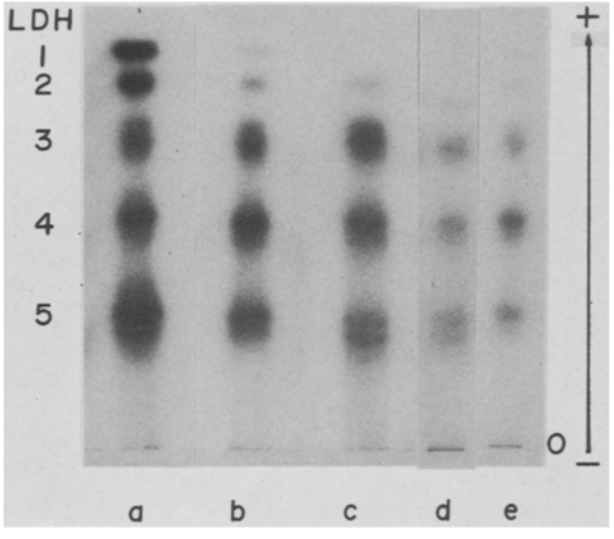
Activity of lactate dehydrogenase in different tissue of esophagus

Type of tissues	Protein (mg/ml)	Specific activity	Presence of LDH forms LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
Striated muscle of the body of the esophagus	9.4	0.41	+	+	+	+	+
Junction of striated and smooth muscle	9.4	0.27	−	+	+	+	+
Smooth muscle from the body of the esophagus	10.6	0.24	−	+	+	+	+
Smooth muscle from the GE junction	8.7	0.30	F	+	+	+	+
Esophageal mucosa	8.7	0.15	+	+	+	+	+
Diaphragmatic (striated) muscle	14.9	0.61	+	+	+	+	+

+, present; −, absent; F, faint.

supply, whereas subnuit A is associated with anaerobic glycolysis[3].

In contrast to the striated muscle area which had all 5 LDH isozymes, the smooth muscle of the body of the esophagus lacks in LDH-1 activity suggesting that the smooth muscle in this area carry on more anaerobic glycolysis than the striated muscle segment. In this respect the smooth muscle of the body of the esophagus is similar to intestinal smooth muscle[12]. The smooth muscle from the esophago-gastric junction, on the other hand, revealed some LDH-1 activity indicating that the muscle of the esophago-gastric junction carry on both anaerobic as well as aerobic metabolism. Synthesis of the anaerobic LDH fraction is stimulated by a low oxygen tension and its biological function is to maintain activity even with an excess of lactate[13]. This metabolic concept is compatible with the idea that the lower esphageal sphincter in the gastroesophageal junction is designed to maintain a constant state of activity in contrast to the body of the esophagus which has no tone under basal conditions. The present study lends support to the observations made by others[6,7] that the myogenic active tension of the lower esophageal sphincter is more dependent on aerobic metabolism as compared to the body of the esophagus.



Starch gel electrophoretic patterns of LDH isozymes in different tissues of opossum's esophagus (0, origin). Samples are *a* diaphragram striated muscle, *b* smooth muscle of GE junction muscle, *c* smooth muscle of the body of the esophagus, *d* junction of striated and smooth muscle, and *e* esophageal mucosa.

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2 M.C. Blanchaer and W.M. Van, Am. J. Physiol. *202*, 827 (1962).
3 A.C. Wilson, R.D. Cahn and N.O. Kaplan, Nature *197*, 331 (1963).
4 F.C.A. Romanul, Archs Neurol. *11*, 355 (1964).
5 F.C.A. Romanul, Archs Neurol. *11*, 369 (1964).
6 N.W. Weisbrodt, Gastroenterology *72*, A125 (1977).
7 D.C. Templeman, Gastroenterology *72*, A166 (1977).
8 J. Christensen and G.F. Lund, J. clin. Invest. *48*, 408 (1969).
9 C.R. Shaw and R. Prasad, Biochem. Genet. *4*, 297 (1970).
10 O.H. Lowry, M.J. Rosebrough and A.L. Farr, J. biol. Chem. *193*, 265 (1951).
11 H. Harris, The principles of human biochemical genetics. American Elsevier Publishing Co. Inc. New York 1971.
12 P. Keller, J. comp. Path *84*, 467 (1974).
13 D.M. Dawson, T.C. Goodfriend and N.O. Kaplan, Science *143*, 929 (1964).

Ultrastructural correlation of water reabsorption in isolated rat cauda epididymidis[1]

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Summary. Electron microscopic study was made on the water reabsorption of the epithelial cells of the rat cauda epididymidis. It was shown that when the epididymal duct was reabsorbing water at a maximal rate, widely dilated intercellular spaces were seen. It is suggested that the standing gradient model of water reabsorption first proposed for the gall bladder may also operate in the cauda epididymidis.

There is evidence that fluid reabsorption takes place in the rat cauda epididymidis in vivo[2-5] and in vitro[6,7]. Fluid reabsorption is secondary to a net transepithelial transport of sodium ions, which is an energy requiring process. Like many epididymal functions, the process is androgen-dependent, since castration in rats diminished the ability of the cauda epididymidis to reabsorb fluid[8]. In this study, an attempt has been made to show by electron microscopy the

morphological correlation of water reabsorption in the isolated duct of rat cauda epididymidis.

The rate of fluid reabsorption in isolated duct of rat cauda epididymidis was estimated using a visual method as described previously[7]. In brief, segments of cauda epididymidis were dissected out free of connective tissue and incubated in a small bath (vol. 10 ml) filled with normal Krebs bicarbonate solution maintained at 35 °C and bubbled continuously with 5% CO_2 in O_2. The lumen was flushed free of sperm and filled with normal Krebs bicarbonate solution (0.25 µl). The luminal diameter of the lumen was measured at intervals of 0.4 mm along the length of the sac under a microscope (magnification ×40) and volume of the duct calculated. A reduction in the luminal volume indicated a net reabsorption of fluid in the duct. The rate of fluid reabsorption was expressed in µl fluid reabsorbed/cm^2 of tubule in 30 min. The Krebs bicarbonate solution used had the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.56; $MgSO_4$, 1.13; NaH_2PO_4, 1.17; $NaHCO_3$, 25; glucose, 11.1. When gassed with 5% CO_2 in O_2, it had a pH of 7.4.

After various reabsorption studies, the epididymal ducts were fixed with both ends ligated, in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 for 4 h. After fixation, the ducts were trimmed and postfixed in 1% osmium tetroxide for 2 h. The tissues were dehydrated and embedded in Epon for electron microscopy.

The epididymal epithelium is made up of a single layer of epithelial cells resting on a basal lamina. Hamilton[9] has classified the cells into 4 types, viz. the principal cells, basal cells, halo cells and clear cells. Externally the epididymal duct is surrounded by a thick layer of smooth muscle coat. It is worth noting that the principal cells and clear cells participate in the formation of the junctional complexes, but basal and halo cells do not. The cells illustrated here are principal cells. The luminal surface is characterized by long microvilli which have a core of fine filaments running longitudinally through the villus and often continuing for some distance into the apical cytoplasm (figure 1). The epithelial cells are separated from each other by narrow intercellular spaces, except at the luminal border, where the cells are held by tight junctions (figure 2). Desmosomes are scattered along the lateral surface of the cells. The endoplasmic reticulum is very abundant in all regions, but rough endoplasmic reticulum is more concentrated at the basal region of the cell. The Golgi complex is large and consists of several stacks of cisternae (figure 1). Mitochondria are numerous, and they appear to be more concentrated towards the luminal pole of the cells. This lumen to base gradient of distribution may be of physiological significance[10].

In 9 experiments, the epididymal lumens were filled with normal sodium containing Krebs bicarbonate solution. Water reabsorption was measured by the technique described. In these epididymal sacs, the rate of fluid reabsorption was found to be 2.15 ± 0.09 µl/cm^2/30 min SEM. After 30 min of incubation in the bath, the ducts were removed and fixed and prepared for EM study. The epithelia from these preparations always showed widely dilated lateral intercellular spaces (figure 3). The mean intercellular space width was 0.92 µm. The adjacent epithelial cells were in contact only at the luminal borders, where they were held by tight junctions and scattered regions of desmosomal structure along the lateral surfaces. Some experiments were performed in which fluid reabsorption was abolished by filling the lumens with a sodium-free solution (NaCl substituted by choline chloride)[6]. The rate of fluid reabsorption in these ducts was reduced to 1.31 ± 0.19 µl/cm^2/30 min SEM ($n = 8$) ($p < 0.001$). Under EM the lateral intercellular space was found to be markedly reduced and the average width was 0.28 µm.

Although morphological studies of the mammalian epididymis have been undertaken[9,11,12], very little attention has been focussed on the structural-functional relationship in this transporting tissue. This work has produced evidence for an intercellular route of water transport in the rat cauda

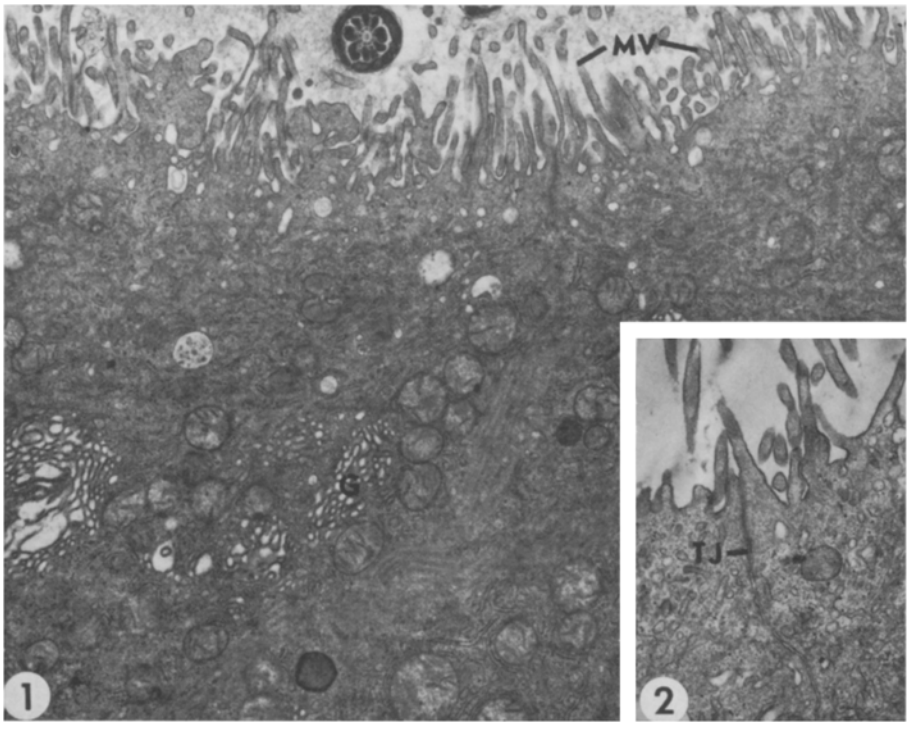

Fig. 1. EM of normal cauda epididymidis in vivo. Note the long microvilli (MV) of the principal cells at the luminal border. The Golgi (G) apparatus is large and consists of stacks of cisternae. The endoplasmic reticulum and mitochondria are prominent in the apical cytoplasm. × 4800.

Fig. 2. Higher magnification to show that the adjacent epididymal cells are held by tight junction (TJ) at the luminal border. × 8900.

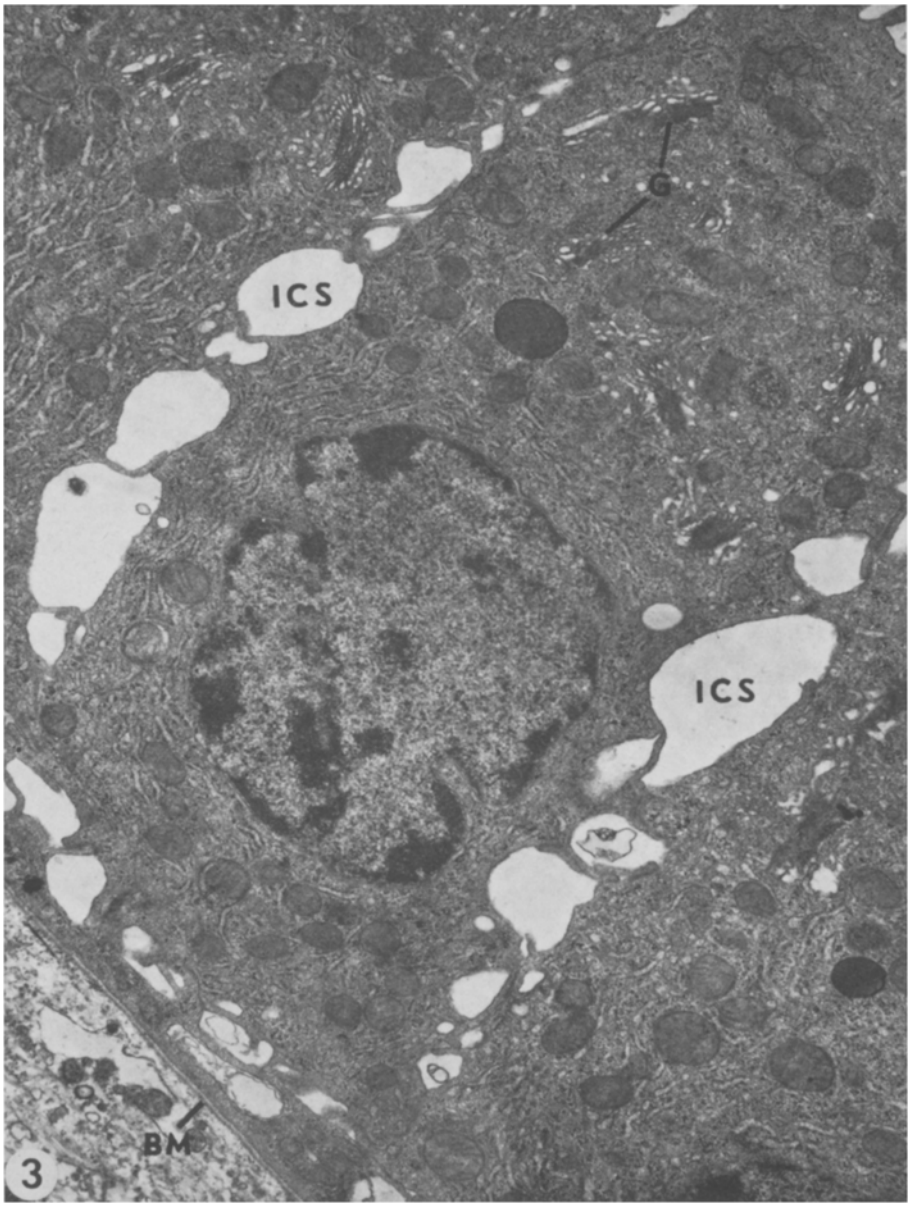

Fig. 3. Electron micrograph of epididymal duct after 30 min of incubation in Krebs bicarbonate solution. Note the markedly distended intercellular space (ICS), probably due to active water reabsorption into the intercellular space. BM, Basal lamina; G, Golgi. × 6000.

epididymidis, although we cannot preclude the possibility that reabsorption may also occur intracellularly by other mechanisms. The intercellular spaces were found to distend in the ducts known to be actively absorbing water. This observation is consistent with fluid transport in the gall bladder[13], collecting duct of the mammalian kidney[14], and amphibian epithelia[15]. It is likely that fluid reabsorption in the rat cauda epididymidis may conform to the standing gradient model as proposed by Curan and MacIntosh[16] and Diamond and Bossert[10].

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2 B. Crabo and B. Gustafsson, J. Reprod. Fert. *7*, 337 (1964).
3 N. Levine and D.J. Marsh, J. Physiol., Lond. *213*, 557 (1971).
4 P.Y.D. Wong and C.H. Yeung, Proc. int. Union Physiol. Sci. *13*, 818 (1977).
5 P.Y.D. Wong and C.H. Yeung, J. Physiol., Lond. (1977).
6 P.Y.D. Wong and C.H. Yeung, J. Reprod. Fert. *49*, 77 (1977).
7 P.Y.D. Wong and C.H. Yeung, Br. J. Pharmac. *58*, 529 (1976).
8 P.Y.D. Wong and C.H. Yeung, J. Endocr. *72*, 12P (1977).
9 D.W. Hamilton, in: Reproductive Biology, p. 268. Ed. H. Balin and S. Glasser. Excerpta Medical Foundation, Amsterdam 1972.
10 J.M. Diamond and W.H. Bossert, J. gen. Physiol. *50*, 2061 (1967).
11 D.W. Hamilton, in: Handbook of Physiology Section 7: Male Reproductive Physiology vol. 5, p. 259. Ed. D.W. Hamilton and R.O. Greep. Williams and Wilkins Co., Baltimore 1975.
12 L. Nicander and T.D. Glover, J. Anat. *114*, 347 (1973).
13 J.M. Diamond and J. Mc. D. Tormey, J. gen. Physiol. *50*, 2031 (1967).
14 J.J. Grantham, C.E. Ganote, M.B. Burg and J. Orloff, J. gen. Physiol. *41*, 562 (1969).
15 D.R. Dibona, Nature *238*, 179 (1972).
16 P.F. Curan and J.R. Macintosh, Nature, Lond. *193*, 347 (1962).