Distribution of Inulin Space in the Rabbit Thoracic Aorta[1]

When a drug is added to the oxygenated physiological saline bathing an isolated organ in a tissue bath, it will mix rapidly with the saline and then more slowly penetrate the tissue or organ. If the rate of penetration of the drug is to be related to its pharmacological effect, the dimensions of the extracellular space in the tissue must be known. Recent studies using an isotope frozen-section technique have described the movement of tritiated norepinephrine through the wall of a blood vessel in particular the rabbit thoracic aorta, during the time course of contraction. After 1 min exposure to the drug, the media contains less than 25% the amount of norepinephrine present in a similar volume of the bath solution, and this is unevenly distributed[2,3]. Although values of the extracellular space of vascular tissue have been reported[4-8], they are not known in sufficient detail for an analysis of drug distribution under these circumstances.

In this paper, the dimensions of the inulin space in the rabbit thoracic aorta, its distribution along the course of the vessel from the aortic arch to the diaphragm, and its variation through the thickness of the vessel wall are described. The aorta was chosen for this study because it represents the most commonly used in vitro preparation.

Methods. The thoracic aorta from the aortic arch to the diaphragm from rabbits weighing 2.0–2.7 kg was used. The inulin space was determined using (carboxyl-^{14}C) inulin, 2.48 and 2.7 mc/g, obtained commercially. In brief, after equilibration in Krebs bicarbonate solution maintained at 37 °C, the tissues were placed in fresh solution containing 1 μc/ml of ^{14}C-inulin and after a prescribed period of time removed and their radioactivity assayed.

Inulin uptake was determined under a variety of circumstances for the following specific purposes. a) The time course of inulin uptake; 6 rings were prepared from each of 6 aortae, and exposed for times ranging from 5 to 120 min. b) The distribution of inulin uptake along the length of the aortae; 16 segments of equal length were prepared from 7 aortae, and exposed to ^{14}C-inulin for 1 h, the previously determined equilibrium time (see results). c) The distribution of inulin uptake between the adventitia and media prepared by the stripping technique[9]; this procedure was carried out in 7 strips after 1 h incubation with ^{14}C-inulin.

After incubation each tissue specimen was blotted on Whatman No. 1 filter paper previously moistened with the salt solution, its sides trimmed with a razor blade and weighed.

d) The distribution of the inulin space through the depth of the aorta wall; tissues previously incubated in ^{14}C-inulin were frozen in a flat plane on a special tissue holder. Segments of approximately 25 mm^2 were sectioned parallel to the intima at 24 μm. For details of this isotope-frozen technique see previous description[2].

The radioactivity of all tissue specimens was determined by scintillation spectrometry. Uptake was expressed as ml of bath fluid cleared per g tissue wet weight (ml/g).

Results. The rate of ^{14}C-inulin uptake by the rabbit thoracic aorta was rapid at first, and then declined exponentially with time. The value of the ^{14}C-inulin clearance was 0.43 ± 0.04 ml/g after 30 min and 0.48 ± 0.02 ml/g after 1 h (M ± S.E.M.: $n = 7$). This latter value was unchanged after 2 h. Since a plateau was reached at 1 h or less, this was taken as the standard incubation time in all subsequent experiments.

It is clear from Figure 1 that inulin uptake is uniform along the whole length of the aorta, from its cardiac to abdominal end. The uptake is not evenly distributed between the tunica adventitia and media. This was demonstrated in the stripping experiments in which the uptakes were 0.56 ± 0.02 and 0.42 ± 0.01 ml/g for adventitia and media respectively (M ± S.E.M.: $n = 7$; $P < 0.001$).

The inulin space is fairly evenly distributed through the media thickness, at least when this tissue is considered in 24 μm increments (Figure 2). Mean values for adventitial and medial uptake calculated from mean section values are 0.59 and 0.39 ml/g respectively. For technical reasons, the first tissue slice, that containing the first

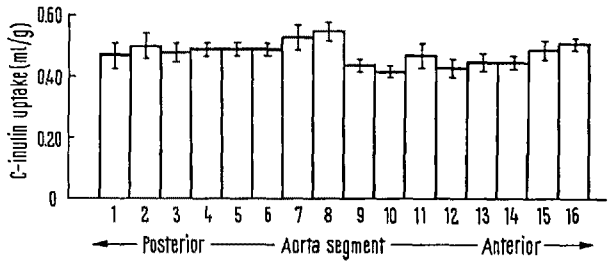

Fig. 1. Uptake of ^{14}C-inulin by aorta segments after 1 h exposure. Segments were taken from different positions of the aorta (number 1), the most posterior and adjacent to the diaphragm; number 16, anterior and adjacent to the aortic arch. Each column is the mean result of corresponding segments for 7 different rabbits.

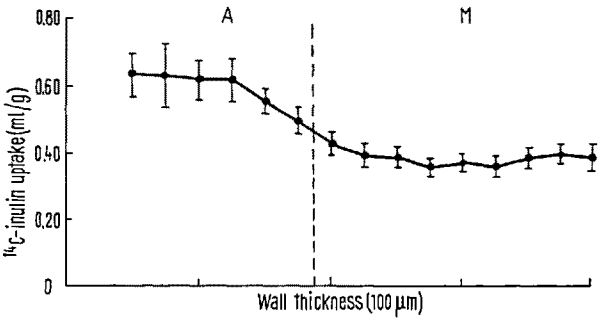

Fig. 2. Distribution of ^{14}C-inulin space through the thickness of the rabbit thoracic aorta. Uptake of the ^{14}C-inulin expressed as ml of bath fluid cleared per g tissue (ml/g) is placed against the thickness of aortic wall. The vertical bars represent standard errors of the mean.

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part of the intima was not included in the distribution curves. The transition from uptake values around 0.40 to 0.60 ml/g takes place fairly abruptly at the adventitio-medial junction.

Discussion. With the exception of methods based upon electron microscopy, only indirect methods employing large molecules, which presumably do not enter the cell, are available to measure the extracellular space. This space in vascular tissue is exceedingly complex and is not easily amenable to planimetric measurement.

The homogeneity of the inulin uptake values along the course of the vessel allows data determined from one part of the vessel to be compared directly with that from other parts, and is consistent with previous although less extensive studies[10]. A difference between the magnitude of the space in the 2 tunicae has been noted before in the carotid artery[6]. This finding implies that, since the space in the adventitia is approximately 30% greater than that in the media, overall measurements of the inulin space are not valid if precise analysis of the distribution and movement of substances in the separate tunicae are to be made. The even distribution of the space through the media is in agreement with histological findings of the uniformity of the elastic lamellae in this tunica[11].

Differences in values for extracellular space are due not only to different tissues but to the substances used. In the same vessel for example the sucrose space is larger than that for inulin[6]. This is not unreasonable when it is realized that the molecular weight of sucrose (342) is only 1/15 that of inulin (5000). Because of such considerations and as neither of these substances can be excluded from intracellular sites[12,13], the quantitative values of the inulin space do not necessarily precisely reflect the extracellular distribution of added foreign agents. However there is no reason to doubt that they form a reasonable (and the best available) approximation.

Zusammenfassung. Die ganze Länge und Dicke des extrazellulären Raumes der thorakalen Kaninchenaorta wurde gemessen. Der Raum ist unverändert die ganze Länge des Blutgefässes hindurch und entspricht 0,59 ml/g 0,39 ml/g für die Adventitia und die Media. In jeder dieser Tunicae ist der Raum durch ihre ganze Dicke gleichmässig verteilt.

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Micro-Diver Studies on Isolated Intestinal Cells

It is firmly established that the epithelial lining of the gastro-intestinal tract is constantly being replaced[1]. The turnover rate in the rat jejunum is 1–2 days and it is brought about by a rapid cell renewal at the bases of the crypts. The newly formed cells apparently move from the crypts up along the villi to the tips where they die and are discarded into the intestinal lumen. Thus, at any given moment, the cells at the different levels from the bases of the crypts towards the tips of the villi are of different age and at different stages of development. It would therefore be of interest to be able to study epithelial cells taken from different levels along crypts and villi. In this preliminary report techniques are described for isolating and studying the respiration of intestinal epithelial cells from the bases and from the tips of the villi of the jejunum of the rat.

Methods. Rats of the Sprague-Dawley strain, weighing about 200 g, were used. The animals had been deprived of food for approximately 24 h. They were sacrificed by cervical fracture. The abdomen was immediately opened in the midline and a segment (about 5 cm long) of the jejunum just distal to the ligament of Treitz was chosen for the experiment. The intestinal segment was flushed with 10 ml of cold (+2 to +6 °C) Tyrode solution, cut open and again rinsed twice in cold Tyrode solution to remove any remaining intestinal content adhering to the mucosal surface. The segment was kept in the Tyrode solution on ice.

A small piece of the jejunal segment, approximately 5 × 5 mm, was placed in cold 0.25 *M* sucrose solution or Tyrode solution. Single villi were carefully dissected free from this tissue section under a Zeiss stereomicroscope by means of a scalpel and stainless steel needles of various sizes. In doing so, the lamina propria of the villus was apparently freed from the intestinal epithelial cells and their basement membrane. It was then possible to isolate a cluster of epithelial cells from the tip and/or from the base of an isolated villus. The size of the isolated cell cluster was estimated by an objective with a measuring grid.

The oxygen uptake of the intestinal cells was determined by the micro-diver technique originally described by ZEUTHEN[2]. The intestinal cells were introduced by suction into divers made of Pyrex glass together with approximately 0.5 µl medium (see below) after which the tip of the diver was sealed with bees wax heated to its melting point. The diver was adjusted to floatation equilibrium in 0.1 *M* phosphate buffer, pH 7.4 and transferred to a floatation vessel containing the same buffer solution. The vessels were immersed in a thermostatically regulated waterbath of 37 °C. After a temperature equilibration period of 30–40 min, the oxygen consumption was measured manometrically for 2–3 h. The pressure change was plotted against time and was found to be linear. Blank divers containing incubation medium but no cells showed no oxygen consumption. Oxygen consumption was calculated according to ZEUTHEN[2].

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