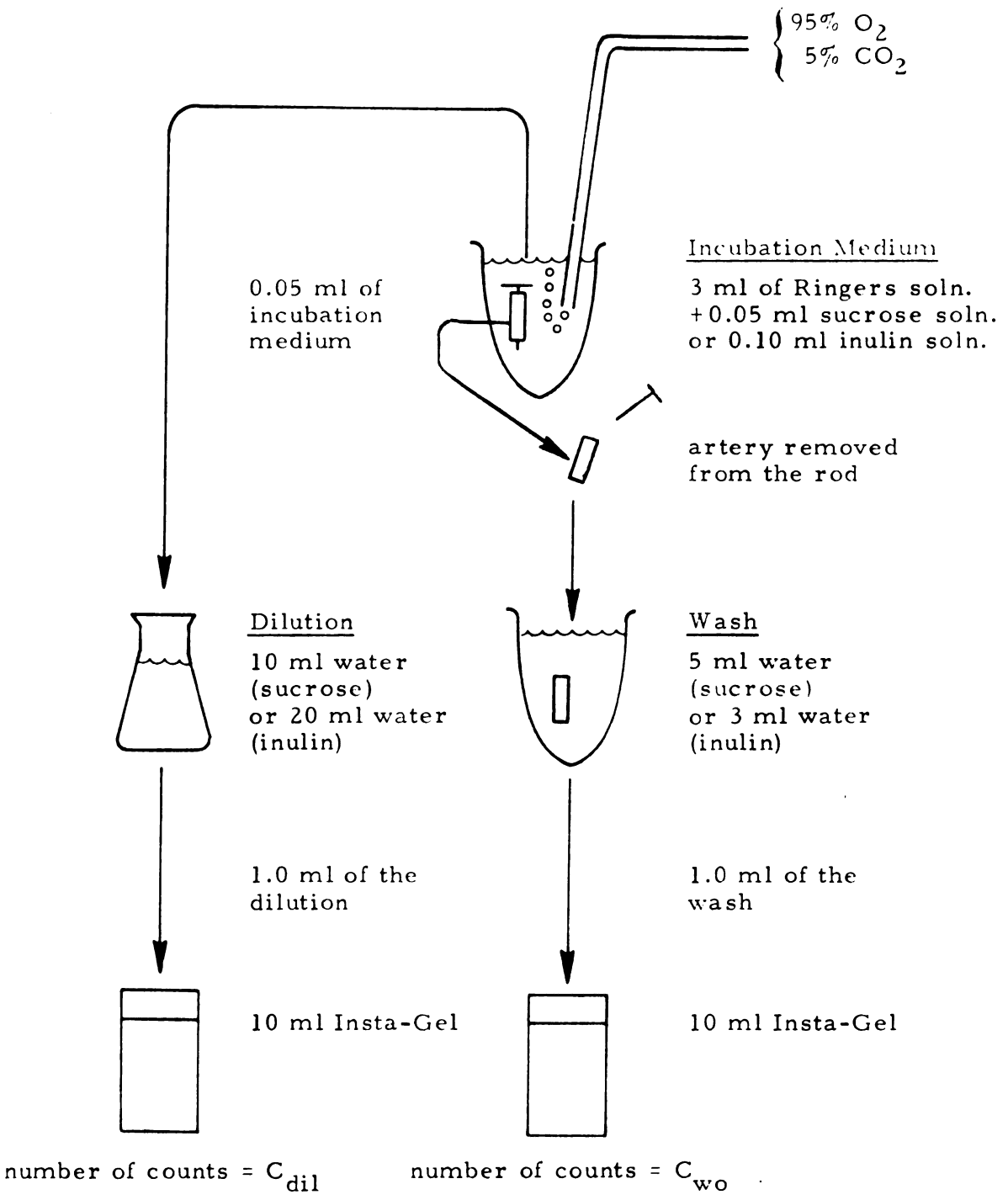

Figure 2.--Flow diagram of the experimental procedure.

either 3 or 5 ml (depending on whether inulin or sucrose was used) the number of counts in the tissue is approximated by equation 3.1.

$$C_t = C_{wo}(I) \qquad 3.1$$

C_t = the number of counts in the tissue and C_{wo} = the number of counts in the washout aliquot. I = 5 if sucrose was used or I = 3 if inulin was used. This equation assumes that the amount of tracer left in the tissue is negligible (for an artery 1.5 cm in length and 1.5 mm in diameter mounted on a 0.9 mm diameter rod, the total tissue volume is 0.34% of the washout medium in a sucrose solution or 0.57% of the washout medium in an inulin solution).

The Number of Counts in the Incubation Medium. A 0.05 ml aliquot of the incubation medium was diluted with 10 ml of water if sucrose was used, or 20 ml of water if inulin was used. A 1.0 ml aliquot of this dilution was then mixed with 10 ml of Insta-Gel and gave approximately the same total counts as the washout sample. The number of counts in the incubation medium is a function of the number of counts obtained from this dilution. Since there was 3.05 ml in the incubation medium and a .05 ml aliquot was removed this relationship is given by equation 3.2.

$$C_{im} = C_{dil}(D)\ (3.05/0.05) \qquad 3.2$$

C_{im} = the number of counts in the incubation medium and C_{dil} = the number of counts in the dilution aliquot. D = the dilution, and is 20.05 ml for an inulin experiment or 10.05 ml for a sucrose experiment.

Calculation of ECS. If it is assumed that at the end of the 30 minute incubation period, the incubation medium is in equilibrium with the ECS, then the concentration of tracer in both volumes must be equal as in equation 3.3.

$$C_t/V_o = C_{im}/3.05 \text{ ml} \qquad 3.3$$

V_o = ECS and 3.05 ml = the volume of the incubation medium. Once V_o is known, V_i (the intracellular volume) can be calculated by equation 3.4.

$$V_i = V_t - V_o \qquad 3.4$$

V_t = the total water volume, obtained by weighing.

The Time Studies. Time studies were performed with both tracers to determine how long incubation must proceed in order to equilibrate the tracer between the incubation medium and the ECS. These studies indicated that 30 minutes was sufficient for both sucrose and inulin, see Figures 3 and 4. In these experiments a tissue was incubated for 5, 10, 15, 30 and 60 minutes with a 60 minute washout in Ringers solution in between each incubation.

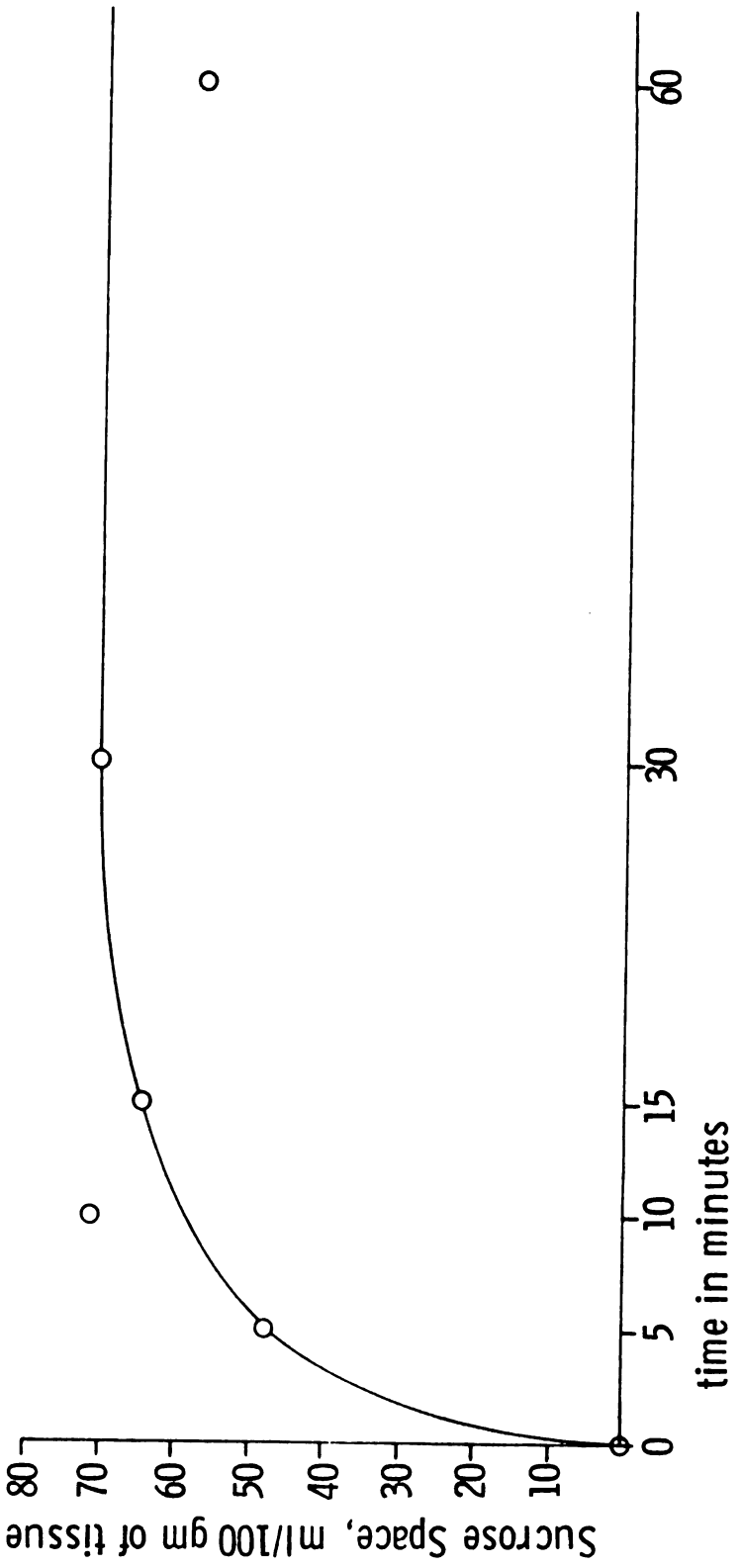

Figure 3.--Uptake of sucrose ^{14}C as a function of time in canine femoral artery.

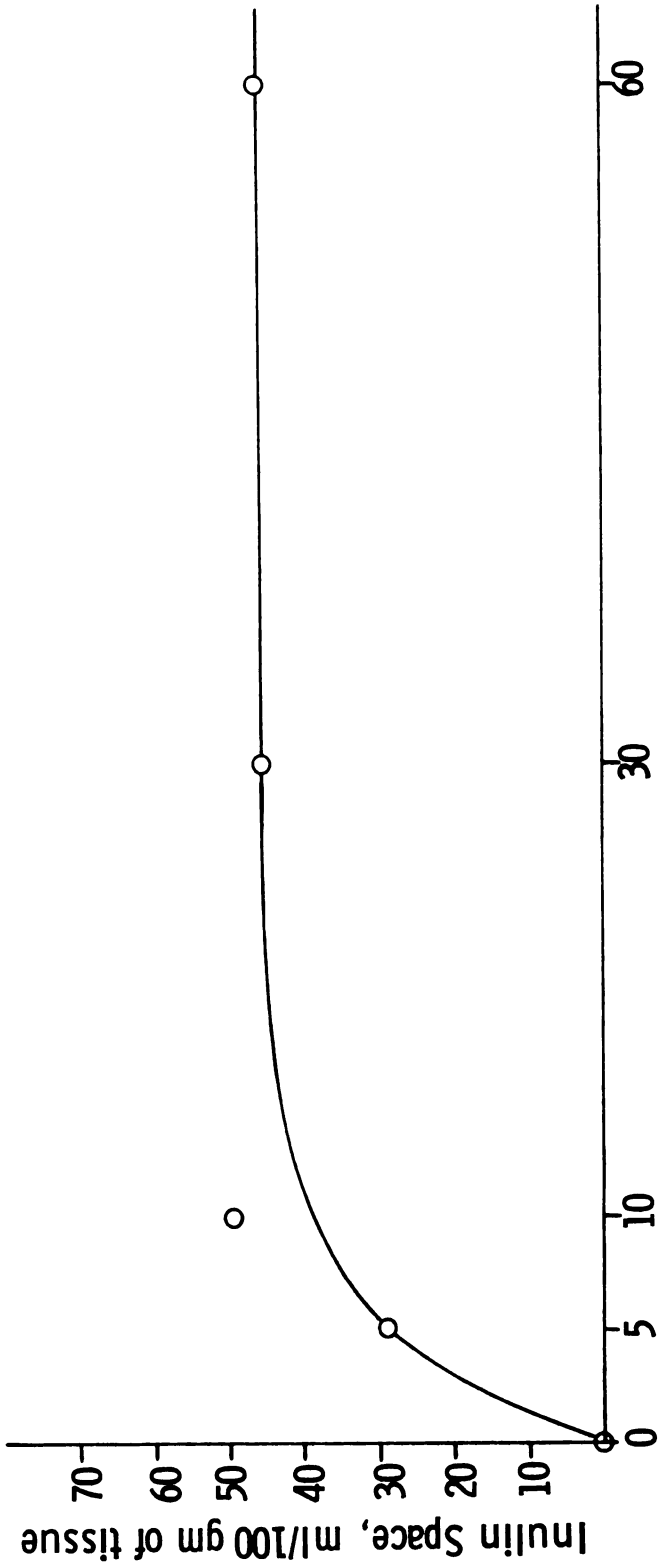

Figure 4.--Uptake of inulin ^{14}C as a function of time in canine femoral artery.

These results are similar to those obtained by Arvill (1) for sucrose.

Apparatus and Solutions

Stainless steel rods were fashioned from syringe needles ranging from 15 to 24 guage. The tips were cut off and both ends were filled with solder. The tips of the rods were then polished smooth to prevent damage to the tissue during cannulation.

The composition of the normal potassium Ringers solution used was as follows: 7.66 gm NaCl, 0.316 gm KCl, 0.141 gm $MgCl_2$, 1.9 gm $NaHCO_3$, 1.0 gm glucose, 8.33 cc 10% calcium gluconate, brought to one liter with water. For low potassium Ringers the solution was made isosmolar with NaCl. These solutions were oxygenated by bubbling with a mixture of 95% O_2 and 5% CO_2 for 30 to 60 minutes.

Stock sucrose solution was made up of 0.00714 millimoles of a 14 mCi/millimole sample of ^{14}C labelled sucrose purchased from ICN Pharmaceuticals. This gave a concentration of 0.00143 millimoles/ml when diluted with 5 ml of water. Stock inulin solution was made up of 50 mg of a 1.0 μCi/mg sample of ^{14}C inulin (also purchased from ICN) diluted with 10 ml of water to give a concentration of 5 mg/ml.

RESULTS

The Total Water Space

The total water space remained the same regardless of which tracer was used, the degree of tension, or the concentration of potassium. The total water volume determined from all the experiments of this study (n = 75) was 76.0 gm/100 gm tissue (± 3.1 s.d.). This value agrees with the water space reported for canine lingual artery (75.7 gm/100 gm tissue (17)).

Sucrose Space as a Function of Tension

It became apparent after several experiments that, not only did the rod in the lumen of the artery change the measured sucrose volume, but the tissue was extremely sensitive to the degree of tension under which it was placed. When tension was removed, i.e. the artery was judged to be "relaxed," the sucrose space averaged 44.6 ml/100 gm tissue (± 0.08 s.d.), see Figure 5. This value closely approximates the sucrose space measured by Arvill et al. (45.4 ml/100 gm tissue (1)). Assuming a water

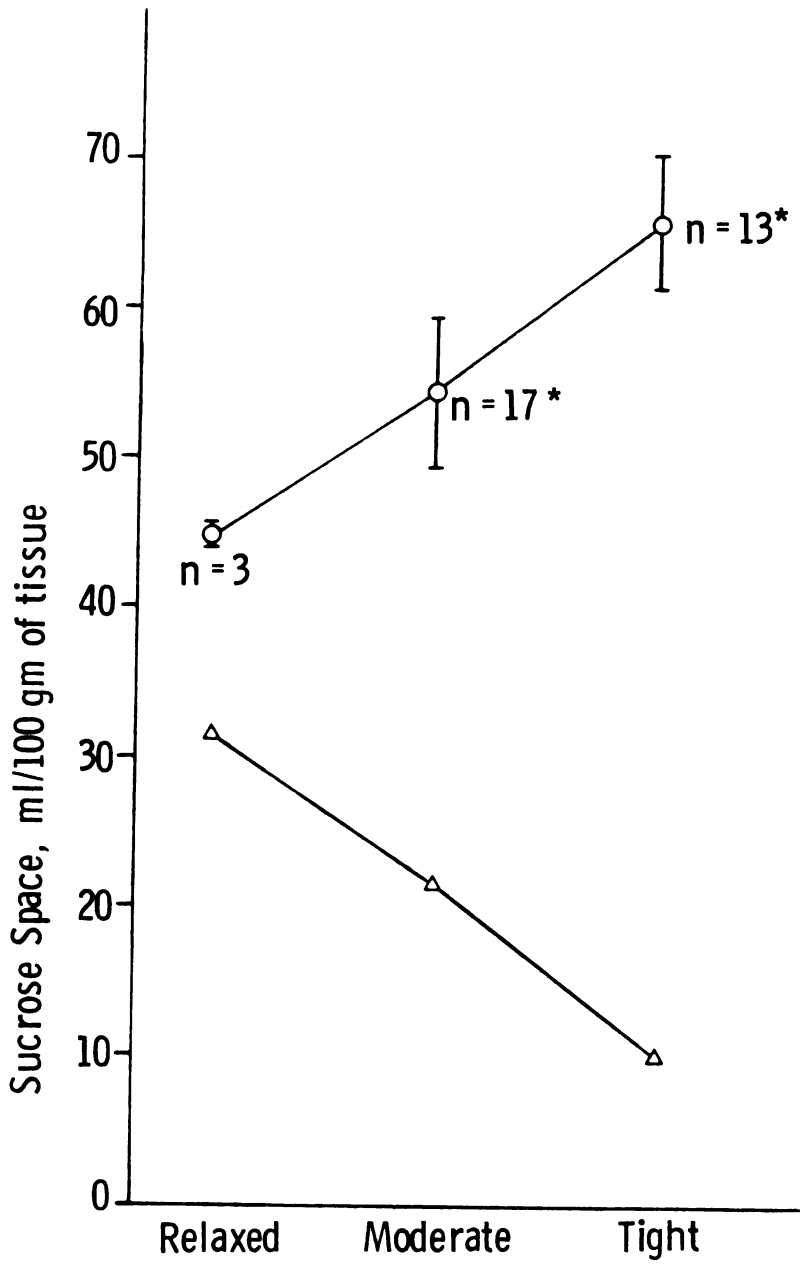

Figure 5.--Sucrose space as a function of tension in canine femoral artery. o, extracellular; Δ, intracellular (calculated by difference). *, statistically different from control at P < .002.

space of 76.0 gm/100 gm tissue, this would leave an intracellular volume of 31.4 ml/100 gm tissue. Arteries which were defined to be under "moderate" tension had an average sucrose space of 54.4 ml/100 gm tissue (± 5.0 s.d.), which gives an intracellular volume of 21.6 ml/100 gm tissue. Tissues which were judged to be "tight" gave even larger sucrose volumes. The average was 66.0 ml/100 gm tissue (± 4.6 s.d.). The corresponding intracellular volume was 10.0 ml/100 gm tissue.

In three experiments, two pieces of tissue were taken from a single animal and the arteries were cannulated with rods having different outside diameters. This was an attempt to find pairs of arteries which were as close in size as possible. In all three cases, the artery cannulated with the larger rod (and thus under a greater degree of tension) gave a larger sucrose space. There was no apparent effect of varying the placement of the larger rod (i.e. in the distal or proximal piece of tissue), see Figure 6.

Inulin Space

The inulin space measured under "moderate" tension was 40.5 ml/100 gm tissue (± 4.8 s.d.). This volume could not be differentiated to a 95% confidence level from the inulin space measured under the "tight" condition, which was 44.9 ml/100 gm tissue (± 4.1 s.d.). If the "tight" and "moderate" data is combined, the inulin space becomes

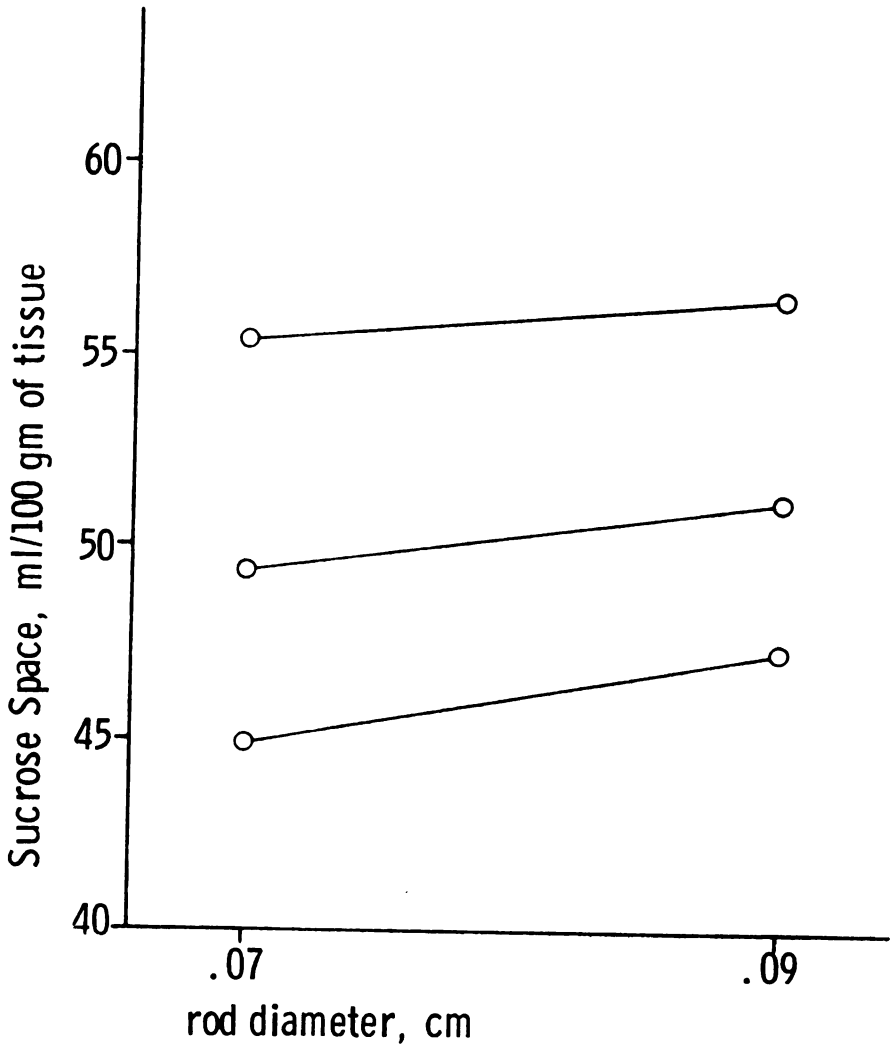

Figure 6.--Sucrose space as a function of rod diameter for samples of canine femoral artery. Points connected by a line are measurements taken from two segments of the same artery mounted on different rods.

41.3 ml/100 gm tissue (± 4.9 s.d.) which leaves an intracellular space of 34.7 ml/100 gm tissue.

In eight experiments a direct comparison of inulin and sucrose volume was attempted (see Figure 7). In seven of these, two adjacent sections of an artery were cannulated with a pair of rods which had the same diameter. One of these tissues was then incubated in a sucrose solution, and the other in an inulin solution. The respective volumes measured with different tracers could then be compared. In the eighth experiment, four sections of a single artery were cannulated by two pair of rods of two different diameters. The results showed the sucrose space to be greater than the inulin space in each of the nine pairs of tissue. The "tight" arteries, as expected, had greater sucrose volumes than the "moderate" tension vessels. This trend was not as well defined with the inulin space however.

The Effect of Low Potassium

Sucrose Space. The sucrose space, measured for "relaxed" tissue in a low potassium environment (1.0 mEq/l) was 45.9 ml/100 gm tissue (± 6.2 s.d.), which would give an intracellular volume of 30.1 ml/100 mg tissue. The sucrose space measured in this way could not be differentiated from the 44.6 ml/100 gm tissue which was determined with normal potassium.

When the tissue was placed under tension from the rod on which it was cannulated (no distinction was

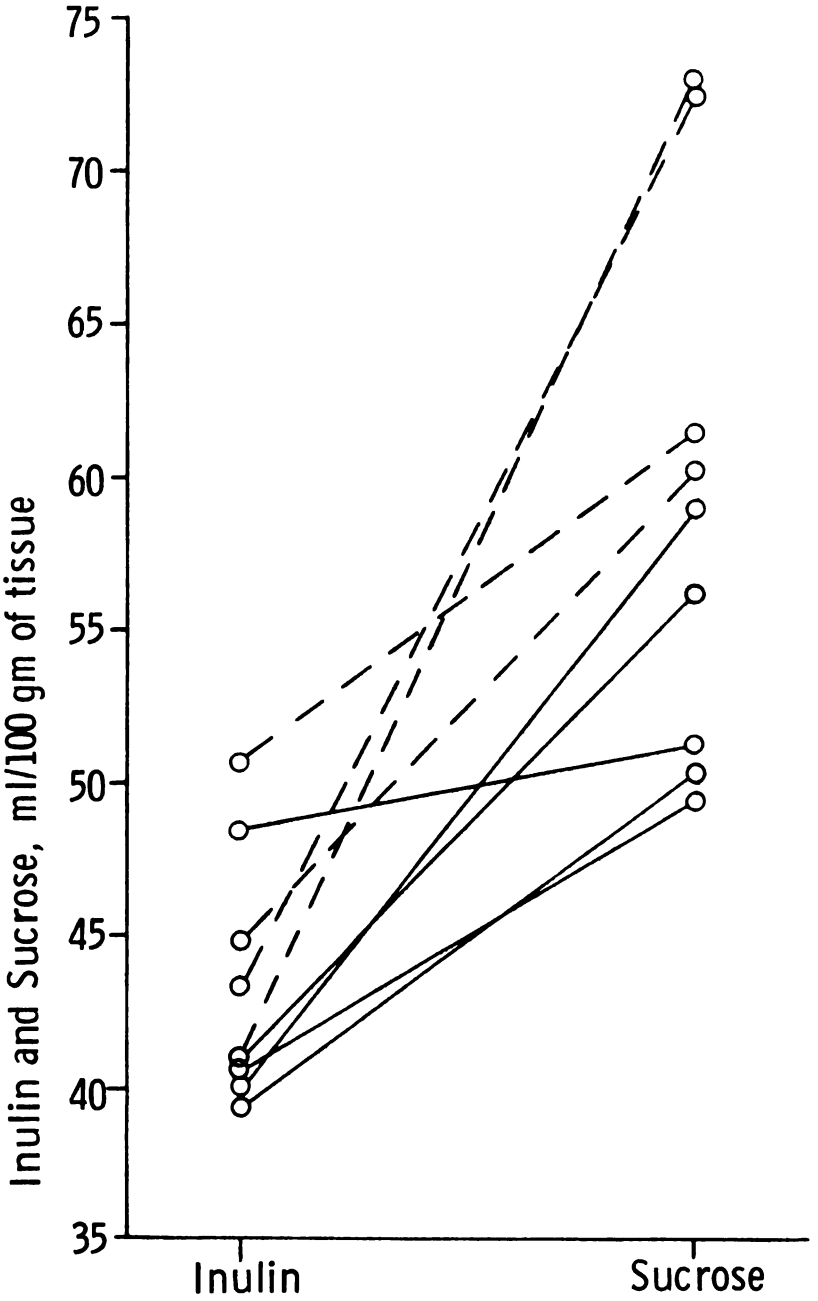

Figure 7.--Direct comparison of inulin and sucrose spaces in canine femoral artery. Connected points are from adjacent sections of the same artery. Dashed lines correspond to arteries under "tight" tension and continuous lines correspond to arteries under "moderate" tension.

attempted between "moderate" and "tight" in this study) and in a low potassium environment, the sucrose space became 59.3 ml/100 gm tissue (± 8.2 s.d.). This would leave an intracellular space of 16.7 ml/100 gm tissue. This sucrose volume was also indistinguishable from that obtained from the normal potassium tension data (59.4 ml/100 gm tissue ± 7.5 s.d.). These results are given in Figure 8.

Inulin Space. Unlike the sucrose space, the inulin space in a low potassium environment differed significantly from the normal potassium data. With just "moderate" tension considered, the low potassium inulin space was 36.0 ml/100 gm tissue (± 4.1 s.d.) which yields an intracellular space of 40.0 ml/100 gm tissue. The inulin space under "moderate" tension in a normal potassium environment was 40.5 ml/100 gm tissue which differs from the low potassium value with a level a significance of 0.013. These results are shown in Figure 9.

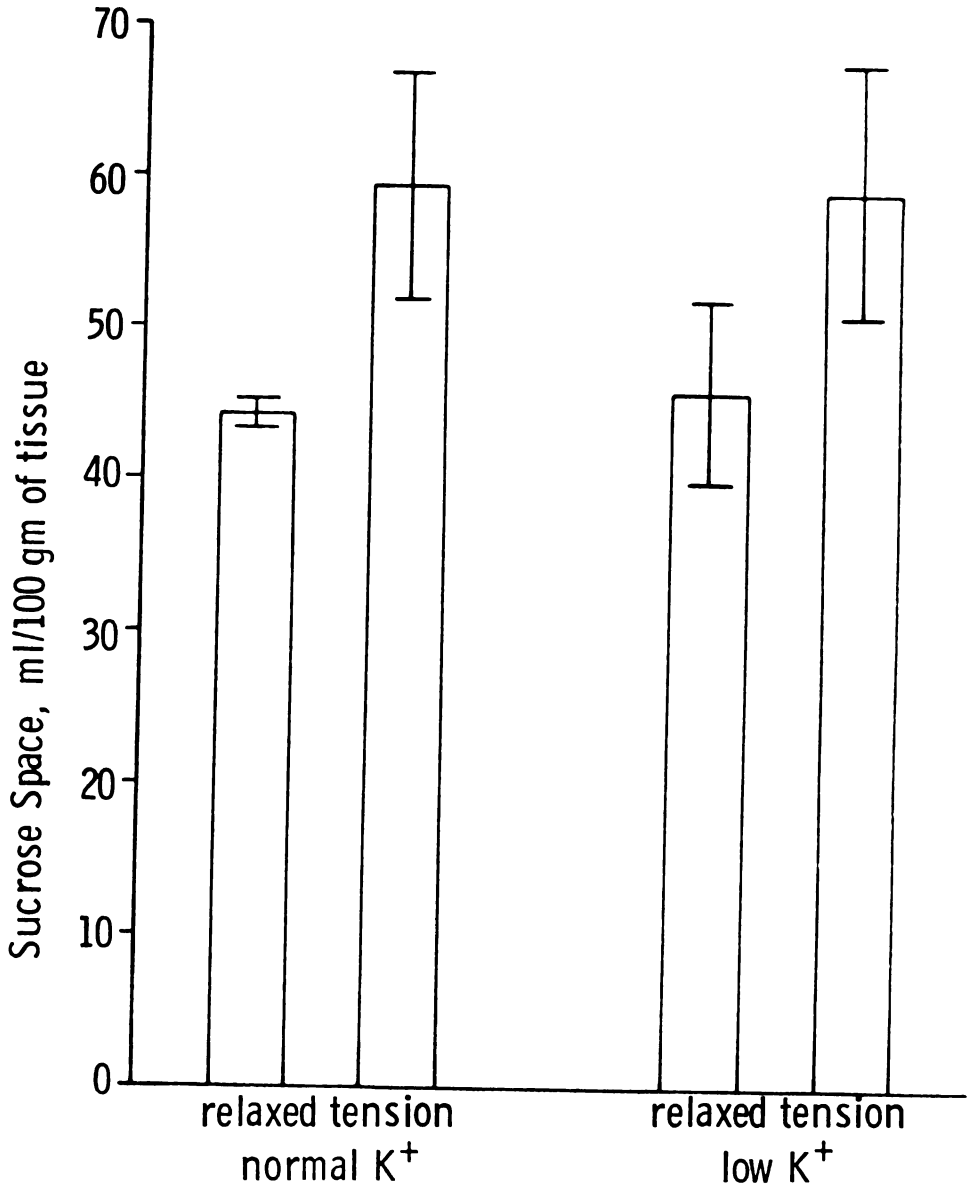

Figure 8.--Sucrose space as a function of the potassium concentration in canine femoral artery.

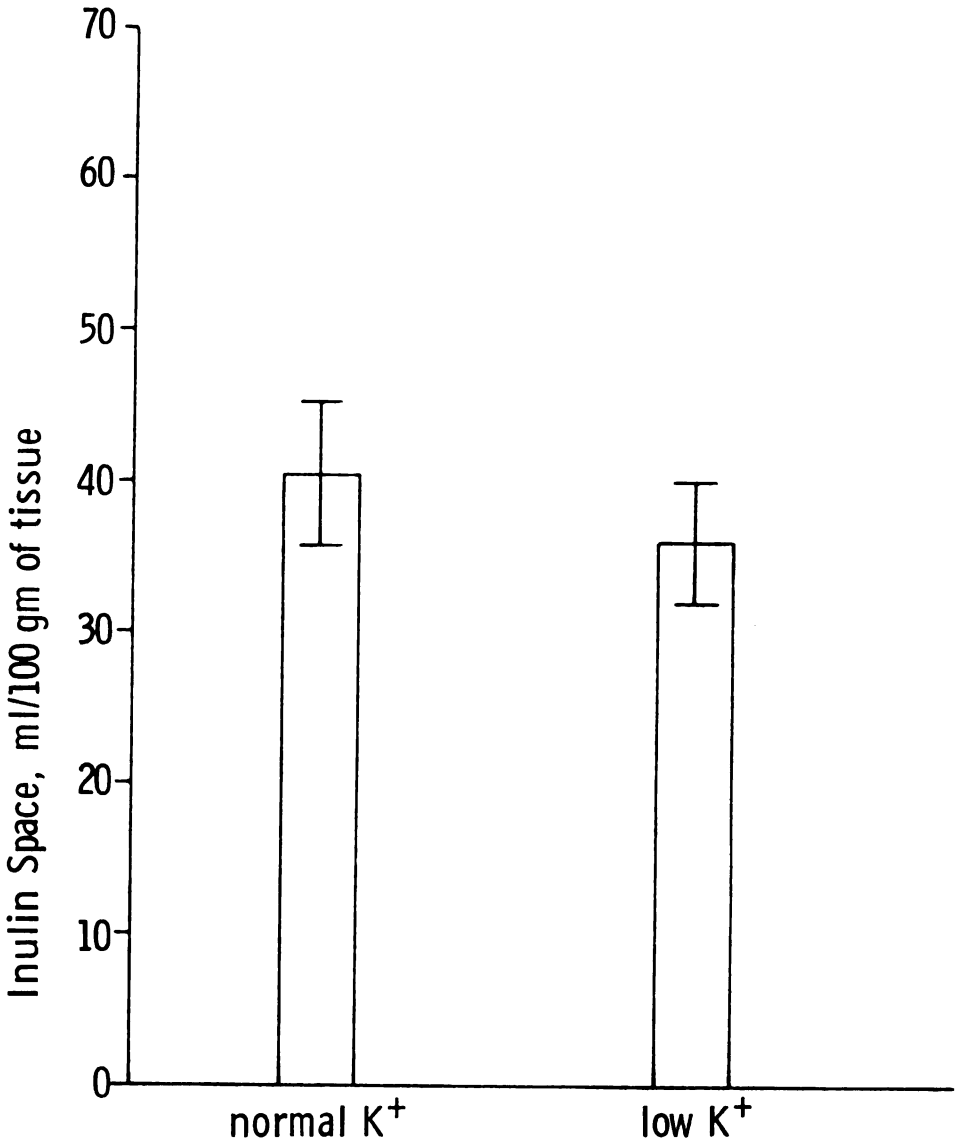

Figure 9.--Inulin space as a function of the potassium concentration in canine femoral artery.

DISCUSSION

In one experiment, the sucrose space measured for a "tight" vessel was 73.0 ml/100 gm tissue in contrast to a water space of 76.0 ml/100 gm tissue. If sucrose does not enter the muscle cells and is not bound, then the intracellular space was 3 ml/100 gm tissue or approximately 3% of this particular piece of tissue. These proportions are hard to believe. In an investigation of canine carotid artery, Villamil et al. (25) have shown that binding of sucrose is unlikely, since the sucrose space never exceeded the total tissue water when the cellular barriers were abolished by metabolic inhibition. Sucrose has, however, shown evidence of entering smooth muscle cells (1, 6, 9).

Bozler and Lavine (6) for example, measured sucrose volumes as high as 67-85% of the muscle weight in frog stomach (the water space averaged 81%). These large volumes were measured in tissue that had previously been incubated in a 2mM calcium chloride solution which caused the muscle cells to swell. It is known that vascular smooth muscle contracts in low potassium (14). This study

indicates a decrease in inulin space in a low potassium environment, with the water space remaining constant implying an increase in the cell volume. If the muscle cells swell with contraction they could be exhibiting the same effect achieved by Bozler and Lavine with calcium chloride. Certainly the increase they observed in the sucrose space is similar to the increase seen in this study with tissue under tension.

Cell swelling with low potassium contraction can be explained by considering the action of the Na-K pump. This pump is believed to transport more sodium ions out of a cell than it transports potassium ions into the cell. Since this active transport mechanism is slowed down by lowering the external potassium concentration, it seems likely that an excess of ions would accumulate internally. This would increase the osmolarity inside the cell and consequently water would be drawn in and effect cell swelling.

In contrast to sucrose, inulin space remained relatively constant with respect to tension. A summary of the data is given in Table 3. It seems likely that if sucrose is entering the muscle cells under tension while the inulin space remains unchanged under the same conditions, that the inulin molecule is sterically inhibited from entering the cells. The molecular weight of inulin is 7000 compared to 342 for sucrose. The inulin space (41.3 ml/100 gm tissue) corresponds quite well to other values reported for comparable tissues, see Table 2.

TABLE 3.--Summary of the results.

Potassium	Tension	ECS	SD	SEM	n
		Sucrose			
normal	relaxed	44.6	0.8	0.5	3
normal	moderate	54.4	5.0	1.2	17
normal	tight	66.0	4.6	1.3	13
normal	moderate & tight	59.4	7.5	1.4	2
low	relaxed	45.9	6.2	4.4	2
low	moderate & tight	59.3	8.2	2.6	10
		Inulin			
normal	tight	44.9	4.1	2.1	4
normal	moderate	40.5	4.8	1.1	17
low	moderate	36.0	4.1	1.4	9
normal	moderate & tight	41.3	4.9	1.1	21
Water Space--76.0 ml/100 gm tissue, SD 3.1, SEM 0.4					

ECS, ml/100 gm tissue

SD, standard deviation

SEM, standard error of the mean

n, number of points

Villamil et al. (25) combined light and electron microscopy of canine carotid artery to calculate an extracellular volume (including solids) of 55.7 ml/100 gm tissue which they called "anatomical space." These investigators measured the water fraction of collagen and elastin fibers at 70% and concluded that if the ECS contained only elastin and collagen then the accessible volume would be 39 ml/100 gm tissue. They then called this number the "lowest possible estimate" of the ECS. Extrapolating this to the water space measured in this study (76.0 gm/100 gm tissue) and using the same anatomical space, a value of 42.3 ml/100 gm tissue is obtained. This could be considered an "upper estimate" if the true extracellular water fraction was between 70 and 76%.

CONCLUSION

It is apparent that sucrose is an unreliable measure of ECS in canine femoral artery when the tissue is placed under tension with a cannulating rod. Of the two tracers tested, inulin appears to be the best choice when measuring ECS with the artery under tension. The inulin space of 41.3 ml/100 gm tissue corresponds quite well to the "lower estimate" of 39 ml/100 gm tissue suggested by Villamil et al. (25), and the "upper estimate" of 42.3 ml/100 gm tissue determined in this work. The inulin tracer also exhibited sufficient sensitivity to changes in the potassium environment. The cell volume increased from 35.5 ml/100 gm tissue to 40.5 ml/100 gm tissue when subjected to a potassium concentration in the surrounding medium which was 25% of the normal concentration.

APPENDIX

TABULATED EXPERIMENTAL DATA

Exp. No.	V_o/100g	water vol / wet wt.	V_i/100g calculated	V_i/V_o calculated
	Sucrose, Normal Potassium, Relaxed Tension			
26	45.20	0.8031	35.11	0.7768
29	43.70	0.7799	34.70	0.7847
32A	44.94	0.8152	36.58	0.8139
	Sucrose, Normal Potassium, Moderate Tension			
27	57.04	0.8088	23.85	0.4181
28	57.17	0.7884	21.67	0.3791
32B	47.31	0.7876	31.44	0.6646
33	63.88	0.7825	14.36	0.2248
34	61.94	0.7815	16.21	0.2616
35A	55.42	0.7736	21.94	0.3958
35B	56.53	0.7574	19.21	0.3399
36A	50.33	0.7654	26.21	0.5208
37A	58.93	0.7650	17.57	0.2982
41A	56.23	0.7554	19.31	0.3434
43A	51.23	0.7448	23.25	0.4538
43B	49.39	0.7536	25.96	0.5256
46B	56.75	0.7622	19.46	0.3430
47B	47.80	0.7227	24.47	0.5118
48B	54.18	0.7583	21.66	0.3997

Exp. No.	V_o/100g	water vol / wet wt.	V_i/100g calculated	V_i/V_o calculated
50B	54.28	0.7375	19.47	0.3587
51B	46.68	0.7734	30.66	0.6568
	Sucrose, Normal Potassium, Tight Tension			
3	61.67	0.7302	11.35	0.1841
5	67.26	0.7532	8.05	0.1197
7	63.00	0.7870	15.70	0.2492
22	65.84	0.8158	15.74	0.2390
23	65.33	0.7865	13.32	0.2039
24	59.78	0.7811	18.32	0.3065
30	67.30	0.8024	12.94	0.1923
31	70.76	0.7834	7.58	0.1071
38A	72.43	0.7432	1.90	0.0262
39A	60.23	0.7447	14.24	0.2364
40A	73.00	0.7361	0.61	0.0084
42A	61.35	0.7414	12.78	0.2084
45A	69.37	0.7381	4.44	0.0640
	Sucrose, Low Potassium, Relaxed Tension			
11	41.56	0.8255	40.99	0.9861
14	50.30	0.8447	34.17	0.6794

Exp. No.	V_o/100g	water vol / wet wt.	V_i/100g calculated	V_i/V_o calculated
Sucrose, Low Potassium, Moderate and Tight Tension				
8	49.55	0.7940	29.85	0.6024
9	57.49	0.7978	22.22	0.3878
10	48.48	0.7874	30.25	0.6240
12	52.90	0.7871	25.81	0.4879
16	75.56	0.7863	3.07	0.4060
17	56.11	0.7500	18.89	0.3367
18	60.23	0.7600	15.77	0.2618
19	65.07	0.8026	15.20	0.2335
20	63.99	0.7888	14.89	0.2327
21	63.15	0.7727	14.13	0.2237
Inulin, Normal Potassium, Moderate Tension				
36B	38.38	0.7761	39.23	1.0221
37B	39.87	0.7769	37.81	0.9483
41B	41.08	0.7681	35.73	0.8697
43C	48.44	0.7588	27.44	0.5663
43D	40.41	0.7419	33.78	0.8359
44A	37.15	0.7473	37.58	1.0117
44B	45.07	0.7621	31.14	0.6910
44C	49.70	0.7651	26.81	0.5395
53B	38.28	0.7619	37.92	0.9906
54B	33.31	0.7350	40.19	1.2065

Exp. No.	V_o/100g	water vol / wet wt.	V_i/100g calculated	V_i/V_o calculated
55B	45.06	0.7333	28.27	0.6275
56B	45.05	0.7125	26.20	0.5815
57B	37.97	0.7222	34.25	0.9022
58B	33.22	0.7059	37.33	1.1251
59B	36.02	0.7260	36.58	1.0156
60B	38.96	0.7238	33.42	0.8577
61B	40.01	0.7050	30.49	0.7620
	Inulin, Normal Potassium, Tight Tension			
38B	43.27	0.7451	31.24	0.7220
39B	44.79	0.7285	28.07	0.6267
40B	40.98	0.7510	34.12	0.8327
42B	50.62	0.7425	23.63	0.4668
	Inulin, Low Potassium, Moderate Tension			
53A	32.72	0.7692	44.20	1.3506
54A	41.57	0.7179	30.23	0.7272
55A	34.05	0.7050	36.45	1.0706
56A	37.85	0.7240	34.55	0.9127
57A	36.28	0.7194	35.66	0.9830
58A	31.12	0.7220	41.08	1.3202
59A	30.85	0.7358	42.73	1.3851
60A	41.46	0.7286	31.40	0.7572
61A	38.36	0.7036	32.00	0.8341

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