Letter to the Editor regarding Julio-Kaljzic et al (2018)

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Dear Madam

Thank you for letting me comment on the paper, selected for commendation by the subject editor, by Julio-Kalajzic & others (2018) who identify potassium ion channels in the enterocyte that might facilitate fluid secretion. Whilst applauding the undoubted technical expertise in cell biology and genomics that was brought to bear, I find myself at odds with all the conclusions drawn regarding intestinal secretion.

Secretion is fluid movement from behind the enterocyte into the intestinal lumen, supposedly generated by processes within the enterocyte. Chloride ion secretion is the driving force for fluid secretion, with maintenance of membrane potential difference through potassium ion channels conjectured to be essential for secretion. Blockage of the appropriate potassium ion channel could therefore prevent secretory diarrhoeal disease.

Mass transport is the only way secretion can be correctly assessed. Deduction of secretion using Fick's principle with non-absorbed markers will be false if absorption causes marker loss, as occurs for all markers currently in use. Deductions from 'unidirectional' fluxes of chloride isotopes are also false as the correct solution to mathematics of the Ussing chamber (Lucas, 2005) disallows partition into unidirectional fluxes, since fluxes are reciprocally linked. *In vivo* experiments that include interstitial volume (Eklund et al., 1985) mislead since increased extracellular volume will resemble but not actually be luminal secretion. Finally, short-circuit current is not chloride ion and hence fluid secretion, although this technique is now the mainstay of investigations into secretion. Its inherent ambiguity is that an increase in current could be an outward chloride ion or an inward sodium ion current. Changes in short-circuit current show only that normal tissue responds to challenge by enterotoxins or pharmacological agents.

When confronted with evidence from *in vivo* perfusion experiments, deductions deriving from short circuit current experiments, however closely and logically argued, fail to be validated. Short-circuit current changes are coincidental phenomena resulting from interrupted fluid absorption clearly seen when *E.coli* STa enterotoxin is the perturbing agent. STa causes a large increase in short-circuit current (Young et al., 1990) in rat jejunum and ileum but does not cause intestinal fluid secretion (Lucas et al., 2005) *in vivo*. STa toxin

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will elevate short circuit current in isolated cells (Rufo et al., 1997) which is reduced by glibenclamide and clotrimazole. When tested *in vivo*, these compounds do not reverse STa mediated fluid absorption inhibition (Lucas et al., 2011). It is possible to dismiss findings of a mismatch between inhibition of short circuit current and lack of effect *in vivo* since they stem from one laboratory but while STa does not cause secretion, adverse osmotic forces, *E.coli* labile toxin (LT) and *C. difficile* toxin A all show net secretion when measured in the same laboratory. The maximum permissible inference from the *E.coli* experiments is not that there is never fluid secretion; it is that if secretion occurs as with other toxins, it is not explained by fluid secretion directly from the enterocyte and cannot be measured by short-circuit current measurements.

Is there a similar mismatch between current measurements *in vitro* and fluid movement *in vivo* in the Julio-Kalajzic paper? When this concept is tested by examining secretion after exposure to cholera toxin *in vivo*, the potassium channel mutants fail to show the anticipated reductions in secretion, confirming similar negative findings (Preston et al., 2010) with other mutants. The channel mutant values for cholera induced secretion are uncorrelated with the size of secretion that is expected from changes in short-circuit currents often exceeding 100 uA/cm^2. If secretion is isotonic, Faraday's Law indicates that 50 uA/cm^2 equals about 12 ul/cm length/hour of secretion. The short-circuit current indicated a weight to length ratio of 0.1 g/cm should arise after 5 hours, with the cholera stimulated loops having a ratio of 0.28 g/cm, two to three times what was found. It seems unlikely that short-circuit current and fluid movement are directly related but the contemporaneous occurrence of both arises from altered fluid uptake.

The authors conclude that the correct potassium ion channel eludes identification. Yet, some enterotoxins elevate short-circuit current, fail to instigate fluid secretion and their actions *in vivo* are unaffected by potassium channel blockers. This is now the pattern emerging with potassium channel mutant studies since short-circuit current can alter but there is no evidence of fluid secretion being induced or accelerated; indeed, the discussed paper supports the view that aspects of the enterocyte secretion hypothesis fail when tested *in vivo*. The interesting paper by Julio-Kalajzic and others inadvertently provides further evidence that the enterocyte secretion model is defective and that secretion is caused by a combination of vasodilatation and increased hydraulic permeability, supplemented by interruption of fluid absorption.

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