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EFFECTS OF STRETCHING AND STIRRING ON WATER AND GLUCOSE ABSORPTION BY CANINE MUCOSAL MEMBRANE

BY JUI S. LEE

From the Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455, U.S.A.

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SUMMARY

1. A 'mini' canine mucosal membrane preparation permitting simultaneous determination of water (J_v) and glucose (J_g) absorption rates, microscopic examination or micropuncture of the villi was used in this study.

2. The small membranes were more stretched than the large ones, with more than a one-fold increase in both J_v and J_g, apparently due to a change in architectural orientation between the villi and subvillous supporting tissue so as to facilitate water transport via the lymphatic system.

3. During stirring of the bathing solution, the villi in the small membranes were widely separated from each other with more to-and-fro swaying movements than in the large ones. Stirring was seen to cause up-and-down movements of the loosely suspended large membranes but not the small ones. In the small membranes stirring caused no change in J_v but an increase in J_g due to the increase in glucose concentration in the absorbate, while in the large membranes both J_v and J_g were greatly increased. It is thus considered that the increase in absorption in the large membranes caused by stirring is mainly due to the increased membrane movements promoting lymph flow.

INTRODUCTION

It is well known that the villi of the canine small intestine exhibit lengthening, shortening, and swaying movements. The role of villous movements in absorption, long an issue of interest, remains uncertain. Kokas & Ludany (1933) and Mahler, Nonnenbruch & Weiser (1932) have reported that villous movements augment the rate of glucose absorption, while Wells & Johnson (1934) found no correlation between villous activity and transport. In the intestine *in vitro*, active villous movement ceased (for literature, see Verzar & McDougall, 1936), but gas bubbling or stirring of the mucosal bathing fluid agitated the villi, causing passive rapid to-and-fro swaying movements. In the canine mucosal membrane preparation of Hakim, Lester & Lifson (1963) it was seen that gas bubbling of the bathing solution caused some rapid up-and-down movement of the whole membrane somewhat similar to spontaneous movements of the intestinal wall. It was also found that when the membrane was mounted onto the end of a glass holder or tubing of smaller diameter it was stretched, as indicated by the smaller number of villi per unit gross mucosal area; and the villi were widely separated from each other.

The present paper describes the effects of stretching the mucosal membrane and of stirring the bathing solution on water and glucose absorption by 'mini' canine mucosal membrane preparations.

METHODS

Male dogs weighing 16–25 kg, fasted for 18–24 h, were anaesthetized by an intravenous injection of sodium pentobarbitone (30 mg/kg). Over a 5 h period about thirty small pieces of mucosal membrane (without muscular coat) were removed from four to five short consecutive segments of a 40 cm section of upper jejunum the upper end of which was about 10 cm from the ligament of Treitz. Two sizes of membranes were used. The small and large membranes were mounted onto the end of glass tubing of outer diameter 3 mm (inner diameter $\approx$ 1·8 mm) or outer diameter 10 mm (inner diameter $\approx$ 8 mm) as described previously (Lee, 1972). For small membranes the glass tubing (4 cm long) was attached to and passed through the centre of a glass dish (outer diameter $\approx$ 62 mm; depth, 18 mm) containing about 45 ml of Krebs–Ringer solution. For large membranes the other end of the glass tubing was closed with calibrated graduations for measuring the volume of the absorbate. The surface of the membranes was about 5 mm below the surface of the fluid to minimize the hydrostatic effect. The dish with the small or large membrane was placed in a warm double-jacketed glass chamber. The total volume of the mucosal fluid was about 200 ml, the bulk of which was in a mucosal fluid reservoir about 20 cm above the dish. It was gassed with 5 % carbon dioxide in oxygen, and flowed under gravity at a rate of about 30 ml/min via a heat exchanger into the dish; from there it overflowed into the warm chamber, and was then returned to the reservoir by a pump. This set-up permits prolonged microscopic observation, micropuncture or photography of the villi, because the villi were not disturbed by gas bubbling of the mucosal fluid. When stirring of the mucosal fluid was required, a gas bubbler or a glass stirrer was placed in the dish. The glass stirrer was made of a glass rod (diameter 3 mm) with a bent end 7 mm in length, driven by a motor with a frequency of 1600 per minute. The mucosal bathing fluid was Krebs–Ringer solution containing (mM): NaCl, 116; KCl, 5·8; $NaHCO_3$, 25; $CaCl_2$, 1·8; $MgSO_4$, 1·2; NaH_2PO_4, 1·2; glucose, 27·7. The osmolarity was 300 mosmol. The solution was saturated with 5 % CO_2/95 % O_2 (pH 7·55 at 37 °C) and maintained at 38 °C. The serosal side was moist air without any bathing fluid.

In the small membranes the absorbate remained in the upper end of the glass tubing, and its volume was estimated by the length of fluid column multiplied by its cross-sectional area. In the large membranes the absorbate dripped to the bottom of the glass tubing, where its volume could be easily read from the graduations. The absorption period was 40 min, but the rate of water absorption (J_v) was expressed as microlitres per square centimetre of gross mucosal area per hour (μl/cm^2.h), and that of glucose absorption (J_g) as micromoles per square centimetre per hour (μmol/cm^2.h). For each type of experiment the control rate was obtained from membranes of the same segment of the intestine. In most cases equal numbers of control and experimental membranes were used. Glucose in the absorbate and mucosal fluid was analysed by the fluorometric method of Lowry, Passonneau, Hasselberger & Schulz (1964).

It should be noted that the rate of serosal appearance of glucose is an approximate measure of its net rate of transport because of the possibility that some metabolic changes affect the concentration of glucose in the absorbate.

RESULTS

Effect of stretching on J_v, J_g, and glucose concentration ratio between absorbate and mucosa (A/M)

It was found that when a mucosal membrane was mounted onto the end of a length of small-diameter glass tubing it was automatically stretched to a greater degree than when it was mounted onto the end of a piece of larger-diameter tubing, as indicated by the smaller number of villi per square centimetre of gross mucosal area in the former. The number of the villi was counted from a photomicrograph of the

membrane. As shown in Table 1, there were 446, 806 and 870 villi per square centimetre of gross mucosal area in the 3, 10 and 28 mm diameter membranes, respectively. J_v of the largest membranes (28 mm) was determined by Hakim *et al.* (1963) under practically identical conditions. J_v of the smallest membranes (519 μl/cm^2.h) was 253 % that of the largest membranes (205 μl/cm^2.h). A/M of the small membranes (1·4) was also slightly but significantly ($P<0.01$) greater than that of the large membranes (1·2).

TABLE 1. Comparison of number of villi, water absorption rate (J_v), glucose absorption rate (J_g), and absorbate to mucosal glucose concentration ratio (A/M) of small and large membranes

Membrane diameter (mm)	Number of villi (cm^{-2})	J_v (μl/cm^2.h)	J_v (μl per villus per hour)	J_g (μmol/cm^2.h)	A/M
3	446±21 (33)	519±34	1·2	19·7±1·3	1·4±0·1
10	806±24 (28)	316±12	0·4	11·9±0·5	1·2±0·1
28	870*	205±96†	0·2		

Values are means ± S.E. of the mean. Numbers in parentheses indicate number of membranes. P values between differences in J_v and J_g, < 0·01; in A/M, < 0·02. Mucosal glucose concentration, 27·7 mM. Mucosal fluid was gassed with 5 % CO_2/95 % O_2.

* Hakim & Lifson (1969).

† Hakim *et al.* (1963).

The higher rate of water absorption in the small membranes, where there was more stretching, than that in the large membranes, was not due to any mechanical leak of mucosal fluid to the serosal side. When mucosal fluid was sodium-free Krebs–Ringer solution (sodium chloride replaced by mannitol, lithium chloride or potassium chloride), of twenty-nine small membranes was zero. And when mucosal fluid contained 0·1 % Evans Blue dye, no appearance of dye in the absorbate was ever observed.

Effect of stirring of the villi on J_v, J_g and A/M

When the mucosal fluid was stirred either by gas bubbling or by a glass-rod stirrer, the villi were observed to show rapid swaying movements. In the small membranes all the villi exhibited such movement. However, in the large membranes the villi were closely packed, and those in the central portion of the membrane showed much less movement even at a high rate of stirring of the bathing fluid. Four series of experiments were carried out, and the results are presented in Table 2. The membranes of each series were obtained from the same segment of the intestine but those of different series were obtained from different segments or different dogs. Thus, J_v, J_g and A/M are not comparable between the results of different series. It can be seen that the effects of stirring were similar whether stirring was by gas bubbling or by a mechanical stirrer. J_v of large membranes was approximately doubled by stirring of the bathing solution but J_v of the small membranes was not changed at all (series II). J_g of both small and large membranes was significantly increased by stirring. A/M of both small and large membranes was about 1 (0·9–1·0) without stirring, and increased on stirring to 1·5–1·7 or 1·1–1·3 in the small and large membranes, respectively.

Effect of stirring on fluid exchange rate between intervillous space and mucosal fluid

The rate of exchange of the fluid between the intervillous space and the mucosal fluid was determined by the rate of disappearance of a small drop of Lissamine Green (0·6% in saline) applied by a micropipette ($\approx 10\,\mu$m in diameter) into the space between the bases of the villi of small and large membranes. It was found that in the large membranes the green colour of the dye became undetectable in 0·5 min with stirring, but in 2 min without stirring. In the small membranes (in which the villi

TABLE 2. Effect of stirring on water absorption rate (J_v), glucose absorption rate (J_g) and A/M

Series	Mucosal membrane	Stirring of villi	J_v (μl/cm^2.h)	J_g (μmol/cm^2.h)	A/M
I	Small	By gas bubbling (6)	323±57	14·9±2·6	1·7±0·0
		By stirrer (6)	320±25	13·4±1·0	1·5±0·1
II	Small	By gas bubbling (19)	628±60	27·3±2·6	1·6±0·1
		No stirring (21)	633±61	17·9±1·7	1·0±0·1
III	Large	By gas bubbling (6)	389±40	11·9±1·2	1·1±0·1
		By stirrer (6)	389±50	11·9±1·5	1·1±0·2
IV	Large	By gas bubbling (10)	309±42	10·9±1·5	1·3±0·1
		No stirring (10)	150±15	3·8±0·4	0·9±0·1

Values are means ± S.E. of the mean. Numbers in parentheses indicate number of membranes. Mucosal glucose concentration, 27·7 mM.

were widely separated from each other) dye disappeared in 3–4 s with stirring and in 10–20 s without stirring. The results demonstrate clearly that the effect of stirring may increase the rate of fluid exchange between the intervillous space and bathing fluid. In the small membranes the fluid exchange rate was some 3-fold greater without stirring than that in the large membranes with stirring.

Effect of stirring on mucus removal

For the small membranes, the mucus layer can be mostly removed by gentle flushing with the bathing fluid while the membrane is being set up; but in the large membranes, because of the crowding of the villi, some mucus cannot be removed even with vigorous stirring of the bathing fluid whether by gas bubbling or by use of a glass stirrer, or by flushing the villi with a jet of the bathing solution from a syringe. The presence of an appreciable amount of mucus on the surface of the villi was made evident by the immediate plugging of a micropipette tip ($\approx$ 10 μm in diameter) when the latter was in gentle contact with the villi.

DISCUSSION

A 'mini' intestinal mucosal membrane preparation is here described which can be used for studying water and solute transport, and for microscopic observation or micropuncture of the villi. The small size of the membrane (3 mm in diameter) may prove suitable for transport or morphological studies of the villi of human biopsy specimens. Since the serosal side was moist air without any bathing fluid, it was

possible to determine the net rate of absorption of solutes and water with ease. In a similar set-up it has been found that J_v was the same whether the serosal side was moist air, light mineral oil or Krebs–Ringer solution.

Effect of stretching on J_v

Mounting a mucosal membrane onto the end of a small holder stretches it more than mounting it on a large holder, as indicated by the smaller number of villi per unit area (Table 1). Both J_v and J_g of small membranes are roughly double the values of larger membranes, despite the fact that villus density was greater in the larger membranes. J_v (1·2 μl per villus per hour) of a single villus of the smallest membranes was 6-fold greater than that (0·2 μl per villus per hour) of the largest ones. This seems most likely to be associated with some non-epithelial factors resulting from increased stretching of the membrane; it is unlikely to be due to the increase in the absorbing area of the villus because there were no morphological differences between the villi in the small and large membranes by direct microscopic observation. Possibly stretching of the membrane may alter the orientation of the lymphatics and blood vessels in the subvillous facia-like layer so as to facilitate lymph flow; this accounts for most of the fluid transported to the serosal side in rat and canine intestinal preparations *in vitro* (Lee, 1967, 1969); an obstruction of lymph flow could reduce J_v by more than 80% (Lee, 1963). Further experimentation is certainly needed to elucidate this point.

The following characteristics of the small and large membrane may also contribute to the increase in J_v and J_g. (1) In the small membranes, which were more stretched, the villi were widely separated from each other, and more of the mucus can be removed during the setting up of the experiment by flushing the membrane with the bathing solution; in the large membranes most of the mucus around the villi cannot be removed. (2) The fluid exchange rate between intervillous space and bathing solution was several-fold greater in the small than in the large membranes whether the bathing solution was stirred or not. (3) As has been demonstrated previously (Lee, 1969, 1977), cell extrusion from the villous tips proceeded even under *in vitro* conditions. In the large membranes most of the extruded cells that accumulate around the villous tips can not be removed even by stirring or by gas bubbling.

Effect of stirring on J_v

The results demonstrate that stirring of the bathing solution either by gas bubbling or by a mechanical stirrer doubled J_v in the large membranes but caused no change at all in the small membranes. One possible explanation for this may be proposed as follows. Because the large membranes were loosely suspended over the glass holder, they exhibited rapid up-and-down movements in the turbulence of the bathing solution caused by stirring. No such movement was seen to occur in the small membranes because they were maximally stretched over the end of a length of small-diameter glass tubing (outer diameter 3 mm). Movements of the subvillous facia-like layer of the membrane may facilitate lymph flow to the serosal side. This is in agreement with a previous observation (Lee, 1967) that in the rat intestinal preparation *in vitro*, intestinal motility doubled J_v by promoting lymph flow.

Stirring would certainly accelerate the removal of the mucus around the villi, thin

the unstirred water layer (Dietschy, Salle & Wilson, 1971), increase the rate of fluid exchange between the intervillous space and the bathing solution, and induce passive movements of the villi in both small and large membranes. Although all these factors may affect J_v, it is difficult to explain why stirring caused no change in J_v of the small membranes. A plausible explanation is that in the small membranes, which were subjected to a greater stretch, the rate of water transport by the lymphatic route might have reached its maximal capacity even without stirring of the bathing solution. Hence, the effect of stirring on the increase in J_v of the loosely suspended large membranes could be mainly due to the increased membrane movements promoting lymph flow. If this is the case, the results seem to suggest that neither the unstirred water or mucus layer around the villi nor the fluid exchange rate between intervillous space and bathing fluid is essential in water absorption under many circumstances. This is also supported by the finding that the fluid is almost entirely absorbed from the villous tips as demonstrated by Lee (1969) using a micromanipulative technique. Briefly, when the villous tip was occluded, no absorbate appeared in the villous central lacteal (initial lymphatic) as lymph, but when the villous lower region was occluded the fluid was rapidly absorbed into the central lacteal. Furthermore, Kinter & Wilson (1965) have shown by an autoradiographic method that sugar and amino acids are mostly absorbed from the villous tips. These authors have also demonstrated that the low rate of absorption of the epithelial cells at the basal region is apparently not due to poor contact with the bathing fluid, since [^{14}C]inulin and other radioactive compounds may reach the bases of the villi in less than 1 min. If both water and solute are absorbed chiefly from the villous tips, it would be reasonable to believe that the surface area of the whole villus, the unstirred water or mucus layer around the villi, or fluid exchange rate between bathing fluid and intervillous space may not exert a significant effect on absorption.

Effect of stirring on J_g and A/M

As shown in Table 2, J_g was increased by stirring in both small and large membranes. In the large membranes the increase in J_g was related to a large increase in J_v and a small increase in glucose concentration in the absorbate, while in the small membranes with no change in J_v the increase in J_g was entirely due to the increase in the absorbate glucose concentration, as indicated by a large increase in the A/M ratio (Table 2). The question arises as to why stirring increases glucose concentration in the absorbate or A/M. This is difficult to explain with certainty. Perhaps the increase in glucose concentration in the absorbate may be related to membrane movements and villous movements induced by stirring. Without stirring, A/M of the small and large membranes was 1·0 and 0·9, respectively. With stirring, A/M of the small membranes increased to 1·5–1·7 and that of the large membranes to 1·1–1·3. The greater increase in A/M in the small membranes is apparently related to the more vigorous villous movements induced by stirring. The increase in A/M in the large membranes may be chiefly due to the movement of the whole membrane. This is supported by the observation that in the rat jejunal preparation *in vitro*, intestinal motility increased not only J_v but also glucose concentration in the absorbate collected as lymph (Lee, 1967). Furthermore, Gibaldi & Grundhofer (1972), using an everted sac of rat intestine, found no effect on the transfer of either aniline or

salicylamide by vigorous stirring of the mucosal solution. This may be explained by the fact that stirring may not cause appreciable movement of the thick intestinal wall. The villi of the rat intestine are much shorter (about 300 μm) than those of the canine intestine (about 1000 μm), and were never observed to show any movement even during stirring of the bathing solution (unpublished observations). Therefore, it appears that both villous movement and membrane movement may be involved in the increase in glucose concentration in the absorbate, although the underlying mechanisms are far from clear.

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