## The Passage of Ferritin into Jejunal Epithelial Cells

The possibility of the passage of intact protein molecules into intestinal epithelial cells, and through them into the rest of the body, is of considerable importance for the explanations of the pathogenesis of intestinal allergies. It is often considered that not only are proteins degraded by the intestinal enzymes, but that there is no mechanism by which any of them which remained undegraded could pass into and through the epithelium. Since it is obvious that a few molecules could well remain undenatured, and since it is known that only a little of an antigen is necessary to produce an allergic reaction, the main problem has been to find out if it is, in fact, possible for intact molecules to enter the epithelial cells.

It has been shown[1] that lipid droplets can enter the epithelial cells via small invaginations, between the microvilli, which pinch off, move into the cell, fuse into larger vesicles, and eventually discharge their contents at the base and sides of the cells. It has also been demonstrated that lipids can enter vesicles after passing directly through the plasma membranes of the cells[2], but in addition the purely vesicular path has been confirmed[2,3]. CLARK[4] found that new-born (< 18 days old) rats and mice could ingest proteins and colloids, as well as lipids, via vesicles; older animals could only ingest lipids and had lost the ability to ingest proteins and colloids. However, other cells which have been studied by many workers do not seem to possess such selective uptake by small vesicles. In fact, the small vesicles seem to be entered by anything which is presented to them and is small enough to fit inside their mouths. As lipids can be ingested in small vesicles it would seem highly probable that proteins could be also. Since PALAY and REVEL[3] have shown how difficult it is to be certain of the lack of vesicular uptake in these cells, it was decided to repeat some of CLARK's experiments.

*Procedure.* Adult hooded Wistar rats (4 months, 140 to 150 g) had a loop of jejunum isolated by ligaturing its ends, leaving the blood supply intact. 1 ml of 5% ferritin (N.B.C., cadmium-free) was injected in physiological saline into the loop and left for 3 h. The rats were then

[1] S. L. PALAY and L. J. KARLIN, J. biophys. biochem. Cytol. *5*, 373 (1959).
[2] J. ROSTGAARD and R. J. BARRNETT, Anat. Rec. *152*, 325 (1965).
[3] S. L. PALAY and J.-P. REVEL, *Lipid Transport* (Ed. H. C. MENG; Charles C. Thomas, Springfield, Ill. USA 1964), p. 33.
[4] S. L. CLARK, J. biophys. biochem. Cytol. *5*, 41 (1959).

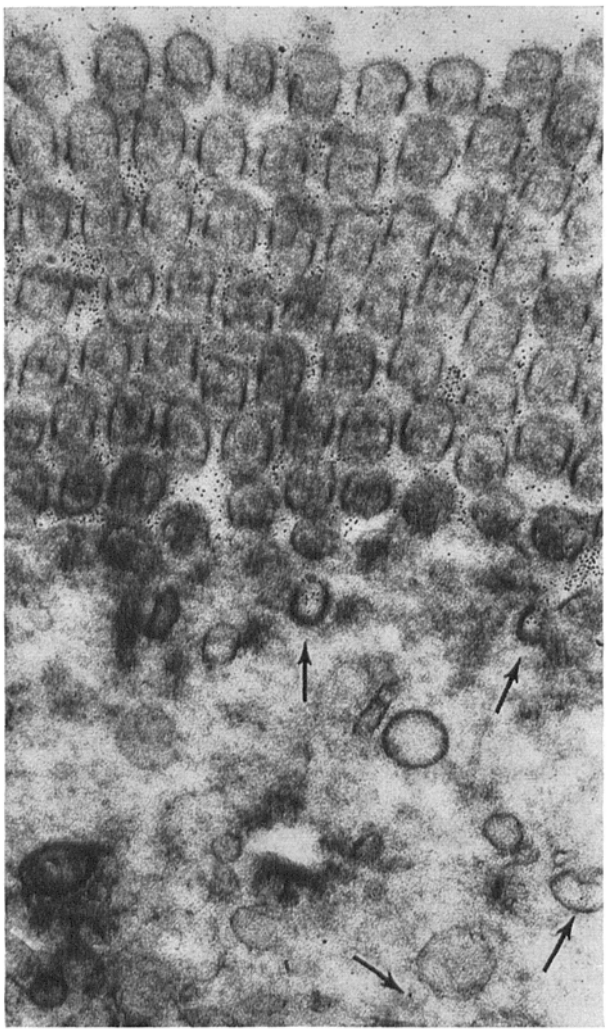

Fig. 1. Portion of an epithelial cell at the distal end of a villus. There are many molecules of ferritin between the long microvilli and a few invaginations and vesicles in the cell (arrows). × 80,000.

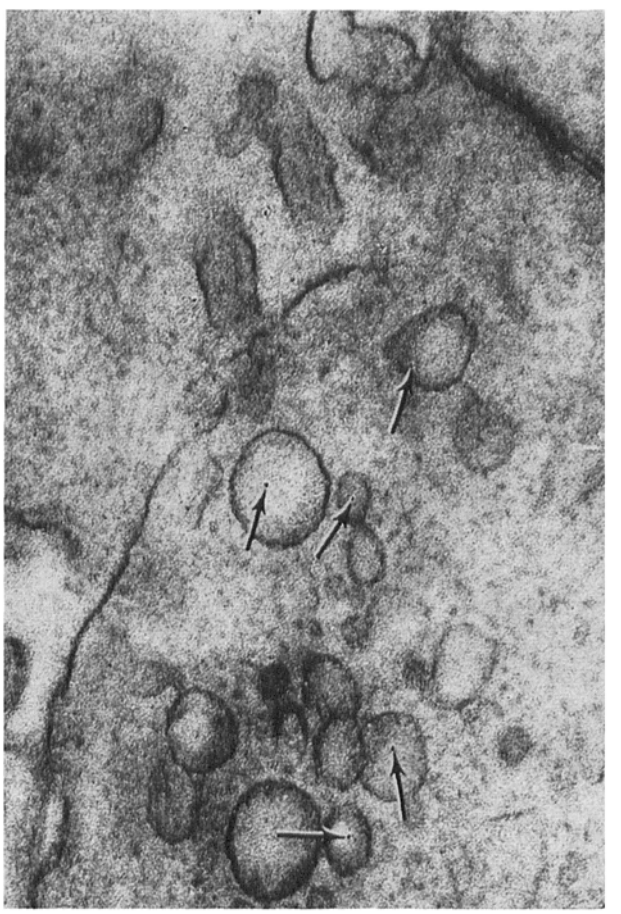

Fig. 2. Portion of 2 epithelial cells at the proximal end of a villus. There are few molecules of ferritin between the few short microvilli, but there are still some in vesicles in the cells (arrows). × 120,000.

killed and 4% buffered glutaraldehyde was used for 30 min to fix the loop. The tissue was then finely diced and post-fixed by buffered 2% osmium tetroxide for 1 h. The blocks were then dehydrated and embedded in Epon by the usual techniques. Sections were stained with lead citrate.

*Results.* The epithelial cells at the distal ends of the villi (Figure 1) had numerous particles of ferritin between their long microvilli. There were occasional small invaginations at the bases of the microvilli; some of these contained ferritin. Small vesicles in the cells also sometimes contained a few molecules of ferritin. They were also present occasionally throughout the cells' membrane systems and, extracellularly, at the base and sides of the cells.

The epithelial cells near the proximal ends of the villi (Figure 2) had much less ferritin adjacent to their small and infrequent microvilli. However, in these younger cells, there were many more small invaginations – perhaps resulting from the much less pronounced terminal web. Hence there were almost as many ferritin molecules in the invaginations and small vesicles in these cells as in the more distal ones. It should be emphasized, however, that in both of these sites the amount of ferritin in the cells was very small, much less than the amount of lipid which can enter the cells.

One could tell that the ferritin was not substantially degraded by observing the micelles, present in the molecules, under high magnification.

*Conclusions.* A small proportion of intact protein molecules can enter adult rat jejunal epithelial cells, via small vesicles, and pass through them into the body. This presumably is the basis of intestinal allergic phenomena. CLARK's[4] results showed that the numbers of molecules which are ingested in this way are much reduced in the adult as compared with the new-born animal. However, he was incorrect in saying that none can enter in this way.

The small amount of intracellular ferritin found in these experiments also supports ROSTGAARD and BARRNETT[2] when they suggest that the passage of lipid via vesicles is relatively much less than directly through the plasma membranes of the microvilli. One cannot, of course, exclude some selective mechanism which would allow much more lipid than ferritin to enter the invaginations. However, it seems unlikely. Indeed, considering the relative sizes of the particles, ferritin should enter them more often than the lipid droplets.

*Résumé.* On a étudié la pénétration de la ferritine dans les cellules épithéliales du jejunum de rats adultes. Quelques molécules ont passé dans les cellules grâce aux petites vésicules. Ce processus est, peut-être, à la base de l'allergie intestinale.

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## Mechanical Prerequisites for Effective Uterine Work[1]

The human uterus is continuously contracting. The contractions accomplish useful mechanical work only if the uterine cavity is sufficiently filled with an incompressible material.

Under normal conditions the intrauterine volume is a variable which influences the uterine performance. The correlation between the intrauterine volume and the uterine performance can be defined with a work diagram. As with the heart muscle[2], this diagram consists of curves for the resting tension, active pressure, and for volume output. The work diagram characterizes the uterine performance even under extreme conditions. In experiments it will be demonstrated that, as the volume is increased, a point is reached at which the prerequisites for effective uterine work are satisfied. Our studies were designed to compare the conditions for the effective work by the pregnant and by the non-pregnant human uteri.

The experiments were performed on a total of 7 pregnant and on 12 non-pregnant uteri obtained by hysterectomy. The uteri were continuously perfused through their normal vascular systems with oxygenated physiological solution containing: NaCl 8.0, KCl 0.4, $CaCl_2$ 0.2, $MgCl_2$ 0.1, $NaHCO_3$ 1.0, $NaH_2PO_4$ 0.05, dextrose 2.0 g aqua dest. ad 1000.0 ml.

The perfusion pressure was kept constant at 200 cm water. At 37 °C the uteri survived for as long as 40 h. A thin-walled rubber balloon was filled with water and inserted into the uterine cavity. The volume of water in the balloon was increased in steps. The intrauterine resting and active pressures were recorded alternately by an electro-mechanical transducer and the volume output was estimated gravimetrically using an electronic balance. The first parameter was measured at constant volume so that the uterus was under isometric tension, and the latter one at constant pressure, i.e. under isotonic conditions. The maximal uterine contractions were induced every 5 min by intraarterial administration of 100 µg acetycholine[3].

In non-pregnant uteri the work diagram was obtained by expansion of the residual volume. Because of the resistance of the uterine muscle to stretch, the resting tension curve was relatively steep. The isometrically measured active pressure reached its maximal value with normal filling. The isotonically determined output volumes were small; they varied from 3 to 5 ml. Additional filling increase the resting pressure, but the active pressure and the output volumes were reduced. In non-pregnant uteri the mechanical work can always be obtained with the residual filling (see Figure).

In early and midterm pregnancy, a uterus containing only the products of conception does not perform any external mechanical work. However, an experimental work diagram can be constructed if the uterine volume is further expanded. Because of increased extensibility of the uterine wall the resting tension curve is considerably flatter. The isometrical work tension reaches its maximal value only with additional volume. The volumes expelled during contractions are larger than in the non-pregnant

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[2] O. FRANK, Z. Biol. *32*, 370 (1895).

[3] K. H. MOSLER, Arch. Gynäk. *202*, 487 (1965).