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ACTIVE F^- TRANSPORT: SPECIES AND AGE EFFECTS WITH RODENT INTESTINE, *IN VITRO*

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SUMMARY

Active F^- transport in a secretory direction across everted sacs of intestinal sections from four species of rodents was observed. Both the F^- specific electrode and microdiffusion methods of fluoride analysis were employed.

1. Net accumulation of F^- in the mucosal fluid of ileal sacs was observed in three of the four species studied. Sacs from gerbil and hamster exhibited the greater rates of active F^- transport.

2. Active F^- transport with gerbil ileum exhibited a unilateral dependency on the presence of Cl^- in the mucosal compartment, as had previously been reported for rat, suggesting that the mechanism was similar.

3. A decrease in active F^- transport was observed within the age range of 4–18 weeks with both rat and gerbil ileal sacs.

4. F^- secretion by active transport was also observed in sacs of jejunum and colon from gerbil, as had previously been reported for rat, confirming that the process occurs along the entire length of rodent intestine.

INTRODUCTION

Active F^- transport in a secretory direction across rat intestine has been reported previously[1,2]. When everted sacs are incubated with equal initial F^- concentrations in both fluid compartments a net accumulation of F^- is observed in the mucosal compartment. An investigation of the intestine of other rodents was undertaken to determine the presence, magnitude and characteristics of active F^- transport.

Studies of F^- metabolism have demonstrated changes with age[3]. Young animals store up to half of ingested F^- in mineralized tissues[4,5]. The remaining F^- is excreted in urine and feces. Older animals show decreased storage and increased excretion of F^- (refs. 6 and 7). Active F^- transport across epithelial membranes is likely to be one of the components of F^- metabolism, and could have a regulatory influence if changes occurred with age. Studies were carried out with rodents of increasing ages to determine if an alteration in the rate of active F^- transport in the intestine would be observed.

Abbreviation: PD, potential difference.

MATERIALS AND METHODS

For the comparison of active F^- transport rates between rodent species male guinea pigs, rats, hamsters, and gerbils 8–10 weeks of age were employed. Cannulated everted sacs[8] of 10 cm length were prepared from sections of terminal ileum. The tissue was incubated in Krebs bicarbonate saline solution[9] gassed with O_2–CO_2 (95:5, v/v) containing 18 mM glucose and 0.5 mM Ca^{2+}, for 1 h at 37°. An equal initial F^- concentration of 2.0 mM was present in both the 5-ml mucosal fluid and the 2.0-ml serosal fluid. The final fluid volumes were obtained by weight, and the tissue was dried for 12 h at 100° in a tared weighing bottle following lipid extraction with a chloroform–methanol solution (2:1, v/v) for 2 h (ref. 10). The amount of F^- present in each compartment was calculated from the F^- concentration and the fluid volume. The results are expressed as the change in F^- content in each fluid compartment per kg of dry tissue weight per min (μmoles/kg/min). Additional details of the procedures and calculations are included in other publications[1,2].

The activity of F^- was measured directly with a F^- specific electrode (Orion 49-09). A sleeve junction calomel reference electrode and a specific ion meter (Orion 401) were used with this F^- electrode. The pH of the medium was buffered at 7.2, furnishing insufficient OH^- to cause interference at the F^- levels employed. Consequently, separate buffering of the samples as suggested by McCANN[11] was not carried out. Standard solutions were made up with NaF and distilled water. Samples of the initial medium and the final fluid compartments were analyzed. This technic was employed in the comparative study.

For the age study and the measurement of active F^- transport along the entire intestine of gerbil the Wharton microdiffusion technic of F^- analysis[12,13] was employed. The results were obtained at 590 nm with a 4-cm light-path cuvette in a single-beam spectrophotometer (Zeiss PMQII).

The electrical potential difference (PD) measurements were performed by placing salt bridges of 3% agar and saturated KCl in PE160 polyethylene tubing into the mucosal fluid and along the cannula into the serosal fluid in the sac. The bridges were connected to paired calomel reference electrodes and a specific ion meter (Orion 401).

The age study was carried out with animals which were furnished at bi-weekly intervals from their breeder. The original litters were isolated and the animals shipped on a schedule so that each group was maintained for a similar period in the shipment facilities and the animal quarters of the investigator. The incubations were with equal initial F^- concentrations on each side of the tissue. Everted sacs of ileum from rats and gerbils ranging from 4 to 18 weeks of age were studied. F^- analysis was performed by the method of WHARTON[12,13].

A unique property of F^- transport by rat small intestine appears to be a dependency on the presence of Cl^- in the mucosal fluid compartment[2,14,15]. Incubation of gerbil ileum with equimolar substitution of SO_4^{2-} for Cl^- was carried out, to determine if the gerbil ileum would display a similar response.

Because the active transport process had been observed along the entire intestine of rat[1,2], the rates of active F^- transport were observed with gerbil jejunum, ileum and colon. An initial F^- concentration of 2.0 mM was employed on both sides of the tissue. F^- analysis was performed by the method of WHARTON.

RESULTS

The net F^- movements with sacs of terminal ileum from the four rodents studied are shown in Fig. 1. Guinea pig exhibits a loss of F^- from the mucosal fluid which is approx. 1/3 of the F^- loss from the serosal fluid. Sacs from rat display a modest net accumulation of F^- in the mucosal fluid, and a loss of F^- from the serosal fluid which is similar to the serosal fluid loss with sacs from guinea pig. A mucosal accumulation and serosal loss of F^- not significantly different than with rat ($P > 0.05$) are seen with hamster. Gerbil exhibits net F^- movements similar to those observed with hamster, and significantly greater than those with rat (P's < 0.005).

The tissue exhibiting the highest active F^- transport rate, gerbil ileum, was compared with the transport rate of rat ileum, employing the method of WHARTON[12,13] for F^- analysis. The microdiffusion method measures the total amount of F^- present, rather than F^- activity. The net F^- movements in all cases are greater than those observed using the F^- electrode. The F^- transport activity of gerbil ileum under these conditions appears to be approximately double that observed with rat (mucosal: 145.0 $\pm$ 14.2 μmoles/kg per min (ref. 17) *vs.* 72.1 $\pm$ 15.0 μmoles/kg per min (ref. 5); serosal: —181.8 $\pm$ 1.8 μmoles/kg per min (ref. 17) *vs.* —75.4 $\pm$ 7.4 μmoles/kg per min (ref. 5)).

Since movement against an electro-chemical potential is one of the criteria for active transport, the PD was measured across gerbil ileum. The mucosal compartment was found to be negative with respect to the serosal compartment. The addition of 2.0 mM F^- does not appear to significantly reduce the electrical potential difference (0 mM F^-: 6.4 $\pm$ 0.2 mV (ref. 4); 2.0 mM F^-: 5.9 $\pm$ 0.6 mV (ref. 6)). These potential differences are in the same range as those observed with rat ileum[1,16,17].

In Table I are reported the net F^- movements measured by the microdiffusion method with the substitution of SO_4^{2-} for Cl^- either on the mucosal side or the serosal side. With SO_4^{2-} substitution on the serosal side, the net F^- movements which occurred in both serosal and mucosal compartments were similar to those observed with the control. However, with the substitution of SO_4^{2-} for Cl^- in the mucosal

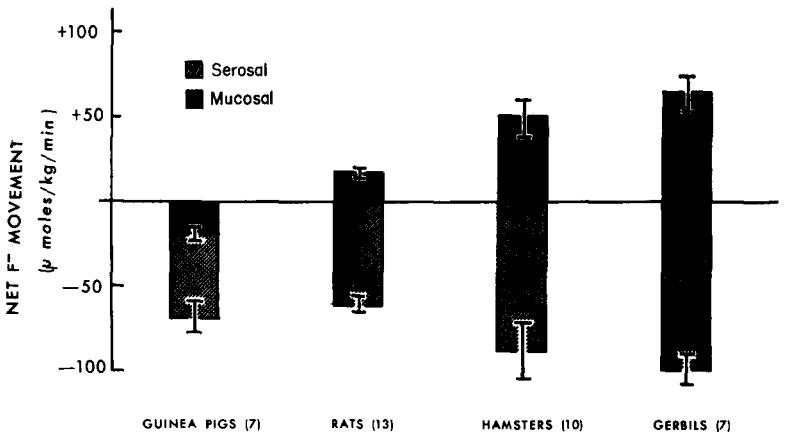

Fig. 1. Active F^- transport by rodent ileum; everted sacs with equal F^- concentrations initially on both sides of the tissue were incubated for 1 h at 37°. The S.E. of the mean is given, the number of animals is in parentheses.

TABLE I

Cl^- SUBSTITUTION WITH GERBIL ILEUM

Everted sacs were incubated with 2.0 mM F^- initially on both sides of the tissue for 1 h at 37°. Equimolar substitution of SO_4^{2-} for Cl^- was carried out on one side only. Animals were 8 weeks old. The S.E. is given.

Medium	*Number of animals*	*Net F^- movement (μmoles/kg per min)*	
		Mucosal	*Serosal*
Control	9	+160.7 ± 17.4	−188.5 ± 18.8
SO_4^{2-} for Cl^-, serosal side	4	+163.2 ± 56.6	−193.5 ± 20.2
SO_4^{2-} for Cl^-, mucosal side	5	− 62.8 ± 15.6	+ 1.3 ± 8.5

TABLE II

ACTIVE F^- TRANSPORT WITH GERBIL INTESTINE

Everted sacs were incubated for 1 h at 37°. Equal initial concentrations of 2.0 mM F^- were placed on both sides of the tissue. The S.E. is given.

Region of intestine	*Number of animals*	*Net F^- movement (μmoles/kg per min)*	
		Mucosal	*Serosal*
Jejunum	8	+ 21.7 ± 5.7	−106.6 ± 10.2
Ileum	9	+160.7 ± 17.4	−188.5 ± 18.8
Colon	5	+ 53.2 ± 23.6	−187.7 ± 24.1

fluid compartment, a net F^- loss is observed in the mucosal fluid, and a significant change is not observed in the total amount of F^- within the serosal compartment.

In Table II are illustrated the net F^- movements with everted sacs of gerbil

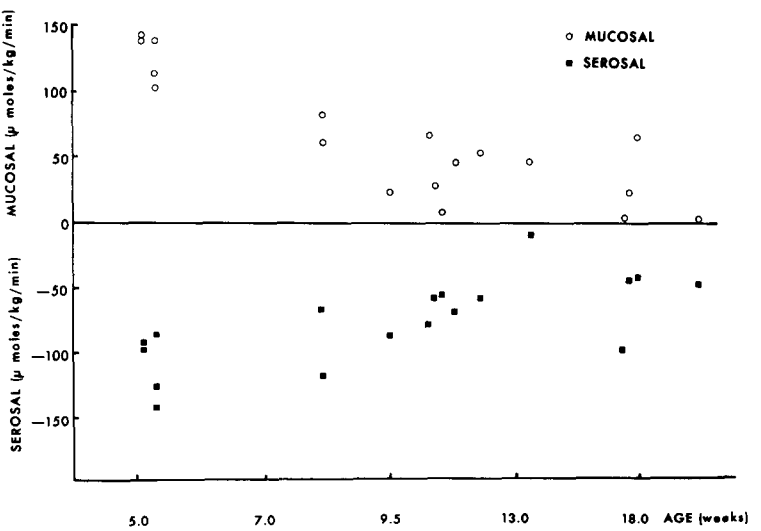

Fig. 2. Effects of age on net F^- movement with rat ileum; everted sacs were incubated for 1 h at 37°. Each pair of values represents one animal.

jejunum, ileum and colon. A F^- accumulation can be seen in the mucosal compartment with all tissues. With gerbil the colon appears more active than the jejunum.

Fig. 2 illustrates the variation in net F^- movements observed with rat ileum from animals in the age range from 4 to 18 weeks. A decrease is observed in the net F^- movements in both serosal and mucosal compartments with increasing age. At 18 weeks active F^- transport at a greatly diminished rate is still found. Fig. 3 displays a similar age study employing gerbil ileum. Again a decrease in the net F^- movements in both the mucosal and the serosal compartments is observed with increasing age. In the cases of rat and gerbil the final rate is significantly different from the initial rate ($P > 0.02$; $P > 0.005$).

DISCUSSION

A variation in the rates of active F^- transport across everted sacs of terminal ileum was observed with the four rodent species studied. Guinea pig did not demonstrate a net F^- accumulation in the mucosal fluid. However, in previous studies[1,2,18] with rat where the active F^- transport process was inhibited equal F^- losses from the fluid compartments on both sides into the tissue were observed. A minimal active F^- transport activity may have occurred with guinea pig ileum.

The results with F^- activity measurements confirm that a true net F^- accumulation occurred against its electrochemical gradient. The F^- electrode as employed only measured F^- that was in the free ionic state. Previous F^- analyses[1,2,14,15,18] were of F^- concentration, and included bound and complexed F^-.

The rates of F^- accumulation observed with sacs of gerbil jejunum, ileum and colon confirm the presence of active F^- transport in a secretory direction along the

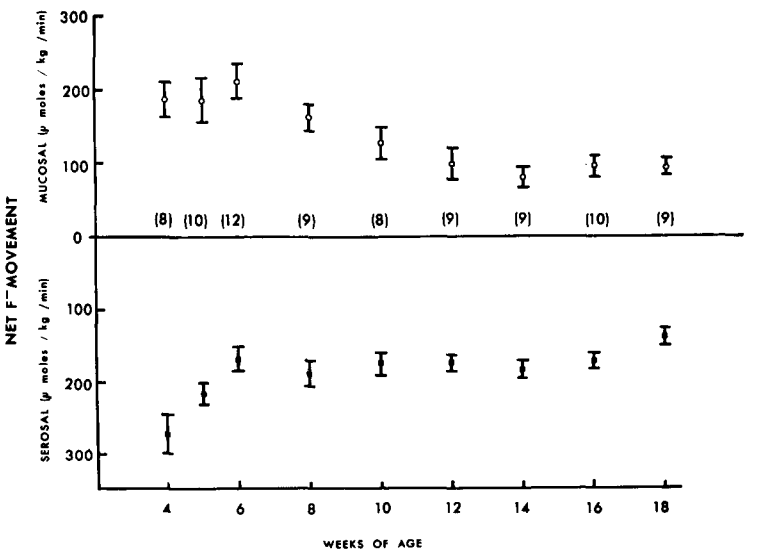

Fig. 3. Effects of age on net F^- movement with gerbil ileum; everted sacs were incubated for 1 h at 37°. The S.E. of the mean is given, and the number of animals is in parentheses.

entire length of the intestine. The activity found with sacs of gerbil colon suggest the previous conclusion[1,2] of active F^- transport with rat colon sacs was correct.

The decrease in the rates of active F^- transport observed with advancing age may be related to the alterations reported in the F^- metabolism with age[3–7]. The nature of this relationship requires further investigation. In particular, the relative amounts of F^- excretion by the urine and feces should be studied. With advancing age the fecal route may decrease in importance. This would offer an explanation for the low fecal excretion found by SPENCER and co-workers[19,20] in older patients.

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