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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A 'two-compartment' diver technique, originally described by HAMBERGER[3], was used to study the respiratory rate of intestinal cells before and after addition of e.g. 3-*O*-methyl-glucose to the incubation medium. This was made possible by placing a capillary tube in the upper opening of the micro-diver. The capillary, sealed in its upper end by wax, contained the solution to be added to the original incubation medium. The 2 solutions were separated from each other by an air lock. Mixing of the 2 solutions was accomplished by lowering pressure in the floatation vessel, which expanded an air bubble in the upper end of the capillary tube and pushed the capillary solution into the incubation medium. The technique has been described in more detail elsewhere[3].

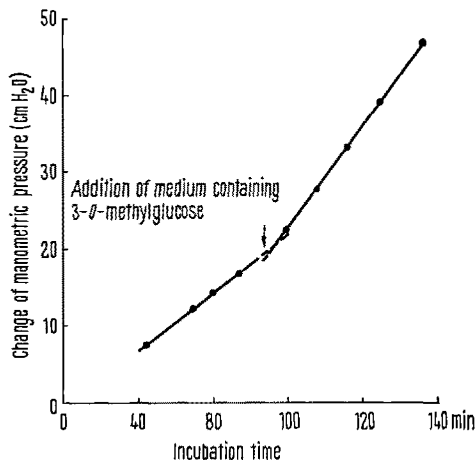
Incubation media: a) Na_2HPO_4-KH_2PO_4 buffer, pH 7.4, 37.5 m*M*; $AlCl_3$ 0.5 m*M*; $MgCl_2$ 0.5 m*M*; Na succinate 25 m*M*; cytochrome c 8.6×10^{-2} m*M*.

b) *Tris* HCl buffer, pH 7.4, 25 m*M*; NaCl 124 m*M*; KCl 5 m*M*; $CaCl_2$ 0.15 m*M*; $MgSO_4$ 0.5 m*M*; Na succinate 6 m*M*.

Results. The respiratory rates of cells from the tips and from the bases of villi were compared in 5 experiments. It was demonstrated that the oxygen consumption per unit area of the cell cluster was approximately twice as great for 'base cells' as for 'tip cells' ($(41 \pm 4.8) \times 10^{-4}$ and $(19 \pm 4.6) \times 10^{-4}$ µl/h × unit area, respectively; mean ± S.E.M., $n = 12$). In these experiments the incubation medium containing cytochrome c was used.

In 4 experiments the effect of adding 3-*O*-methylglucose (20 m*M* solution) to the incubation medium (*Tris* HCl buffer) was investigated. The final concentration of 3-*O*-methylglucose was approximately 5 m*M*. This produced an increased rate of oxygen consumption as illustrated in Figure 1.

Discussion. During recent years several studies on isolated intestinal cells have been reported[4]. However, in none of these previous investigations have cells from different parts of the villi been isolated and studied separately. In this paper such methods are described and it proved possible to isolate and study oxygen consumption of intestinal cells from tips and bases of villi. Furthermore, these cells seem to be fairly intact since 3-*O*-methylglucose, a carbohydrate actively transported into cells but not metabolized[5], added to the incubation medium increased the rate of oxygen consumption of the cells. This may possibly reflect an augmented metabolic activity induced by the active transport of the sugar across the cell wall. With the present technique it thus seems to be possible to isolate and study the respiration of intestinal cells from different levels of the villi from the various consecutive sections of the intestinal tract before and after addition of different solutes[6].



The effect of 3-*O*-methylglucose on the respiratory rate of isolated intestinal epithelial cells. The experiment was performed with a 'two-compartment diver'[3]. The oxygen uptake is expressed as change in manometric pressure.

Zusammenfassung. Der Sauerstoffverbrauch in isolierten Epithelzellen aus dem Dünndarm von Ratten wurde mit dem Kartesischen Taucher untersucht. Die Zellen aus den Villusbasen zeigten einen höheren Sauerstoffverbrauch als Zellen von den Villusspitzen.

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Effects of Drugs and Ions on a Primitive System of Spontaneous Contractions in a Sponge (*Euspongia officinalis*)

In Sponges, contractile responses of the oscular membrane following stimulation have been known for a long time[1–5]. Recent time-lapse cinematography of oscula indicates[6] that besides induced contractions Porifera show two discrete patterns of spontaneous activity: a) Short-term contractions arise here and there on the oscular membrane as rhythmic local pulsations (6 or 7 per h) which are occasionally propagated at 1 mm/min but are usually confined to restricted groups of cells (0.2 mm^2). They last about 30 sec and take the form of slow, localized shrinkings. b) Long-term contractions (5 or 6 per 24 h) are synchronous and bring about tonic closure of the whole osculum for several minutes. Larger areas (5 mm^2) are involved.

Only one type of contractile cells is revealed by electron microscopy[7–9], i.e. the mesenchymatous cells. They have microfilaments and form a network of connected elements[10]. The responses of these cells are reported to be unaffected by acetylcholine and adrenaline[4] and by the substitution of Na^+ by K^+ and of Ca^{++} by Mg^{++} in the external medium[5]. In order to see whether spontaneous and induced contractions behave in the same way, we