protein synthesis and for synthesis of other essential cellular materials.

Another factor that may be important in the in vivo non-reactivity of the lysosomal hydrolases towards other lysosomal enzymes in the lysosomes of normal dividing cells is the possibility that catheptic enzymes exist intracellularly in inactive 'ogen' forms similar to those of the trypsin-trypsinogen and chymotrypsin-chymotrypsinogen system of extracellular digestion. PRESS et al.[8] have isolated several forms of cathepsin from beef spleen. During cell death, changes in the cellular environment or a vacuolar change may bring about modifications in the catheptic structure, resulting in the activation of possible 'ogen' forms.

Binding of the lysosomal enzyme compliment to glycolipid, a negatively charged environment, a high internal pH, and possible 'ogen' forms may contribute to the relative inertness of the lysosomal enzymes to one another and their substrates in vivo. Dramatic increases in lysosomal activity occur when lysosomal membranes are broken or damaged and the lysosomal enzyme complement is spilled out into its external environment. Extracellular factors such as environmental pH and ionic strength both influence lysosomal enzyme activity. When cells die, the environment is thought to become more acidic, probably through an accumulation of short-chain fatty acids. Lysosomal enzymes are known to have their maximal activity at acid pH. Another factor contributing to lysosomal enzyme activation after the lysosomal membranes are broken or damaged may be a sudden increase in ionic strength. It is conceivable that these enzymes exist in an environment of relatively low ionic strength (i.e. bound to glycolipid), and then are activated by exposure to the external environment. Many enzymes are unstable or have little activity in regions of little or no ionic strength. The present author has found that preincubation with bovine uterine cathepsin D at 20 °C in deionized distilled water may reduce the activity of the enzyme as much as 50%. On the other hand, preincubation with this enzyme in 0.01 *M* KCl or NaCl may produce a 2- to 3-fold activation of the enzyme.

In contrast to the dramatic activation of these enzymes when exposed to their external substrates, lysosomal enzymes appear to be inert toward each other even in rapidly regressing tissues. WEBER[9] has reported a nearly 30-fold increase in acid cathepsin activity in regressing tadpole tails, with little net gain in total units of enzyme. Similarly, WOESSNER[10] has found that the total activity of uterine β-glucuronidase remained constant during late pregnancy and several days after parturition. SHAMBERGER[11] has reported striking concentrations of 6 lysosomal enzymes in regressing rat mammary tumors. Preservation of the lysosomal compliment may be important not only in regressing tissues but also in ordinary normal cells. The enigma of lysosomal enzyme preservation may be resolved by determining the turnover rates of some of the acid hydrolases in situations of rapid tissue breakdown.

Greater knowledge of tertiary protein structure and protein chemistry may also yield clues as to why lysosomal enzymes undergo turnover more slowly than do other tissue components. For example, clues regarding the inertness of the lysosomal enzymes towards one another may be gained from the knowledge of the tertiary structure of bovine alkaline pancreatic ribonuclease and papain, which, even though not lysosomal, are nonetheless hydrolytic enzymes.

X-ray diffraction studies show that a molecule of bovine alkaline pancreatic ribonuclease has inactive sulfhydryl bridges and is thus inert to sulfhydryl reagents[12]. In addition, the phosphate ion binding site, which is probably the active center, is located in a cleft of the kidney-shaped molecule. The inertness of the sulfhydryl groups and the masking of the active center, resulting in a sterically hindered molecule, may explain why lysosomal enzymes are unreactive towards one another in regressing tissues as well as in normal dividing cells. Papain has also been shown by X-ray diffraction studies to be a kidney-shaped molecule[13]. The active site of papain appears to be a groove that contains a number of substrate, binding sites, including a sulfhydryl group from cysteine[14]. Histidine may also be a part of the active site. The nature of the tertiary structure of the lysosomal remains to be elucidated. That nature, when determined may explain why these enzymes are inert toward one another.

Zusammenfassung. Die Reaktionsträgheit der Lysosomenhydrolasen gegeneinander in vivo kann aus der räumlichen Hinderung ihrer aktiven Zentren entstehen.

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Epithelial Cell Swelling During Incubation of Rat Small Intestine in vitro

The uptake of fluid by preparations of intestine incubated in vitro is well known[1,2]. The magnitude of this gut fluid uptake (GFU) is proportional to the rate of fluid transport[3,4] and, in contrast to tissues which do not perform transtissue fluid transport, is increased in the presence of metabolizable substrates and reduced by metabolic inhibitors. Although it has been suggested that the fluid taken up occupies a subepithelial extracellular compartment[2], this distribution has not been demonstrated experimentally, and no change in the extracellular space of intestine incubated in vitro could be demonstrated by conventional marker techniques[4] suggesting that the gut fluid uptake may occupy an intracellular compartment. In the present experiments the relation between uptake of fluid by intestine and epithelial cell size is examined.

Methods. The preparation used was the everted sac of rat small intestine prepared from the middle fifth of the combined jejunum and ileum as described previously[5]. Fluid transfer was varied in different experiments by the addition of a metabolizable substrate (glucose) or an osmotic inhibitor (mannitol).

At the end of the incubation the sacs were blotted between damp filter papers, weighed, opened, drained and weighed again. GFU was defined as the increase in weight of the tissue during incubation. After the final weighing the tissue was immediately flooded with osmium/dichromate fixative and sliced into small segments. Following full fixation and dehydration the tissue was embedded in Epon and 2 μ sections made. The sections were examined by phase-contrast microscopy and estimates of cell height and width made using a calibrated micrometer eyepiece. At least 30, and usually between 50 and 70 observations were made on a given sample.

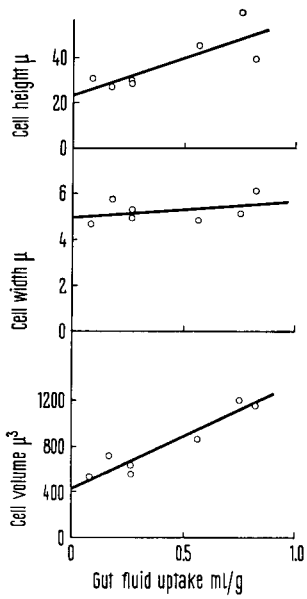

Fig. 1. The relations between gut fluid uptake and cell height, cell width and cell volume. The data are taken from experiments in which gut fluid uptake (GFU) was varied by the addition of glucose or mannitol to the saline bathing the luminal face of everted sacs of rat jejunum.

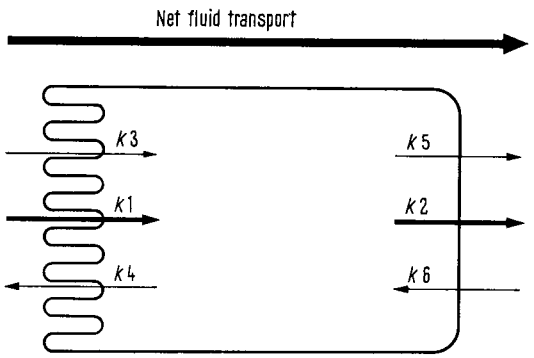

Fig. 2. Parameters of cell volume in an intestine performing net fluid transport from luminal to abluminal surfaces. Cell volume is dependent upon the difference between the net movement of fluid into the cell at the luminal surface (k_1) and the net movement of fluid out of the cell at the abluminal surface (k_2). Each of these net movements represents a difference between opposed unidirectional fluxes at each face (k_3 and k_4, and k_5 and k_6), and a steady state transepithelial fluid movement in the direction indicated can only be maintained when k_1 and k_2 have the same sign.

Results and discussion. The results of these experiments are shown in Figure 1. It can be seen that the height of the cells increased with GFU. Regression analysis of this relation gave a coefficient of correlation of 0.800 indicating statistical significance ($p < 0.05$). In contrast no significant correlation between cell width and GFU was observed. The third part of Figure 1 shows the relation between a calculated cell volume and GFU. The values for cell volume were calculated from the measured cell heights and widths and are based on the assumption that the cell can be considered as a right cylinder. It can be seen that the cell volume calculated in this way is linearly related to GFU. Regression analysis of this relation gave a correlation coefficient of 0.951 indicating a very high degree of statistical significance ($p < 0.001$).

Thus, at least part of the uptake of fluid by the gut wall, during fluid transport in vitro, can be accounted for by a measurable increase in the volume of the epithelial cells. These experiments confirm the earlier suggestion[4] that the intestinal mucosal cells may swell during incubation in vitro, and demonstrate further that this swelling is proportional to the GFU.

The relation of cell volume to GFU is of some interest for the implications it may have in the mechanism of fluid absorption in the intestine. A current hypothesis for transepithelial fluid transport emphasizes the role played by active solute transport at the lateral borders of the transporting cells[6]. Such a mechanism would not explain the present observations without some modifications. Consideration of the parameters governing cell volume in a system performing net transport of fluid from luminal to abluminal compartments (see Figure 2) indicates that an increase in cell volume could be due either to a decrease in the net movement of fluid out of the cell at the abluminal face, or to an increase in the net movement of fluid into the cell at the luminal face. The former explanation is obviously inconsistent with an increase in net transepithelial fluid transport, and the relation between cell volume and fluid transport must be dependent upon a variable process at the luminal face. Since this process has been shown to be linked to the metabolic status of the epithelium[4] the possibility of osmotically active solute pumps at this site requires further consideration[7].

Résumé. L'augmentation du fluide du tissu de l'intestin grêle incubé in vitro s'accompagne d'un accroissement du volume des cellules épithéliales.

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