and Trillwood and were unable to detect any inhibitory effects of F on cultures of HeLa and human esophageal cells, in concentrations of up to 10 ppm. Berry and Trillwood(11) have criticized the findings of Armstrong *et al,* but the latter workers have repeated and confirmed their earlier findings(12) under conditions which have met Berry and Trillwood's criticisms.

*Summary.* A group of rats were placed on a high fluoride diet for 28 days. Portions of their scapulae were then removed and devitalized, and in the meantime the animals were put on a diet of normal F content. A control group of animals were similarly treated, but did not get a high F diet. The scapulae were implanted subcutaneously into the respective donor animals and removed after 2 or 4 weeks, and examined histologically and chemically. Despite an F content 7 to 10 times higher in the experimental compared to the control animals' scapulae, the tissue responses were identical: a fibrous capsule formed around the implants and giant cells appeared and began to resorb the bone. No necrosis or degeneration was seen. It is concluded that, under these conditions, F does not exert any toxic action.

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## Transmural Potentials Across the Small and Large Intestine of the Bullfrog, *Rana catesbeiana.** (30957)

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In contrast to the large number of studies on mammalian intestine(1,2,3) no systematic study of the electrical characteristics of the small intestine of the bullfrog has been published. This report describes results obtained *in vitro* in a study of the electrical potential difference (PD) across both the small and large intestine of the bullfrog *(Rana catesbeiana)* and its modification by the presence of sugars and amino acids in the bathing fluids. A preliminary report of the results has been made(4).

*Materials and methods.* Bullfrogs were obtained from September to July from commercial suppliers. They were stored at 8-12°C for approximately 2 weeks in glass containers containing distilled water that was frequently replaced. Such storage minimizes seasonal changes. After pithing the frog, about 15 cm of the proximal small intestine was removed distal to the entry of the bile duct, everted

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over a glass rod and segments (5 cm) made by closing one end with a ligature and tying the other over a glass cannula. A glass weight attached to the former ligature facilitated suspension in 20-50 ml of either buffered chloride or buffered sulphate saline(5). Large intestine was also removed, everted and similarly mounted. Transmural potentials were recorded by agar salt bridges (polyethylene tubing) in contact with the mucosal and serosal fluids. In the experiments using buffered chloride saline the bridges were made with 3% agar in 1 M KCl while in those using sulphate saline greater stability was obtained if the bridges were made with 3% agar in the sulphate saline. The bridges led to 2 matched calomel cells (Radiometer) connected to a recording potentiometer (Heath) through a Beckman pH meter. The apparatus for recording the PD was found to be very stable and had little drift even after hours of continuous running. Any asymmetry in the circuit (cells and bridges were always matched so that this was below 1 mv) was routinely measured initially and subsequently every 30 minutes by placing both bridges in the mucosal fluid and recording the resultant PD. This PD varied only very slightly during the day. The transmural PD reported here have been corrected for this PD. The incubating fluids contained 5.6 mM glucose unless otherwise stated and were gassed with either 95% oxygen, 5% carbon dioxide (aerobic) or 95% nitrogen, 5% carbon dioxide (anoxic) saturated with water vapor at room temperature.

*Results.* With chloride saline, the PD across the isolated small intestine immediately after setting up was 1.41 ± 0.22 mv (17)‡ serosa positive to mucosa. After 30 minutes this decreased to 0.30 ± 0.17 mv (10) and then slowly increased (Fig. 1). Even after 5 hours incubation, the PD was not greater than 2 mv. Because of the initial small PD in chloride saline, recording the effects of inhibitors and other conditions was technically difficult. Significantly higher potentials were obtained, however, when the incubating medium was changed to a buffered sulphate saline. Typical results are shown in Fig. 1. Both initial (3.7 ± 0.75 mv(6)) and subsequent PD were always higher.

‡ Mean ± S.E. Figures in brackets = no. of experiments.

*Effects of metabolic inhibitors.* In chloride saline, addition of 1 mM dinitrophenol to the mucosal solution caused a rapid depression of PD, but if $5 \times 10^{-4}$ M dinitrophenol was used a small but stable increase was observed. In sulphate saline, 1 mM dinitrophenol caused only a small depression of PD; even after 50 minutes of contact with the drug the PD of most segments was well maintained. Iodoacetamide (1 mM) when added to the mucosal solution in sulphate saline had a biphasic action. After a transient increase in the PD (0.5 mv) lasting for some 5 minutes, there was a slow decrease but if iodoacetamide was added to the serosal solution at this point, a rapid depression of PD occurred. Stronger concentrations of iodoacetamide (10 mM) when applied to the mucosal solution, also had a biphasic action but after the small increase, there was a rapid fall in PD to near zero. Neither fluoroacetate (2 mM) nor ouabain ($5 \times 10^{-4}$ M) greatly affected the PD when applied to the mucosal solution, but ouabain rapidly depressed the PD when applied to the serosal solution. Sodium fluoride (10 mM) did not depress the PD when added to the mucosal fluid in sulphate saline but increasing the concentration to 50 mM caused a rapid fall to zero.

*Effects of sugars.* The effect of the presence or absence of sugars on the PD was tested in experiments similar to that shown in Fig. 2. Everted segments of small intestine were incubated in sulphate saline initially containing no glucose. Even in the absence of exogenous metabolic substrate most segments possessed a measurable PD, serosa positive to mucosa. Addition of fructose (5.6 mM) to the mucosal solution caused a small decrease in the PD but addition of 5.6 mM glucose caused a rapid increase in the PD. Phlorizin ($5 \times 10^{-4}$ M) added to the mucosal solution immediately depressed this glucose dependent potential. A repeated dose had no further effect. The inhibition was reversible on washing out the phlorizin. In similar experiments addition of 5.6 mM glucose to the serosal fluid (whether the intestine was everted or not) did not cause any

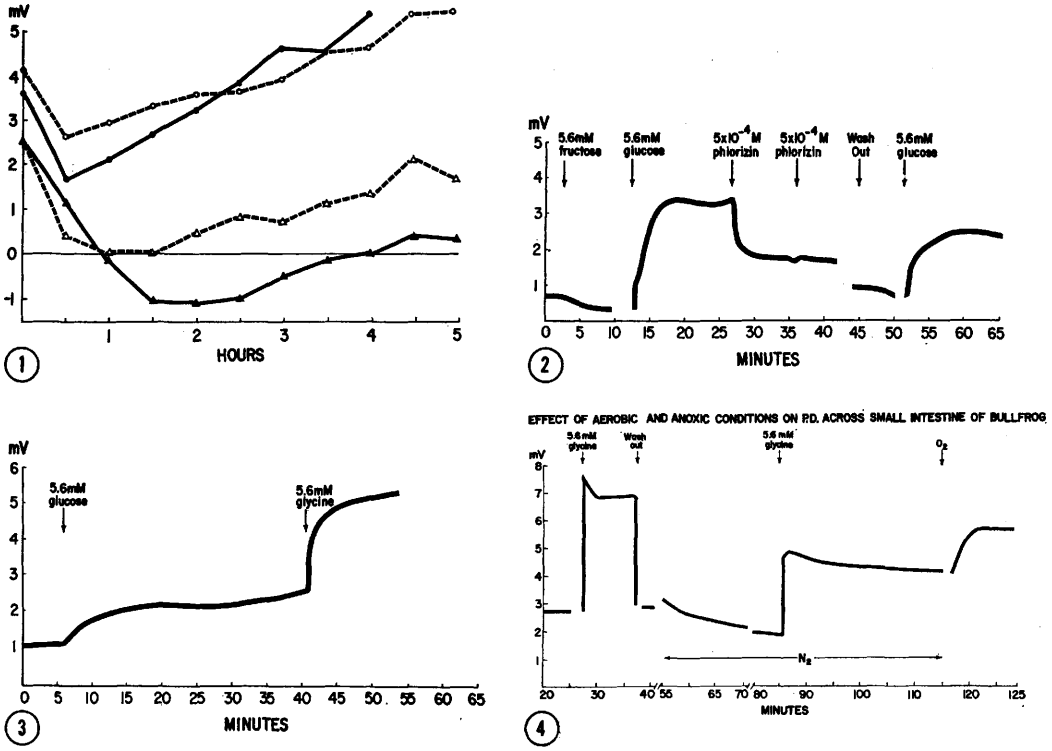


FIG. 1. PD across everted proximal smal intestine of bullfrog, *R. catesbeiana*. Two incubated in sulphate saline (●——●, ○-----○) and 2 in chloride saline (▲——▲, △-----△) containing 5.6 mM glucose and gassed with 95% $O_2$, 5% $CO_2$.

FIG. 2. Effect of fructose, glucose and phlorizin on PD across bullfrog small intestine incubated in sulphate saline gassed with 95% $O_2$, 5% $CO_2$. Breaks indicate where mucosal solution was replaced with fresh sulphate saline.

FIG. 3. Effect of addition to mucosal solution of 5.6 mM glucose and the 5.6 mM glycine on the PD across bullfrog small intestine incubated in sulphate saline gassed with 95% $O_2$, 5% $CO_2$.

FIG. 4. Effect of aerobic and anoxic conditions on PD across small intestine of the bullfrog incubated in sulphate saline. Horizontal arrows labelled $N_2$ indicate that the mucosal solution was gassed with 95% $N_2$, 5% $CO_2$, while the vertical arrow under the $O_2$ indicates the time when 95% $O_2$, 5% $CO_2$ was again admitted to the mucosal fluid.

increase in PD. Both galactose and 3-O-methylglucose increased the PD when added to the mucosal solution (5.6 mM) but neither were as effective as glucose (Table I). Phlorizin ($5 \times 10^{-4}$ M) also depressed the potential caused by these two sugars. Addition of 5.6 mM sorbose, xylose, sucrose, mannitol, urea, ascorbic acid and sodium acetate to the mucosal solution did not cause any increase in PD. In fact most of these solutes gave a depression of PD.

*Effects of amino acids.* Experiments similar to that shown in Fig. 2 demonstrated that the addition of certain amino acids to the glucose-free sulphate mucosal fluid caused a rapid increase in the PD. Addition of 5.6 mM glycine to the mucosal fluid caused a rapid increase in PD (similar to that shown by the actively transported sugars) which stabilized after a few minutes and then gradually in-

TABLE I. Effect of Sugars on PD Across Bullfrog Small Intestine Incubated in Sulphate Saline. Sugar was added to mucosal fluid only (5.6 mM) and the increase in PD after 10 min was measured. Results are expressed as the mean ± S.E. Figures in brackets are No. of experiments.

| Sugar | | Increase in PD (mv) |
|---|---|---|
| D-glucose | (41) | 1.50 ± .14 |
| D-galactose | ( 8) | 1.00 ± .22 |
| 3-O-D-methylglucose | (11) | .54 ± .09 |
| D-fructose | ( 5) | −.35 ± .08 |
| L-sorbose | ( 5) | −.36 ± .12 |
| D-xylose | ( 4) | −.22 ± .10 |

TABLE II. Effect of Amino Acids on PD Across Bullfrog Small Intestine Incubated in Sulphate Saline. Amino acid was added to mucosal fluid only (5.6 mM) and the increase in PD after 10 min was measured. Results are expressed as the mean ± S.E. Figures in brackets are the No. of experiments.

| Amino acid | | Increase in PD (mv) |
|---|---|---|
| Glycine | (23) | 1.71 ± .19 |
| DL-alanine | ( 6) | 1.48 ± .51 |
| L-cysteine | ( 5) | 1.04 ± .14 |
| L-lysine | ( 5) | .90 ± .26 |
| L-methionine | ( 5) | .66 ± .12 |
| L-glutamic acid | ( 5) | .53 ± .12 |
| L-aspartic acid | ( 4) | .46 ± .01 |
| D-alanine | ( 6) | .18 ± .07 |

creased until a new steady state was reached. Replacement of the mucosal fluid with glycine-free sulphate saline usually caused an immediate decrease in PD to the solute free level but in some experiments this took over an hour. The reason for the slow desorption in these intestines is not known. If glycine was added to the serosal fluid, no increase in PD occurred. Phlorizin ($5 \times 10^{-4}$ M) did not decrease the PD caused by glycine; in fact there was evidence of a slight increase after the glycoside was added to the mucosal fluid. The other amino acids that were effective in increasing the potential when added to the mucosal solution are shown in Table II.

*Interaction between amino acid and sugar.* If glucose (5.6 mM) was added to the mucosal solution of an intestine incubated in glucose-free sulphate saline and the resultant increase in PD allowed to come to the steady state, addition of 5.6 mM glycine to the same mucosal solution caused a further increase in PD. The magnitude of this increase was similar to that occurring when the amino acid was added in the absence of glucose (Fig. 3). Similar results were obtained when the glycine was added before the glucose and with galactose and glycine.

*Effects of anoxic conditions.* In chloride saline anoxic conditions gave equivocal results. In some experiments the PD oscillated about zero (± 1 mv) while in others there appeared to be no significant effect. In sulphate saline anoxic conditions when maintained for at least 30 minutes caused a slow decrease in PD. Addition of either glucose or glycine to the mucosal fluid after 30 minutes of anoxia still caused an increase in PD although the increase was less than with aerobic conditions. Glucose was less effective than glycine in maintaining the potential during anoxia. Admission of oxygen to the mucosal fluid after the anoxic period in the presence of glucose or glycine always caused a rapid and sustained increase in PD approaching that of the normal aerobic value (Fig. 4). Thus, unlike mammalian small intestine, the mucosa of bullfrog small intestine does not appear to be functionally damaged by long periods of anoxia.

When sodium fluoride (5 mM) was added to the mucosal fluid during anoxia, little or no effect on the glycine stimulated PD was observed, but if the same concentration was placed in the serosal fluid, the PD fell rapidly to zero. Admission of oxygen partly reversed this fall and allowed a small PD to be maintained. Under aerobic conditions 5 mM fluoride did not depress the PD caused by glycine even if present in serosal, mucosal or both fluids.

*Transmural PD of the large intestine.* The PD across the large intestine incubated in sulphate saline was 47.1 ± 3.3 mv(12), serosa positive to mucosa. Unlike the small intestine this PD was unaffected by the presence or absence of glucose or glycine (5.6 mM) in the mucosal or serosal fluids. Anoxia or dinitrophenol (1 mM), however, caused a rapid depression of the PD to zero. This could be reversed in the former case by admitting oxygen and in the latter by washing out the dinitrophenol. Sodium fluoride (10 mM) had little effect when added to the mucosal solution but a 5-fold increase in the concentration caused a rapid fall in PD. Sodium fluoroacetate (2 mM) when added to the mucosal fluid did not immediately depress the PD but during 30 minutes there was a gradual reduction. Increasing the concentration of this inhibitor to 10 mM caused a more rapid depression of PD. Both iodoacetamide (1 mM) and ouabain (0.1 mM) when added to the mucosal solution had little effect, but if either were added to the serosal fluid, the PD was rapidly abolished.

*Discussion.* Bullfrog small intestine has a measurable PD across the whole wall when

incubated in chloride or sulphate saline containing glucose. This PD is maintained by metabolic activity. With chloride as the major anion in the incubating fluid the PD is small. This result agrees with the unpublished observation of Chalfin, Cooperstein and Hogben(6) that "the electrical potential across the small intestine (bullfrog) is close to zero". The results reported here, however, show that if the larger sulphate ion is substituted for chloride higher potential differences are obtained, presumably because anion movement across the mucosa is more restricted. Similar increases in PD have been noted in a number of tissues(7,8) when chloride is replaced by sulphate in the bathing solution. The PD across bullfrog small intestine was always serosa positive to mucosa when sulphate saline was used. Addition of either of the actively transferred sugars, glucose, galactose or 3-methyl glucose to the mucosal fluid (buffered sulphate saline) caused a rapid increase in the PD which could be blocked by addition of phlorizin to the same fluid. Phlorizin in the serosal fluid had no effect; similarly if the sugars were added just to the serosal fluid no increase in PD was observed. Thus the bullfrog intestine behaves like that of rat and rabbit in regard to the action of actively transported hexoses on the PD. Bullfrog intestine has, however, the advantage over mammalian intestine in its remarkable resistance to deterioration when incubated *in vitro*. Even after 5 hours the PD is well maintained. This contrasts with the rapid fall of PD observed in rabbit intestine; from an initial 9 mv to 4.5 mv within one hour(9). A tissue deteriorating this rapidly is unlikely to be in a steady state. Even with rat intestine there is an obvious but much slower deterioration with time(3). Clearly the stability of the bullfrog intestine *in vitro* will make it a more suitable preparation for measurement of ion fluxes in the steady state condition than the more rapidly deteriorating mammalian intestine. Although the PD recorded across bullfrog intestine in the presence of glucose is lower than the 7-12 mv found in rat(1,2,3) or the initial 9 mv in rabbit intestine(9) direct quantitative comparisons are difficult because of the different glucose concentrations used and the varying temperatures of incubation. Bullfrog intestine is incubated at room temperature (20-22°C) while mammalian gut is usually kept at 37-38°C. Measurements of the PD across the intestine of goldfish(10) between 20-25°C appear to be of the same order (3-5 mv) as found in the studies with the bullfrog. The PD was not only affected by the presence of sugars but also by a number of amino acids when they were added to the mucosal solution. Apart from D-alanine and the dicarboxylic acids aspartic and glutamic acids, all the amino acids used have been reported to be actively transferred across mammalian intestine(11) and it is likely that they are handled in a similar manner by the bullfrog. In the case of alanine, the racemic DL-mixture was far more effective in increasing the PD than was D-alanine. Such specificity for the generation of the PD is similar to the amino acid transferring system which has a striking preference for the L-stereo isomer. The fact that L-aspartic and L-glutamic acids caused a small increase in PD is interesting. Both are transaminated by rat intestine and studies on their entry into mucosal cells are thus complicated. As it appears that only amino acids that are actively transferred cause an increase in PD it might be that in bullfrog intestine glutamic and aspartic acids utilize some aspect of the amino acid transferring system involved in the generation of the PD. On the other hand, transamination might take place and the resultant alanine formed intracellularly could enter the amino acid transfer system and stimulate the PD. Whatever the mechanism involved, bullfrog intestine appears to be helpful in studying the mode of entry and transfer of dicarboxylic acids. Interaction between the PD developed by amino acid and that developed by a sugar was observed in the case of glucose and glycine. Whether the sugar or the amino acid was present first, the PD developed was the approximate sum of the PD developed by either when added alone. This phenomenon has recently been described for rabbit ileum (12). The rapid and sustained increases in PD that occur when actively transported sugars and amino acids are added to the mucosal fluid could be due to an increased trans-

fer of sodium from mucosal to serosal fluid as has been shown in the rabbit ileum(12). Barry, Smyth and Wright(13) using rat jejunum could find no simple correlation between hexose stimulated PD, short circuit current and net sodium transfer. If translocation of sodium from mucosa to serosa is not always the answer another mechanism for the sudden increases in PD caused by addition of the hexoses must be sought. A possible explanation might be that because the actively transported substances are rapidly concentrated in the intracellular fluid, cellular osmolarity is rapidly increased. By the loss of another osmotically active ion (potassium?) the mucosal cell could reduce the osmolarity to normal. This intracellular change in the ionic concentration would affect the diffusion potentials across the luminal or serosal membranes of the cell and hence alter the transmural PD. Obviously further measurements of short circuit current and the fluxes of potassium, sodium and hexose across the intestine of a number of species are necessary before any proposed mechanism can be accepted.

Further indication that the PD is linked to active transfer mechanisms found in the small intestine are the results obtained in the large intestine, a tissue that does not possess active transport mechanisms for sugar and amino acids(11). The presence of glucose or glycine in the mucosal or serosal fluid bathing this tissue had no effect on the PD even though in this tissue it is practically all accounted for by the net transfer of sodium from mucosa to serosa(14). Thus the mere presence of a PD generated by sodium transfer does not give rise to amino acid and sugar dependent PD. A further interesting difference between the small and large intestine is their sensitivity to the effects of anoxia and dinitrophenol. Under anoxia or in the presence of dinitrophenol the small intestine can partially maintain the PD while the large intestine cannot. As the PD in anoxic small intestine was sensitive to fluoride on the serosal side it appears that the energy for this PD comes from anaerobic glycolysis. Such a source of energy for the maintenance of PD is not available to the large intestine.

*Summary.* Transmural potentials across isolated segments of small intestine of the bullfrog, *R. catesbeiana,* were recorded. In sulphate saline the PD was higher than in chloride saline. Metabolic inhibitors depressed the PD. Actively transferred sugars and amino acids increased the PD when added to the mucosal fluid; this did not occur with the large intestine. Anoxia and dinitrophenol reduced the PD of the large intestine more than that of the small. The results indicate that bullfrog small intestine incubated *in vitro* is a stable and useful model for investigating the association between solute transfer, PD and energy sources.

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