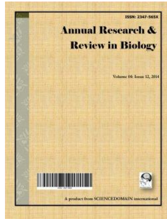

Annual Research & Review in Biology

23(6): 1-27, 2018; Article no.ARRB.38824
ISSN: 2347-565X, NLM ID: 101632869

The Arteriolar Vasodilatation Model of *Vibrio cholerae* Induced Diarrhoeal Disease

Michael L. Lucas[1*]

[1]School of Life Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom.

Authors' contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/ARRB/2018/38824
Editor(s):
(1) Moacir Marocolo Júnior, Professor, Physiology, Institute of Biological Sciences, Federal University of Juiz de For a, Brazil.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) S. Thenmozhi, Periyar University, India.
(2) Nain Taara Bukhari, University of Karachi, Pakistan.
(3) V. Vasanthabharathi, Annamalai University, India.
(4) John-Yew-Huat Tang, Universiti Sultan Zainal Abidin, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/23070

Review Article

Received 5th November 2017
Accepted 18th January 2018
Published 7th February 2018

ABSTRACT

Secretory diarrhoeal disease caused by enterotoxins produced by pathogenic bacteria is characterised by severe fluid loss into the intestine. A prevalent explanation for such high rates of loss, such as occur in episodes of cholera, is that intestinal epithelial cells (enterocytes) actively secrete chloride ion into the lumen. Fluid is drawn into the lumen because of the osmotic pressure difference that is created across the mucosa. Widely proposed as the cause of many forms of secretory diarrhoea, the enterocyte based paradigm displaced an earlier model of secretion i.e. fluid filtration caused by increased capillary hydrostatic pressure, possibly coupled with increased hydraulic conductivity. This would be aggravated by any concurrent inhibition of fluid absorption if it occurred. In the earlier and alternative paradigm, pathophysiological reductions in smooth muscle tone elevate capillary pressure, thereby increasing the hydrostatic pressure gradient that forces fluid from the capillary into the interstitial space and thence into the lumen.
In this review, the present and historical evidence for the vasodilatation view of secretory diarrhoeal

**Corresponding author: E-mail: Michael.Lucas@glasgow.ac.uk;*

disease is presented, together with past challenges of this concept, particularly those involving the erroneous equating of solute permeability with hydraulic conductivity. It can be seen that the physical forces model of altered Starling forces combined with enhanced fluid permeation explains more experimental findings than the cellular based enterocyte model can. Several key past papers advocating enterocyte secretion in which the capillary vasodilatation model was also discounted, were examined for the inherent fallacies within the arguments that were proposed. Where possible, quantitative arguments are proposed that indicate that is it the combination of capillary vasodilatation combined with increased tight junctional hydraulic conductivity that causes profuse secretion, made worse by any concurrent inability to absorb fluid. To assist the general physiological reader, an appendix reviews Bernoulli's principle of flow within tubes and explains the arguably counter-intuitive phenomenon that vasodilatation increases capillary pressure because of a velocity reduction within a dilated segment.

Keywords: Cholera; vasodilatation; diarrhoea; model for secretion.

1. INTRODUCTION

The present paradigm for fluid secretion in diarrhoeal disease emphasises that 'normal' secretion coming from the enterocyte cell of the intestinal mucosal is increased by enterotoxins such as cholera toxin. This places the site of the patho-physiological process as the epithelial cell. In contrast, an older and neglected paradigm is that secretory diarrhoeal disease does not involve epithelial cell based fluid secretion. Instead, vaso-dilatation of intestinal vasculature together with increased hydraulic conductivity leads to the copious fluid secretion characterising cholera. This present review investigates the evidence for the vasodilatation hypothesis, commonly termed the filtration hypothesis in past refutational literature.

It order to present the rival hypothesis in context, it is necessary to review past and even historical literature to indicate points in time at which critical non-sequiturs were made and accepted by the scientific community of the time and perpetuated today. The present review is therefore a critical examination of key research that has steered the intellectual pathway to the present standpoint firmly occupied by the enterocyte secretion hypotheses.

Only by looking at the past work and analysing it critically will it become clear that the enterocyte chloride ion secretion hypothesis is a failing paradigm. It should now be replaced with the vasodilatation model if effective treatment of the acute and life threatening symptoms of many forms of secretory diarrhoeal disease is to be developed.

2. THE ENTEROCYTE SECRETION AND ARTERIOLAR DILATATION MODELS FOR INTESTINAL FLUID SECRETION

2.1 The Enterocyte Chloride Ion Secretion Hypothesis as a Basis for Secretory Diarrhoeal Disease

The current concept of fluid movement from the serosal side of the enterocyte into the lumen is essentially a biochemical intellectual construct since it relies on the internal energy from the epithelial cell to power ion flows across the cell. The ion flows themselves result from specific carriers and channels that move ions in accordance with the formalism of enzyme kinetics as expected from the canonical foundations of biochemistry. Since prior to the enterocyte secretion view, the absorption of nutrients, sodium ion and fluid movement was so well explained by a cellular based view, it is perhaps not so surprising that an explanation for secretion of fluid should also have been based on a biochemical model.

However, in this case, it is evident that biochemistry based models cannot transcend the short-comings of all explanations being cellular based and biochemical in origin. In this case, an explanation must be sought in the physical forces that cause mass transport, as is often the case in physiology. A more likely explanation is that of mass transport of fluid because of physical forces acting within tissues.

The purely biochemical model requires that chloride ion enters the enterocyte cell interior and leaves it through a mucosally sited chloride channel (See Fig. 1 & Fig. 2 Hypothesis A), assuming that the electrochemical gradient is

correctly aligned for chloride ion extrusion into the lumen of the small intestine. The $Na^+/K^+/2Cl^-$ co-transporter in the serosal border allows entry of chloride ion into the cell. This entry is energetically dependent on the ionic gradients for the three ions. The sodium ion: potassium ion ATPase, provides a sodium ion gradient aligned for entry into the cell to pull chloride and potassium ions into the cell interior. Stoicheiometry dictates that two chloride ions enter the cell for every sodium ion returning via the triporter and hence the intracellular chloride ion concentration will increase.

Given that the chloride ion should distribute across the mucosal membrane in accordance with the electrochemical gradient, the assumption is that, at equilibrium, the tendency for chloride ion to enter the cell from the lumen because of the adverse chemical gradient is exactly balanced by the tendency for the chloride ion to exit through the CFTR channel. When chloride ions enter the enterocyte via the triporter, the assumption is that the concentration is slightly above equilibrium and chloride ion will be extruded as electrochemical equilibrium is restored. From this, it can be seen that it is important for chloride ion extrusion that the cell membrane potential difference is maintained, a function attributed to serosal membrane potassium ion permeability.

With the presence of the biochemical machinery within mucosal and serosal cell membranes, routes can be proposed for chloride ion translocation from the serosal to the mucosal surface of the enterocyte, together with

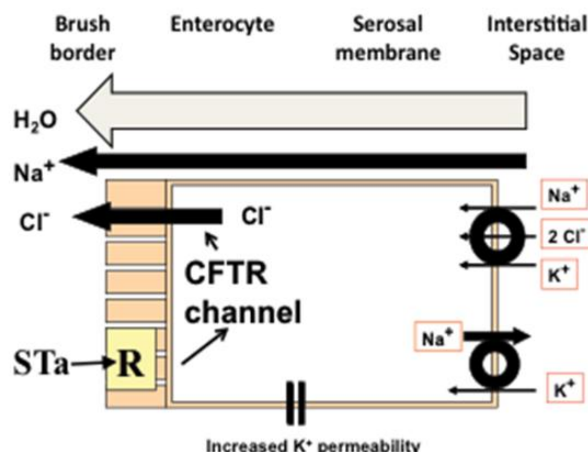

Fig. 1. Enterocyte chloride secretion paradigm

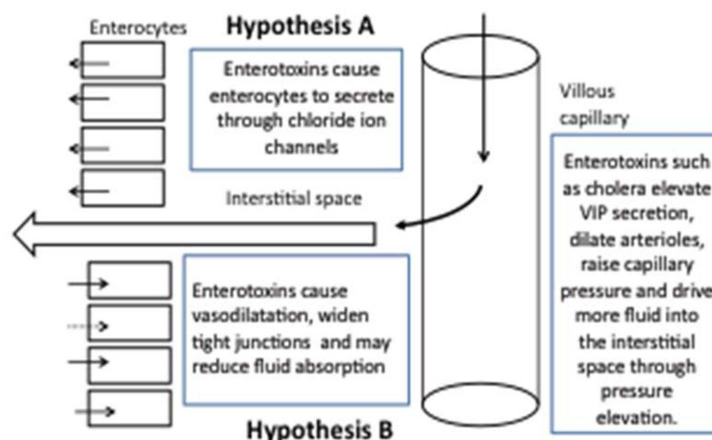

Fig. 2. Enterocyte and vasculature model

appropriately balanced electrochemical forces that should allow chloride ion transmission to the mucosal surface. The extrusion of chloride ion together with sodium ion presumably through the paracellular pathways causes an accumulation of solute near the enterocyte brush border. This provides an osmotic pressure pulling fluid through the lateral spaces, ultimately from the sub-epithelial interstitial space. As a result, chloride ion and water move into the lumen. If this process can be enhanced by enterotoxins, then this would form the basis for enhanced intestinal secretion in secretory diarrhoeal disease.

For illustrative purposes, a receptor, R, for heat stable *E. coli* enterotoxin (STa) is present (Fig. 1) in the brush border. The enterocyte secretion view envisages that STa fits into the luminal receptor and elevates intracellular second messenger concentration, in this case cyclic GMP. This in turn, just as cAMP is thought to do for cholera toxin, acts via protein kinases on the CFTR channel causing it to be become more permeable. Given no change in electrochemical gradients across the luminal membrane, it is possible that more chloride ions are extruded per unit electrochemical gradient and therefore more fluid is secreted.

While intellectually self-contained and satisfying within the constraints of *in vitro* work, the model does not survive challenge when confronted with observations from *in vivo* experiments. The canon of *in vitro* techniques used to measure 'secretion' such as isotope fluxes and electrical measurements are methodologically flawed [1]. *In vivo* experiments not using permeable isotopes to measure fluid secretion indicate that i) *E. coli* STa cannot cause secretion [2] despite a widespread expectation that it should. These and many other observations in the historical literature on the causes of secretory diarrhoea point to an alternative view that has been dormant in the secretion literature but can accommodate the data from the enterocyte secretion model whilst being itself a concept based on physical rather than chemical forces across the mucosa.

The intestinal arteriolar vasodilatation hypothesis as a basis for secretory diarrhoeal disease:

This alternative model for fluid secretion is one that has repeatedly been put forward but has always been overshadowed, more so recently, by the epithelial cell fluid secretion model. This repeated intrusion of the physical forces model is often simply ignored. The physical model is presented (see Fig. 2) together with the biochemical model to facilitate comparison.

The vasodilatation hypothesis (B) relies on the fact that the villous capillary is permeable to fluid that moves into the interstitial space. Starling forces exist across all capillaries and ensure that an excess of intraluminal capillary pressure over interstitial fluid pressure pushes fluid into the sub-epithelial space and greater colloid osmotic pressure of the blood pulls fluid back into the capillary. A steady volume state is achieved when fluid loss into the interstitial space is balanced by the tendency for interstitial fluid to move back into the capillary. A small net driving force for fluid movement into the intestinal lumen would be enough to promote secretion that could be enhanced by increases in the tight junction widths.

It should be remembered that an increase in tight junction width can also serve to promote fluid absorption if the luminal pressure, even if transiently, exceeds interstitial fluid pressure, as it may do in the contracting intestine. If enterotoxins reduced fluid absorption, this model predicts increased seepage of fluid into the lumen since some of the exudate would presumably be reabsorbed under normal circumstances. Compromise of normal fluid absorption would undoubtedly lead to diarrhoea but this would be greatly enhanced by simultaneous loss of interstitial fluid into the intestinal lumen.

Copious and life threatening diarrhoea at the rate that occurs during a cholera episode is likely also to require vasodilatation. It is a physiological and somewhat counterintuitive fact that capillary pressure increases if there is vaso-dilatation of the immediately preceding arteriole. The increase in vasodilatation causes the capillary pressure to rise from average values of 30 - 40 mmHg to whatever the arterial pressure is on the capillary side of the arteriole and may rise to 45 mmHg or more. This increase in hydrostatic pressure forces fluid into the interstitial space and increases fluid secretion into the lumen by physical means. If accessory toxins increase epithelial and capillary endothelium paracellular pathways, as well as inhibit intestinal fluid absorption mechanisms, then this would lead to catastrophic fluid loss, essentially powered by the mechanical force of the heartbeat. It is

unlikely that chemical reactions could match this degree of force or effect changes in interstitial volume in such a short time frame.

3. THE ARTERIOLAR VASODILATATION MODEL IN GREATER PHYSIOLOGICAL DETAIL

To understand why vasodilatation is relevant to the secretory pathology of diarrhoeal disease, an understanding is required of the physical forces that extrude fluid through the capillary wall [3,4]. The central aspect of the concept advocated here is that formation of interstitial fluid is a normal physiological function of the capillaries, with the rate of formation dependent on transcapillary osmotic and hydrostatic pressure gradients. This rate of formation, termed Jv, is positive when interstitial fluid volume increases. The rate of formation is determined by the difference between the hydrostatic pressure gradient across the capillary, (Pc - Pi) extruding fluid from the capillaries and the osmotic pressure gradient across the capillary wall (πc - πi) pulling fluid into the capillary lumen. The normal formulation of the Starling equation which relates fluid secretion to these forces is therefore that:-

$$Jv = Kfc \{(Pc - Pi) - \sigma (\pi c - \pi i)\} \quad (1)$$

where Kfc is the capillary filtration coefficient and σ is Staverman's reflection coefficient. The capillary filtration coefficient relates the rate of flow to the differences in the osmotic and hydrostatic pressure gradients and has units of flow/mm Hg. Staverman's reflection coefficient accounts for the fact that the osmotic pressure exerted across the composite capillary membrane can be less than ideal and takes a value between zero and unity. A capillary almost totally permeable to protein will exert almost zero osmotic pressure, it being understood that it is the difference in protein that exerts the osmotic pressure since the electrolyte content of blood and interstitial fluid should be identical. Changes in the rate of interstitial fluid formation will occur in the short term because of the hydrostatic pressure gradient across the capillary, given that osmotic pressure is unlikely to be altered as blood perfuses the capillary. The rate will also be altered by any change in capillary filtration coefficient, depending on the alignment of the pressure gradients.

Similar arguments can be offered and an almost identical Starling equation can be written for fluid loss through the epithelial tight junctions into the lumen. This would normally be small given that the intestinal filtration coefficient across the tight junction is likely to be far less than that for the fenestrated intestinal capillary. Normal epithelial cell fluid absorption would counteract and therefore normally obscure small fluid losses into the lumen that might only become evident if fluid absorption were to become inhibited.

A consequence predicted by the Bernoulli principle is that vasodilatation of the arterioles preceding the intestinal capillaries would increase the pressure [5,6] within the capillaries (Fig. 3 & Fig. 8 of Appendix). Hence, formation of interstitial fluid increases on vasodilatation, (See [7] and later editions, [8,9].

There are two aspects of note from the point of view of pathological interference with normal fluid absorption by enteropathogenic bacteria. If fluid is secreted into the small intestine, the vasodilatation hypothesis assumes that this is by physical means into the lumen, overwhelming normal fluid absorption. Two sites of action are suggested when the Starling equation is examined: namely that fluid secretion into the lumen could be initiated or if it normally occurs, then enhanced, by elevating the capillary hydrostatic pressure leading to higher rates of interstitial fluid formation. A second mode of action would be increased filtration coefficients at the capillary but more likely at the intestinal tight junctional barrier. This site is exploited by *Vibrio cholerae* which produces a zona occludens toxin (ZOT) that makes the tight junctions more permeable to water.

A combination of vasodilatation, accompanied by an increase in the capillary filtration coefficient i.e. the hydraulic permeability of the capillary junctions or the intestinal tight junctions would dramatically increase the rate of fluid entering the small intestine. This alone might be sufficient to overwhelm the fluid absorption capacity of the intestine and cause secretion. In the event of simultaneous inhibition of any fluid absorption mechanisms, catastrophic fluid losses would arise.

There is a further point of note here: it is evident that it is hydraulic permeability that must increase but not just permeability to any solute of any size. It is possible therefore to fail to detect permeability increases to any solute, usually chosen for experimental convenience and ease of measurement, since these may be too large to

traverse a widened tight junction pore which nevertheless has increased in size leading to an increase in water permeability.

A further consideration is that it is possible that decreases in Staverman's reflection coefficient (σ of equation 1) make the opposing osmotic pressure gradient less effective at counteracting the hydrostatic pressure gradient or that capillary recruitment is increased. Increased intracapillary pressure is therefore the major determinant of fluid loss in cholera, according to the filtration model advocated here.

4. FINDINGS FROM THE EARLY MODERN PERIOD (1738 - 1849) OF INVESTIGATIONS INTO CHOLERA

The origins of physiological research into fluid absorption and secretion in disease states:

One explanation for the extremely high rates of fluid entry into the small intestine in cholera has been the historical one, first proposed by von Haller [10] who assumed that the crypt cells identified by Lieberkühn in 1738 [11] were the source of intestinal fluid secretion that was deemed to be overproduced in this disease. Indeed, the presence of large fluxes of fluid in cholera was the single most persuasive argument for the existence of normal and abnormal intestinal fluid secretion from the enterocytes. Von Haller's assumption of crypt cell production of fluid was therefore one of speculative theorising that remained dominant for a surprising length of time and overshadowed the concept of physical forces leading to fluid secretion.

In the following sections, the evidence for abnormal capillary function in cholera is reviewed from the early modern historical period onwards until 1980 during which time, the enterocyte secretion hypothesis in its present form was first proposed [12] After this date, the enterocyte secretion hypothesis rapidly became the dominant explanation for secretion such that by 1980, there seemed to be no dissenting views, with a few notable exceptions, concerning this concept. Instead, there was a large increase in supportive research that contained profound errors of logic regarding the source of secretion. For this reason, key papers are reprised here where they are relevant to the filtration secretion model.

5. EVIDENCE THAT CHOLERA FLUID WAS EITHER A TRANSUDATE OR EXUDATE OF PLASMA FLUID (1849 - 1974)

The concept that cholera fluid was a transudation from the blood was first proposed by Bequerel [13] who posed the question "ne peut-on admettre qu'une certaine portion de matieres solides du serum du sang, et en particulier de l'albumine, soit exhale par les membranes muqueuses?" Could not the intestinal cholera fluid be an exhalation of blood through the mucosa? Whether or not this loss of fluid into the lumen was through exudation because of loss of epithelial cells or was a transudate through an intact cellular mucous membrane would be a second implied question. At that time, when experimental transmission of the secretory toxins to animals to study pathophysiology was not yet feasible, observations derived mainly from gross anatomical and histological studies of material from patients dying from cholera and undergoing autopsy or from examination of content of collected faecal fluid.

It is at this time that a major and possibly the most egregious *non sequitur* was formulated by the advocates of epithelial secretion. In particular, Julius Cohnheim [14,15] was one of many physicians who sought but did not find sloughed epithelium in rice water stool collections from cholera patients and challenged anybody ("Wer das verfechten will") to contradict the fact that this fluid obviously came from the epithelium.

It is the case that cholera fluid is not simply plasma fluid that leaks into the lumen because of the partial denudation of the mucosa of epithelial cells. Cholera fluid is still produced when the epithelium is intact and attached to the mucosa. However, it is emphatically a *non sequitur* to conclude that because the epithelium is intact, cholera fluid must emanate from the epithelial cells. This false conclusion has misdirected cholera research for well over a century. During this period, evidence for vasodilatation was also assembled.

The collected post-mortem studies by Wendt [16] assembled just prior to another cholera pan-epidemic sweeping the world, indicated that hyperaemia was a predominant feature of the disease. In several tissues outside the intestine, the gross anatomical feature of redness was found. Such redness or hyperaemia occurs after

additional capillary recruitment and also by arteriolar vasodilatation as both phenomena allow more blood to flow through a capillary bed per unit time. The tissue surrounding the capillaries can itself also swell because of the consequent enlargement of the sub-epithelial spaces by increased interstitial fluid formation.

General vasodilatation would also explain why almost any chosen capillary bed can exhibit signs of oedema, including cerebral vasculature, laryngeal vasculature leading to *vox cholerica*, and the vasculature at the sphincter of Oddi leading to bile fluid in the ducts held back by the sphincter, swollen to the point of closure. Pathological vasodilatation would also explain the now almost forgotten observation that both women of child bearing age and post-menopausal women exhibited menstruation-like symptoms when the disease was well advanced.

The widespread occurrence within the body of hyperaemia after general vasodilatation is not the expected physiological response after severe loss of fluid. The normal compensatory response to volume loss is generalised vasoconstriction to maintain mean arterial blood pressure. Vasodilatation in capillary beds elsewhere would be a paradoxical response to the intestinal fluid losses caused by cholera. Detailed pathological studies by Störk [17] confirmed the widespread hyperaemia caused by cholera and led to the hypothesis that oedema occurred in many organ systems because of vasodilatation. In his careful post-mortem study, Störk emphasised the need for immediate autopsy if possible because the intestinal hyperaemia diminished very rapidly post mortem. Many of his autopsies were carried out within a half hour after death and perhaps accounted for his ability to detect the inflammation which often eluded contemporaries. Although this idea was retained within the research record by Pollitzer [18] Störk's work is rarely cited, perhaps because scientific and intellectual continuity was abruptly severed by the First World War.

In the period between Koch's proposal [19] of a cholera poison, and Störk's pathology studies also indicating that there could be an intraluminal toxin causing vasodilatation, opinion was that no enterotoxin was produced but instead endotoxins were released on disruption of the Vibrio organisms. Eventually, cell free culture filtrates from *Vibrio cholerae* were produced. In 1959, De and colleagues demonstrated [20] that culture filtrates of cholera caused swelling of a tied rabbit loop *in vivo*. Later, fatal, dehydrating diarrhoea was shown [21] when similar filtrates were introduced into the rabbit loop.

A phenomenon similar to the swelling of the loop occurred [22] when the culture extracts were injected sub-cutaneously as skin capillaries became more permeable to intravenously injected dye. Later, 'induration' of the skin of guinea pigs or rabbits occurred when cholera stool filtrates were injected sub-cutaneously but not when stool filtrate from patients with non-cholera watery diarrhoea were tested [23] Capillary permeability to the blue dye, Ponceau blue, also increased with stool filtrates that caused induration, leading to the conclusion that the cholera enterotoxin changed capillary permeability and caused fluid loss certainly in skin and maybe even intestinal capillaries. Craig's studies were criticised at the time because the fluid itself did not have a high protein concentration while fluid derived from intestinal lymph during cholera secretion had a higher concentration. This may only reflect the fact that skin capillaries are not as permeable to protein as intestinal capillaries while both undergo increased hydraulic conductance. Blister fluid is known to have a lower colloid osmotic pressure of 5 mmHg [24] than intestinal interstitial fluid of about 10 mmHg [25].

It is noteworthy that cholera stool filtrates affect skin and other capillaries. Studies done later in another context [26] reprising the concept of pseudo-menstruation found in about two thirds of women cholera victims in the 19th Century [18] showed enhanced endometrial vascular permeability after intravenous 125-I-labelled bovine serum albumin injection in the uteri of rats exposed to cholera toxin. It is clear that cholera toxin increases permeation to fluid and dye stuff in non-intestinal capillary beds which perhaps the focus on intestinal pathology caused to be overlooked.

A direct demonstration of vasodilatation with vascular leakage in intestinal capillaries within the villi was achieved soon after the discovery of cholera enterotoxin in crude filtrates. The villi of rabbits given i.v. iron dextran, ferritin and carbon black (Indian ink) and exposed *in vivo* to choleragen showed iron deposits on the venular side of the vasculature, and also ferritin throughout the villus, although carbon black did not accumulate [27,28].

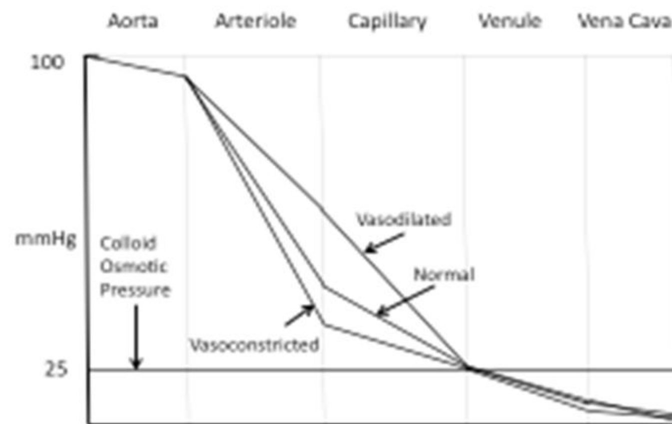

Fig. 3. Hydrostatic and osmotic pressures within the vasculature

Sub-epithelial capillaries in the villus tips though not in the crypts were also found to be dilated in dog loops exposed to live vibrios with some villus oedema, although attributed to i.v. saline perfusion. The mucosa was intact and there was no substantial protein loss from out of the capillaries as lymph protein concentrations were similarly low [29]. The authors did not emphasise the significance of capillary dilatation but instead focused on the lack of damage to the mucosa and the lack of protein in the exudate.

6. THE PERSISTENCE OF AN INTACT INTESTINAL EPITHELIUM DURING PERIODS OF FLUID SECRETION

The intact epithelium in cholera did disprove the exudate theory but not the transudate theory of the origin of secreted fluid. A convincing display of the ability of cholera toxin to cause vasodilatation of submucosal capillaries occurred in guinea pigs that were exposed to Cholera vibrios *in vivo* [30]. Tissue taken for electron microscopy showed dilatation of sub-mucosal capillaries with the epithelium completely intact, even when so much fluid had been secreted that the haematocrit was raised. Between the epithelium and the dilatated capillaries were areas of fluid consistent with increased formation of interstitial fluid and swollen vesicles within cells, giving the whole tissue a water-logged appearance. This is more consistent with a pathological entry of fluid into the post-enterocyte space because of raised capillary pressure that eventually increases interstitial space pressure to an extent that separation of the lamina propria from the epithelium arises. The likelihood that interstitial fluid pressure is very close to intracapillary pressure can be calculated from a knowledge of the hydraulic conductivity of the capillary relative to the epithelium.

A less likely explanation for the existence of sub-epithelial spaces is an accelerated formation of fluid by the enterocytes. Secretion might be expected to pull fluid away from the sub-epithelial space and reduce interstitial fluid pressure, with no obvious reason for the accumulation of cellular free spaces below the epithelium. Suction of fluid from that space by excess osmolarity from chloride secreted at the brush border should reduce not increase interstitial pressure.

The same paper details five cases of cholera in infants in whom congestion of the blood vessels “in the lamina propria and the sub-mucosa was the rule”. Similarly, congestion of the pulmonary capillaries without pulmonary oedema was common to all cases, again implying a widespread vascular event after Vibrio exposure rather than an effect of cholera solely on the intestinal epithelium.

The absence of dilatation in crypt capillaries and its presence in villus capillaries was proposed [29] to be consistent with fluid secretion from the crypts “which represents the chief site of net fluid loss in cholera”. Similar mid villus expansion of inter-epithelial spaces was detected in biopsies from humans [31] but capillary vasodilatation was only confirmed in human studies relatively recently [32], too late to have influenced the consolidation of opinion behind the view based on research after 1971 that secreted fluid came from the enterocytes.

7. INTERPRETATION OF RESEARCH PRIOR TO 1975 IN THE CONTEXT OF THE STARLING FORCES

It is useful to review here the factors in Starling's equation that are relevant to an assessment both of the filtration concept of pathological fluid secretion in diarrhoeal disease and the evidence against it. A second major false assumption that permeated secretion research during this period was that if filtration forces and increased permeability accounted for fluid secretion, then it should be possible to detect enhanced permeability using marker substances. The false assumption is to equate the permeability of solutes, however large their molecular diameter, to solvent permeability (hydraulic conductivity). This then completely obscures the fact that large increases in pathway diameter that facilitate fluid movement do not facilitate automatically the movement of any solute whose molecular diameter exceeds the relevant pore size.

7.1 Assessing Solute Permeability as a Proxy for the Hydraulic Permeability of Capillaries (Kfc) and Tight Junctional Conductance

In order for fluid to move from the capillary to the lumen by physical means at the rates that characterise cholera, there must be an overall increase in hydraulic conductivity. As the capillary pore and the tight junctions are in series, the increased hydraulic conductivity would have to occur at one or both sites, most probably at the tight junctions between the enterocytes. These pores should not allow protein past the luminal surface but it is not a necessary corollary that there should be demonstrable permeation of solute.

An increase in the capillary filtration coefficient, Kfc, might be required in order to increase the rate of formation of interstitial fluid, at no additional elevation in capillary pressure. The hyperaemia and vasodilatation in cholera sufferers means that arteriolar dilatation has occurred and as a consequence, mean capillary pressure in the intestinal capillaries within the villi would be higher than normal (see Fig. 3 and Appendix for explanation). There is also known to be a specialized microscopical architecture in the fenestrated intestinal capillaries in that the large gaps in them are orientated facing the epithelial cells [33,34]. It is the case that rapid fluid movement into the capillaries occurs on absorption depending on the pressure gradient but equally rapid loss will also occur given an appropriately aligned but reversed pressure difference. This arrangement of fenestrated capillaries is also found in salivary glands where large rates of fluid secretion can also occur.

A more likely site for enhanced hydraulic conductivity is the tight junction between neighbouring enterocytes, on the evidence of marker experiments. The observed increases in horse radish peroxidase [35] iron dextran [27] pontamine sky blue 6B dye [23], ferritin [28] and Evans Blue [36] permeation into the sub-epithelial space all imply an increased movement of marker substance from blood to interstitium in cholera. It is noteworthy that no marker enters the lumen. However, this does not prove that the filtration hypothesis is false.

The maximum inference from any of these studies is that large marker molecules do not pass more easily between the enterocytes. It is incorrect to conclude that there has been no increase in hydraulic conductivity. Any increase in the gap between epithelial cells, as tentatively found by Mathan and her colleagues [32] in cholera patients, may increase hydraulic conductivity but not necessarily increase permeability to larger molecules. An increase in Kfc, effectively analogous to the permeability coefficient for water, or increase in capillary pressure would be definitive but a lack of increase in the permeability of marker is insufficiently definitive to reject the filtration hypothesis. In addition, later work described increased intestinal permeability to fluorescent dextran after cholera toxin exposure in the rat [37]. Exposure to cholera toxin may eventually therefore increase capillary permeation to large molecules.

7.2 Physiological Control of Hydraulic Conductance

In the literature on marker permeation, experiments are often interpreted in terms of alterations in permeability while the variable that alters may in fact be a transcapillary pressure difference, rather than capillary or tight junctional permeability. Luminal perfusion with Evans Blue dye led to some absorption [38] that altered on intravenous injection of adrenergic agents. Uptake was enhanced by guanethidine and by terbutaline with the guanethidine increase prevented by propranolol (beta-blocker) but not by phentolamine (alpha-blocker). The common

feature here is vasoconstriction since terbutaline causes systemic vasodilatation that may be countered by intestinal vasoconstriction. Guanethidine does eventually lower blood pressure but there may be either an intestinal response of vasoconstriction to counter the lowered blood pressure or a direct effect since guanethidine competes for the re-uptake transporter and may potentiate adrenergic vasoconstriction. In these cases, the increase in Evans Blue dye absorption may result from vasoconstriction mediated enhanced fluid absorption and therefore entrainment in the fluid stream into the mucosa rather than any increase in permeability. It is therefore beyond the maximum permissible conclusion to assume that permeability has increased.

The concept of a direct effect of sympathetic nerves on hydraulic conductance had previously been considered but with the same neglect of the trans-capillary to lumen pressure gradient as a possible determining parameter [39]. In the Gothenberg preparation, varying the height of a fluid reservoir added to the luminal perfusion pressure or reduced it to suction pressure whenever the reservoir gantry was lowered below the level of the intestine. Fluid secretion fell on i.v. hexamethonium, fell on mesenteric nerve stimulation but was moderately reduced when mesenteric nerve stimulation was inhibited by perfusion of phentolamine.

Hydraulic conductivity was estimated from loop weight changes and step changes in luminal pressure, not from the essential but unmeasured transmural pressure gradient between lumen and capillary. The neglect of intracapillary pressure explains why sympathetic regulation of hydraulic conductance was thought to occur [39]. In reaching this conclusion, fluid secretion was divided by externally applied luminal pressure alone to estimate hydraulic conductance.

As clearly seen from Darcy's Law:-

$$F = C_{fc} \times (P_1 - P_2) \quad (2)$$

It is evident that the pressure difference between the mucosa and the capillary pressure is the crucial variable that has to be used.

This can be assessed if the capillary and likely interstitial pressure can be estimated (Appendix). From their data, it can be seen that zero net fluid movement occurs or can be estimated by extrapolation for all the circumstances that were examined. At zero net fluid movement, inward movement because of colloid osmotic pressure will match the difference between capillary pressure and applied luminal pressure. Assuming colloid osmotic pressure is 25 mmHg, capillary pressure can be estimated to be 38 mmHg in normal tissue, 33.4 mmHg when hexamethonium was given, 30.5 mmHg on mesenteric nerve stimulation and 31.5 mmHg when phentolamine was administered during mesenteric nerve stimulation.

Fluid flows therefore generally follow the calculated changes in capillary pressure. When the intestinal hydraulic conductivity is then estimated by substituting into the Starling equation, estimates are very similar for most of the experimental conditions indicating that the observed changes in the rate of fluid secretion could be better explained by the familiar actions of the sympathetic nerves altering arteriolar diameter. Exceptionally good fits to the presented data can be obtained by assuming a capillary filtration coefficient of 6.0 ul/min/100 cm^2 per mmHg.

It is the case that when suction pressure is applied, the calculated hydraulic coefficients are 50% higher than their positive pressure counterparts. However, it only takes a similar and slight increase in capillary pressure to reduce these estimates to their positive pressure counterparts. It seems very likely that applied sub-atmospheric pressure would draw fluid off quickly into the lumen leading to vasodilatation by physical means as the interstitial pressure fell. The widening of the capillary would mean that capillary pressure would rise. The apparently increased hydraulic conductivity is likely therefore to be increased capillary pressure caused by the non-physiological circumstance of suction in the small intestine.

It seems very likely that nor-adrenergic control of hydraulic conductance does not occur and that what is being shown is the dependence of the rate of fluid flow on capillary or interstitial pressure. In fact, what the experiments show is that jejunal fluid flows are very susceptible to internally and externally applied changes in the transcapillary hydrostatic pressure gradient, leading to fluid secretion that is not mediated by the enterocytes.

The ability of a capillary to lumen transmural pressure gradient rather than enterocyte

secretion to cause net fluid movement is supported by work on spontaneously hypertensive (SHR) rats. The secretion into the lumen was not inhibited by chloride channel blockers furosemide and bumetanide nor by acetazolamide [40] contradicting the chloride secretion hypothesis for secretion of enterocyte origin.

7.3 Inferences about Secretion from Altered Blood Flow

During this period, several attempts were made to relate blood flow to the effects of cholera secretion mainly to refute the filtration hypothesis. In this area, another logical error was to treat blood flow as synonymous with blood pressure and to fail to draw a distinction between arterial input and venous output.

From an ideal point of view, the capillary and the interstitial fluid pressure should be measured in normal and in cholera challenged intestine to give an accurate assessment of the driving force for volume flow. Measurements of mesenteric artery blood flow, even with inferences about the partition of flow into the sub-mucosal and mucosal component within the small intestine are not necessarily helpful here since these are not the pressure measurements that are required. In addition, it is possible for venous outflow not to change while loss of fluid into the lumen increases along with increased arterial (input) blood flow into the intestinal vasculature. This can be seen from the conservation equation for flow in the steady state since input (arterial) flow, F_1, would have to equal flow of fluid into the intestine, F_2, combined with output (venous) flow F_3. Since:-

F_1 (arterial inflow) = F_2 (fluid movement) + F_3 (venous outflow),

it is clear that F_3 can remain unchanged while F_2 and as a result, F_1 have increased. This relationship is not an abstraction that could in theory happen but in practice does not. Differences between arterial inflow and venous run-off have been detected in the intestine [41] with the difference closely correlated to whether the tissue absorbed or secreted fluid.

Measuring venous outflow alone is unhelpful and allows support for the cellular basis of secretion to be wrongly adduced when there is in fact no evidence for it. Flow translates into accurate estimates of pressure only when all other relevant hydrodynamic parameters are known, which they are not in the small intestinal vasculature. Measurements of capillary filtration coefficients based on knowledge of the pressure gradient are also required and this is also something unobtainable from marker substance permeation. Finally, post-arteriolar or mean capillary pressure requires also to be known, since inferences about likely capillary pressure based on the admittedly low mean arterial blood pressure in cholera, may mislead as may comparisons with other pathologies, such as portal hypertension. Mean arterial pressure can be severely reduced but capillary pressure can still be elevated above normal values. Hence, vasodilatation will still elevate intra-capillary pressure even when mean arterial blood pressure is pathologically low – so low, in fact, that a radial pulse can be absent and blood pressure cannot be measured.

7.4 Lack of Secretion in the Absence of Transmucosal Pressure Gradients

The converse experimental circumstance of the effect of a complete absence of capillary pressure on cholera filtration is provided by *in vitro* experiments. Loops exposed to cholera toxin *in vivo* showed net fluid secretion into the loop whereas control loops had no fluid in them. Everted sacs then made from cholera treated loops were unable to secrete fluid *in vitro* and absorbed fluid as well as normal tissues [42]. The secretion that was found *in vivo* was not found *in vitro*. The implication is that cholera secretion can only take place *in vivo* in the presence of a correctly aligned pressure gradient from capillary to lumen. The ability to detect changes in fluid absorption *in vivo* that cannot be reproduced *in vitro* also arises when the effect of shock or blood loss is investigated. In this case, loss of blood causes increased fluid absorption *in vivo* in the rabbit ileum [43]. This phenomenon also could not be reproduced *in vitro* using the everted sac technique [44]. It seems likely that the lack of correspondence is because perfused vasculature is absent in the *in vitro* preparation and no disadvantageous or even advantageous transmucosal pressure gradients can be generated.

7.5 Direct Assessment of Hydraulic Conductivity Rather than Marker Permeability

Since demonstrably increased mucosal hydraulic permeability is one factor that would help to verify the fluid filtration hypothesis of cholera fluid production, an attempt to assess this was made by the process of osmotically loading the lumen. The expectation was that with an osmotic load in the lumen, there would be more entry into the small intestinal lumen of fluid during an episode of cholera than would normally be the case. When mannitol was chosen, fluid accumulation in a cholera treated loop was about five times the rate in a normal loop exposed to the same osmotic load [45,46].

While the experimental principle is correct, cholera with an osmotic load was not compared with cholera without osmotic load and so the experiment is confounded by the fact that the cholera treated tissue would be secreting fluid anyway. However, smaller solutes had a lesser effect on fluid entry because they failed to exert their maximum osmotic effect as their reduced size began to approximate the pore diameter of the intestinal tight junctions. In effect, the reflection term, σ, gets lower for smaller solutes, and as solute penetrates the 'pore', it is not as effective an osmotic load as when it is able to exert its full osmotic strength. This aspect of osmosis is best understood by the equation that relates solute flow entrained in a fluid stream and to Staverman's reflection coefficient, σ, which is the ratio of real osmotic pressure to theoretical osmotic pressure of a given solute traversing a given membrane. In this case

$$J_i = J_V \{1- \sigma \} C_i \quad (3)$$

and if the solute expresses its full osmotic pressure at that membrane, then σ is 1.0 and there is no fluid entrainment of solute. As membranes become leakier, σ declines and more fluid entrainment is possible. The expectation was that σ declines on exposure to cholera enterotoxin.

In the case of cholera treated loops, it is likely that the intestinal or capillary intercellular pore radius had widened. This differential permeation because of molecular size is not unusual in physiological system: urea does not permeate renal membranes because they have a small pore size, whereas it readily permeates red cells. Red cells burst in the presence of external urea, acting as if the urea were exerting no osmotic pressure at all, since the reflection coefficient of the urea with that particular membrane is very low. If Love's findings are accepted, with some reserve, then they indicate an increase in tight junction permeability during an episode of cholera. This was also found less ambiguously in a study comparing the effect of a luminal hypertonic with a hypotonic mannitol load in cholera treated loops and in normal loops [47].Solute change was greater in hypertonic and hypotonic solutions in cholera treated loops compared with control loops and indicated a greater permeability in the cholera loop in both directions. There was also greater volume change in the cholera treated but hypertonically perfused dog loop implying greater hydraulic conductivity after cholera exposure.

8. KEY RESEARCH FROM 1960 ONWARDS ATTEMPTING TO FALSIFY THE FILTRATION HYPOTHESIS

8.1 Mesenteric Blood Flow and Secretion *in vivo*

During the mid-1960s, the relationship between intestinal blood flow and electrolyte loss in cholera was examined since the mechanism of fluid and electrolyte loss had not yet been 'clearly delineated' [48]. As a result, the relationship between superior mesenteric artery blood flow and electrolyte loss was measured with pre-implanted blood flow sensors in anaesthetised dogs given cholera enterotoxin. Inflow of fluid into the small intestine (Fig. 4) occurred in every case with a rise in haematocrit and a fall in plasma bicarbonate concentrations. However, superior mesenteric artery flow rates declined by about 70% while mean arterial blood pressure was approximately 30% of control values.

As the rate of production of fluid was independent of mesenteric blood flow and mean arterial blood pressure, the authors concluded that 'passive movement of fluid from blood to gut lumen by a filtration mechanism is not an important mechanism of loss of fluid in canine cholera'. An inspection of Starling's equation readily shows this to be a *non sequitur*. Superior mesenteric artery blood flow is a measured volume variable that does not report on capillary pressure, the essential variable that must be known before falsifying the filtration hypothesis.

Given secretion of fluid into the lumen, as occurred in every dog, the consequence of fluid loss from the circulation would be a fall in mean arterial blood pressure that would undergo compensation. To maintain arterial blood pressure, peripheral resistance would have increased which reduced but could not stop fluid secretion into the lumen. With the intense vasoconstriction that should occur with fluid and therefore circulating volume loss, the lower capillary pressure (Figs. 3 & 4) would produce less interstitial fluid. In every case, a dramatic rebound of secretion occurred when intravenous fluid was given i.e. when administration of i.v. fluid restored blood volume and pressure, there was no longer the physiological necessity of intense vasoconstriction to maintain blood pressure.

The relationship between mean arterial blood pressure and capillary pressure is not made clearer by measuring superior mesenteric artery blood flow. The fluid filtration hypothesis requires a positive outwardly directed gradient of pressure, with luminal pressure less that sub-epithelial pressure which must itself be less than intra-capillary pressure. With complete vasodilatation after cholera toxin exposure, capillary pressure should rise to resemble the severely reduced mean arterial blood pressure even if this is pathologically low at, for arguments sake, 40 mmHg or so, such that an external pulse is no longer measurable at the wrist in a human patient. The gradient for secretion of fluid is still correctly aligned to allow fluid loss into the lumen.

Even with compensatory vasoconstriction, if this were possible in the cholera challenged arterioles, the intracapillary pressure might fall to a low value but this is still likely to exceed luminal pressure. Only when there was no hydraulic conductivity would the gradient no longer drive water movement. All other states allow fluid movement downhill in a passive system even when the upstream pressure is low. The very important studies of Carpenter and colleagues [48] are a milestone in the historical sequence of significant *in vivo* studies on cholera action but were incorrectly interpreted at the time because of the assumption that measurement of mesenteric blood flow could give insight into capillary pressure.

8.2 Morphological Change in Cholera Treated Loops and in Normal Loops after Applied Pressure Gradients

The emphasis on the falseness of the filtration hypothesis of cholera secretion and its replacement by an enterocyte based model is arguably attributable to the advocacy of Hendrix [12] against filtration and also the large volume of *in vitro* experimental work done by Field [49] and colleagues in favour of the alternative model of secretion emanating from the enterocytes. Restricting discussion for the moment to the arguments marshalled against filtration, these can be tested against the expected outcomes predicted by the Starling equation. Blood volume expansion by jugular vein infusion in rabbits caused secretion of fluid into the lumen but not to

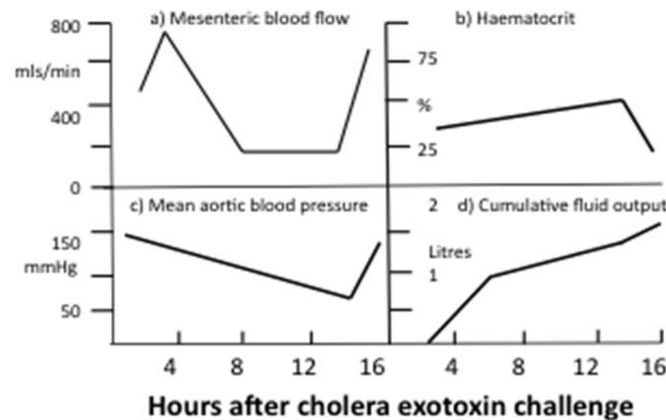

Fig. 4. Effect of cholera toxin on intestinal blood flow, blood pressure, haematocrit and secreted fluid in the dog intestine [48]

the extent that cholera toxin could achieve in non volume-loaded rabbits [50]. Gaps were found between the enterocytes in the volume expanded animals but not at all in those perfused with cholera toxin.

These morphological changes were deemed to be incompatible with toxin-stimulated fluid movement by increased hydrostatic pressure. However, it seems likely that applied pressure by volume expansion in normal loops with applied pressure would increase the sub-epithelial volume and pressure in the mucosa, pushing cells apart slightly. Analogous with the Kfc term in the Starling equation for capillaries, a modest increase in the hydraulic permeability constant at the intestinal tight junctions in cholera would allow fluid to flow past the epithelial cells and not form spaces between the enterocytes. There would not be sub-epithelial volume expansion that was sustained by fluid entry into the interstitial space but against closed tight junctions in the normal intestine. In the cholera challenged intestine, fluid would flow into the lumen at faster rate, given opening of tight junctions, until the pressure gradient across the epithelium had become nil. At this point, intra-loop pressure in tied-off loops would be close to sub-epithelial space pressure and this could be anywhere between 40 to 45 mmHg, representing a standing column of 60 cm of water would undoubtedly cause an intestinal loop to bulge. This phenomenon [51] is often presented visually as incontrovertible proof of enterocyte secretion, especially compelling when neighbouring loops do not swell. However the point made here is that this swelling is dependent on capillary pressure and cannot be reproduced *in vitro*. It can only be generated *in vivo* (Fig. 5) and displayed *in vitro* but there are no purely *in vitro* experiments of closed intestinal loops swelling with secretion to the extent that loops become distended.

Enhanced passage of fluid between capillaries and the interstitial volume need not cause the epithelial cells to be pushed apart. With an increase in hydraulic conductivity that is now known to occur in cholera and is caused by zona occludens toxin [52] an increase in sub-epithelial pressure is not sustainable if there is leakage of fluid into the intestinal lumen. For this reason, dilated lateral spaces are not detected in cholera treated intestine and the imposed pressure gradient not leading to increased inter-villus width is an inappropriate analogy. Instead, there is rapid equalisation of pressure as fluid is extruded into the loop. This manifests itself as copious diarrhoea in open loop preparations of intestine but also as bulging loops in experimental loops that have been tied off. The swollen state of the tied-off loop

Fig. 5. Effect of cholera toxin *in vivo* on rabbit ileal loops

The lower loop (N) was left unfilled. The loop marked (S) was initially filed with saline. The two loops (C) above loop (S) were filled with cholera toxin and became distended. From [51], with permission

in vitro reflects the fact that the volume expands until loop lumen pressure and sub-epithelial pressure equalize, not because active fluid secretion from the enterocyte takes place.

8.3 Marker Experiments

Further objections to filtration included the observation that intravenous injections of mannitol in cholera patients did not lead to greater concentrations of mannitol in the stool when compared to normal subjects [53]. It is again difficult to agree with the authors that this is strong evidence against filtration because measurement of increased hydraulic conductivity is required not the intestinal permeability of an arbitrarily selected solute that is larger than water. We are left with the faulty argument that because no change in mannitol permeability was detected, no change in water permeability occurred.

An aspect of the work of Gordon et al. [53] was that mannitol in the lumen drew fluid into it but only to the extent that the assumed hydraulic conductance was 0.3 mls/min/milliosmole and that an osmotic force of 20 milliosmoles would be required to drive this extent of fluid movement into the lumen. The estimate of water permeability is taken from Fordtran et al. [54] but is at least an order of magnitude higher than the stated values. In addition both groups seem to be assuming that the net fluid uptake is very small in the absence of glucose. In fact, when fluid absorption from isotonic and non-glucose containing perfusates in human intestine was studied, net fluid absorption was found to be 1.8 mls/cm/hr [55] or 54 mls per hour in a 30 cm loop.

In the case of imposed mannitol hypertonicity, the water permeation constant was calculated to be 0.005 mls/min/milliosmole [54] for the mid-jejunum. In this case, 0.45 mls/hour/milliosmole was drawn back into the lumen by the osmotic load for every milliosmole of adverse osmotic gradient. In contrast, an absorbing loop would remove 54 mls per hour. The adverse osmotic load changes net fluid flow by a factor of 100. A recalculation of the water permeability coefficient would then be 0.5 mls/min/milliosmole or 45 mls/hr per 30 cm loop. One milliosmole of osmotic difference could achieve this. Converting osmotic pressure to hydrostatic pressure difference gives a value of 17 mm Hg needed to achieve this extent of fluid movement.

According to Hendrix, reprising Gordon's [53] calculations, the necessary hydrostatic pressure gradient would be 380 mmHg which was clearly not feasible as this is about three times normal systolic pressure. Gordon's argument rested on the assumption that a perfusate without glucose would achieve close to zero absorption and that it was unlikely that there was any significant absorption against which mannitol exerted its osmotic strength. In fact, as others have shown, fluid absorption from an isotonic perfusate not containing glucose was substantial - sufficient to have caused Gordon to underestimate his hydraulic permeability by a factor of 10. This would reduce his calculated required osmotic equivalent pressure to 38 mmHg which is arguably within the range of intra-capillary pressure, after vasodilatation. Given that cholera toxin is likely to enhance hydraulic conductivity, the required equivalent pressure might be as low as 10-12 mmHg. It is evident that the slender numerical argument proposed above for the required capillary pressure to be 380 mmHg could only be accepted at a time when the enterocyte secretion paradigm was unchallenged.

Similar permeability arguments [56] were proposed for relative absorption rates of arabinose and urea in cholera patients but the lack of difference in ratios of solute absorption again does not disprove increased hydraulic permeability. In this case, Renkin's equation relating solute molecular diameter to pore size was used in an attempt to determine the intestinal pore size that urea and arabinose would pass in control patients and in those suffering from cholera.

The assumption is that there is one pore only, not several pores in series, and also that both solutes pass through this common pore. The proof of no change in solute permeability was the fact that amphotericin B increased the arabinose to urea permeability ratio while cholera did not. Notwithstanding minor errors in their table (Table 1 of their paper) which shows small changes in permeability ratio, the data can be reanalysed through an analysis of sensitivity to fluid entrainment since cholera causes fluid secretion. It can be seen that urea permeability is more strongly linked to fluid transport than arabinose, implying dependence on fluid entrainment for urea perhaps between cells whereas arabinose is only weakly sensitive to fluid movement, implying permeation through the cell membranes. The increase in urea permeation

after amphotericin B perfusion is almost exactly that predicted by reliance for the increase on fluid uptake through entrainment while for arabinose there is a discontinuity and an increase in permeation consistent with a new pore being formed.

Hendrix attempted to resolve the permeability question by giving intravenous injections of pairs of radio-labelled markers, including creatinine and tritium, urea and tritium, and lactose and tritiated water in normal, mannitol loaded and cholera treated intestine [57]. For the creatinine: tritium pair (Fig. 6), control and hypertonic mannitol perfused intestine give parallel values and it seems unlikely that mannitol itself affected permeability.

However, in the Hendrix data, it can be seen that the slope of the cholera treated regression line is shallower. What this also implies, contrary to the authors' proffered view, is that for any given rate of creatinine entry into the lumen, there is a greater intestinal lumen to plasma ratio of tritium, implying a greater rate of water entry – hence there may have been increased hydraulic conductivity in the cholera treated loops but without increased permeation of the creatinine marker. This can be seen from (Fig. 6) by drawing a line horizontally across the regression lines representing a fixed creatinine intestinal to plasma ratio, in this case arbitrarily at a ratio of 0.05 and noting that the associated tritium ratio is 0.42 in the normal loop. The same creatinine ratio projects to 0.56 for the cholera treated loops regression line. Hence for a given concentration of tritium label in the plasma, there is a greater concentration of label in the intestine, implying a one third increase in tritium (hydraulic) permeability.

No significant change in hydraulic conductivity in cholera has sometimes been reported [58] but this is inferred from the ratio of permeation into the lumen of two solutes. The properties of solute are not relevant to hydraulic i.e. solvent conductivity, but led to the fallacious proposal that solvent conductivity did not change. In addition, conductivity was not measured directly through precise knowledge of interstitial space to intestinal lumen pressure difference.

It is particularly striking in the Sherer [57] data (Table II) that the lactose intestine to plasma concentration ration is unaltered by cholera treatment and an osmotic load of mannitol in the lumen but the tritium intestine to blood label ratio increases very significantly by 50% after cholera exposure and to a lesser extent by mannitol luminal loading (column 6 of Table II). Similarly, the increase in tritium but not lactose label flux implies that cholera toxin increased paracellular pathway width sufficient to facilitate water but not disaccharide permeation.

In conclusion, lack of increased hydraulic conductivity and lack of increased capillary pressure after exposure to cholera toxin were often claimed during this period of investigation. This was on presented evidence that would not now be considered to have offered definitive proof that increased hydraulic conductivity was not involved.

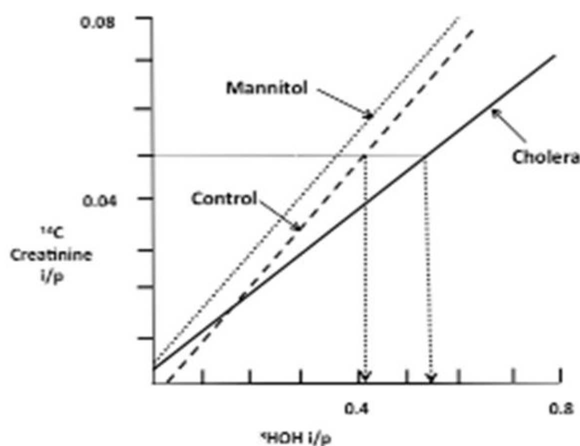

Fig. 6. Effect of cholera toxin on the creatinine to tritium intestine to plasma ratios (from Hendrix 1974)

The presented data indicate that increased hydraulic conductivity may well have arisen as was seen from tritium studies. This was not inferred from the studies on solutes unrelated to the solvent but tritium must be a better isotope to use to mirror fluid movement as it is a hydrogen ion isotope. The permeability studies that did not therefore seem to present a challenge to the enterocyte secretion concept in fact offer support for the concept of increased hydraulic conductance.

9. HENDRIX'S 1984 REBUTTAL OF THE VASO-DILATATION HYPOTHESIS

Before leaving the concept of work done prior to 1974, it is of interest to examine in detail, a comprehensive rebuttal written by Hendrix at a later date [59,60] essentially attempting to rebut the vasodilatation hypothesis once and for all. After that critique of the evidence for and against the filtration hypothesis, there were no further critiques of it and almost all work that was done, with notable exceptions, centred around validation of the enterocyte secretion hypothesis. The critical objections are detailed below.

With respect to 'increased calibre of epithelial "channels" (sic)', the observation that hypersecretion induced by cholera toxin is not associated with any change in the ionic composition of intestinal fluid is not compatible with the notion that cholera toxin increases the caliber of epithelial pores'. However, it is possible for intact epithelial to accelerate absorption and change ionic content whilst at the same time being challenged by secretion of plasma like fluid. The extent of alteration must depend on relative rates of selective absorption and non selective fluid secretion.

There is 'no difference in clearance of mannitol' but as already discussed, this cannot rule out an increase in hydraulic permeability. There is no 'change in permeability when comparing the ratios of two small uncharged water-soluble solutes' but again the same objection can be raised that solute permeability is not relevant to hydraulic conductivity. Similarly, the ratio between solute diffusional clearance and its convective permeability were unchanged in cholera but again why should it as again it is solute that is being studied? Finally, 'osmotic forces should change the composition of secreted fluid' but it is hard to see why this should be expected if all electrolyte movement is not hindered on the basis of molecular diameter in the normal and also the cholera treated pore.

Hendrix argued that osmotic forces can pull fluid out of the plasma into the lumen yet increased blood osmolarity does not pull fluid back towards the blood in the cholera state. Again, one must examine the Starling equation to see this as a non-sequitur because net flow is always a balance between osmotic and hydrostatic pressures and it is eminently possible that a higher capillary pressure extrudes fluid into the lumen against an adverse osmotic gradient, depending on the relative magnitude of both forces.

'Increased hydrostatic pressure leading to filtration through the epithelium has not been believed to play a role in the pathogenesis of cholera-induced secretion because in clinical cholera, diarrhea persists in the face of extreme hypovolemia and hypotension'. Yet it is clear that hypotension refers to mean arterial hypotension and the cited reference does not show a 30% reduction in mesenteric pressure but in flow. It is possible for mean arterial blood pressure to fall in cholera but for capillary pressure to rise because of vasodilatation in the cholera state, in accordance with the Bernoulli equation (Fig. 8). Finally, the solute argument is again reprised in that large molecules such as ferritin do not enter the intestinal lumen in cholera. This is answering a different question, namely is the increase in tight junction pathway size so big that it permits very large solute molecules to enter the lumen of the intestine? The answer may well be in the negative but it diverts from the fact that hydraulic conductivity may well have changed.

The existence of vasodilatation existing in cholera was conceded as well as increased mucosal blood flow but these changes are not associated with increased capillary permeability. This is a curious and erroneous conclusion to draw but one based firmly on non-sequiturs from the past literature. To understand this, we need look no further than Darcy's law which is a simplification of Starling's equation. Darcy [61] proposed that flow in pipes was determined by a resistance term and the pressure gradient along the pipe: -

$$F \text{ (flow)} = K \times (P_1 - P_2) \tag{4a}$$

where flow has the units of volume per time and pressure has the units of mmHg, with the proportionality term having dimensions of

mls/time/mmHg. When considering flow through rather than along capillaries and with the external pressure, P2, being the extra-capillary pressure, equation (3a) can be rewritten as:-

$$\text{Flow (into interstitial space)} = K_{fc} \times \Delta P_{c} \quad (4b)$$

From this, it can be seen that flow is the product of capillary filtration coefficient (often referred to as cfc in the Scandinavian literature) and capillary pressure gradient. It is only possible to specify a value for the capillary coefficient if the trans-capillary pressure gradient is known by measurement. Likewise it is only possible to specify what the capillary pressure gradient is if the capillary filtration coefficient or change in it is known. In the absence of actual measurement, it is insufficient to declare that one variable is constant and, in doing so, derive values for the other variable. This would be essentially presenting hypothetical values as data. However, this is an aspect of the literature that is cited by Hendrix [60].

An attempt was made to estimate the changes in capillary filtration coefficient [62] in normal and cholera treated denervated loops of cat intestine. Capillary filtration coefficient was stated to rise only slightly during cholera treatment but it is clear that the filtration coefficient could only be estimated, after assuming (section D of that paper) that the intracapillary pressure was 20 mmHg. It is of course possible that one or both factors changed but without capillary pressure measurements rather than inferences, the proposition that one variable changes while the other stays constant is impossible to verify.

The *non-sequiturs* are again evident when Darcy's equation is examined. There is no need for the capillary filtration to increase if capillary pressure increases. An increase in the driving force is enough to cause secretion, without need for the capillary constant to increase. In addition, the critical filtration coefficient is likely to be at the intestinal tight junction and not at the level of the capillary.

A final fallacy of multiplying entities without need is the statement that increased blood flow is a response to a requirement to keep the intestinal secretory mechanism supplied with fluid rather than a by-product of cholera-induced secretion. Since vasodilatation leads to increased capillary pressure and extrusion of fluid into the interstitial space, there is no evident need to supply with fluid a possibly non-existent active fluid secretion mechanism. A canal sluice gate needs only to be opened to flood a lock with water, there is no requirement for active pumping of water, given the existence of freely available energy in the form of hydrostatic pressure gradients.

Hence, a discussion of the likely purpose of vasodilatation and its possible requirement to supply a water requiring secretion mechanism requires unknowable intent. Just as likely is that Vibrio induces local VIP production to cause secretion to have a congenial environment.

No further authoritative opposition to the vaso-dilatation hypothesis seemed to arise after 1984 or is at least not known to this author but in retrospect, one recognises the *Leichtfertigkeit* associated with the above arguments. The exact rendering of this word is problematical but it conveys the concept (easily dispensed with) of a principal antagonist being easily satisfied with any arguments at all, however flimsy, against a disturbing idea that could nevertheless not convincingly be rebutted.

10. RELEVANCE OF THE STARLING FORCES TO CHOLERA AND OTHER DIARRHOEAL DISEASES

The filtration hypothesis for production of copious amounts of fluid that eventually enter the small intestine requires that the average hydrostatic pressure within the capillary rises above normal values. This causes more fluid than usual to enter the interstitial space and the assumption is also that increased hydraulic conductivity at the tight junctions may occur, allowing greater than normal fluid entry into the lumen. In the case of exposure to cholera toxin, the assumption is that arteriolar vasodilatation increases capillary pressure. This section considers the feasibility of pressure being a driving force for fluid secretion. This is compared with the metabolic cost of tissue that requires to secrete fluid at excessive rates and the likely energy demands made on such tissue. Finally, for the filtration mechanism to work, all that is required is that intracapillary pressure rises.

Capillary pressure could arise by blockage of the venules or veins by unicellular or multicellular parasites. If unicellular parasites cluster near the arteriole, the downstream capillary pressure is likely to be sub-normal and facilitate uptake of fluid from the interstitial volume. Blockage nearer the venular end of the capillary is likely to raise intracapillary pressure and cause secretion.

Worm infestations where the parasite resides in the mesenteric veins are also likely to cause secretion. Although this section only reviews aspects of cholera, the relevance of filtration to haemorrhagic and other tropical diseases is also evident.

10.1 Pressure and Flow Considerations

The fluid filtration hypothesis for genesis of secreted fluid assumes that the driving force for secretion is the transcapillary hydrostatic pressure gradient provided it exceeds the osmotic driving force drawing fluid back into the capillaries. Although the immediate proximate cause is the capillary hydrostatic gradient, secretion ultimately depends on the mechanical power derived from the heart and not on electrochemical energy originating within the epithelium cell. Cardiac output is 5 litres per minute at rest in the average person or about 7200 litres per day, of which 1800 litres can be expected to perfuse the intestine. As fluid production of about 12 litres per day is anticipated during a severe cholera episode, this does only represent leakage of about 0.67% of any volume of blood flowing per day through the intestinal vasculature. On an hourly basis, 0.5 litres of exudate should be produced, representing a 100 cm length of human intestine producing about 100 mls of fluid per hour or about 1000 ul/cm/hr. It seems therefore that passive filtration is sufficient to account for secretion and could be the sole means of achieving the high rates of fluid secretion of fluid secretion found in some diseases.

10.2 *In vivo* Experiments to Date

Preliminary studies on the relationship between arterial and therefore capillary pressure and fluid secretion [63] have demonstrated an excellent correlation between these variables, exactly as predicted by a consideration of the Starling forces across the intestinal capillaries.

In this study, fluid movement was measured in the rat proximal jejunum (Fig. 7). The possibility of fluid absorption in the jejunum *in vivo* in the anaesthetised rat was minimised by perfusing isotonic solutions in which all sodium ion was replaced by choline ion in order to prevent sodium ion absorption related fluid movement. In addition, the lumen was perfused with ethyl-iso-propyl-amiloride (EIPA) to prevent fluid uptake caused by sodium ion diffusion from the interstitial space into the lumen acting as a source of sodium ion for sodium ion dependent fluid absorption. Under these circumstances, fluid absorption was close to zero. If normal fluid secretion existed, dependent on sodium and chloride ion being available at the serosal pole of the enterocytes, then fluid should also move into the lumen. Under these circumstances, there was fluid absorption at about 20% of the normal value at normal diastolic and mean arterial blood pressure.

Diastolic blood pressure was then slowly reduced by intra-venous infusions by micrometer gauge of vasodilators (vasoactive intestinal polypeptide, acetyl--methyl choline and phentolamine) and a vasoconstrictor (arginine vasopressin), each of

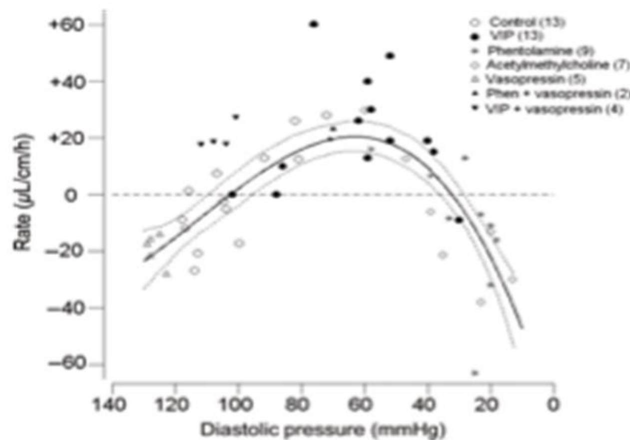

Fig. 7. The relationship between reduction in diastolic pressure *in vivo* and fluid movement in the non-absorbing small intestine

which acts through a different cellular mechanism. As the diastolic blood pressure fell to a lower value, fluid absorption changed to net fluid secretion. As diastolic pressure was lowered even further to stable values below 40 mmHg, the net fluid secretion reverted again to net absorption. The relationship between diastolic pressure and inferred capillary pressure was parabolic with secretion only occurring at lower than normal arterial pressures.

These results can be explained on the basis that vasodilatation increased the intestinal capillary pressure causing net filtration of fluid from the mesenteric capillary bed. Capillary pressure rose with falling diastolic pressure and arteriolar vasodilatation, exactly in accordance with Bernoulli's principle. With continuing and maintained vasodilatation but with further lowered diastolic pressure, capillary pressure fell again since, in a passive system, it cannot exceed arterial pressure and will fall as arteriolar pressure falls.

At low arterial and hence capillary pressures, oncotic pressure again exceeds capillary pressure and fluid movement caused by the change in Starling forces is inward again, instead of outward when capillary pressure exceeds the oncotic pressure. In essence, capillary pressure rises, as mean arterial pressure falls because of vasodilatation. A maximum in capillary pressure is reached after which capillary pressure falls as mean arterial blood pressure falls further. The parabolic nature of the relationship between declining mean arterial blood pressure and direction of fluid movement can be entirely attributed to the inferred capillary pressure changes.

The flow of fluid into the lumen became positive or negative depending on the diastolic arterial blood pressure. Secretion flow reaches a maximum after administration of vasodilator agents and declines linearly as diastolic pressure declines. A reasonable assumption is that while capillary pressure is normally a fraction of diastolic pressure on progressive vasodilatation, it will increasingly resemble this pressure as diastolic pressure falls. Hence, from the low pressure part of the relationship (Fig. 7), the intestinal membrane filtration coefficient is estimated to be 1.25 ul/cm/hr/mmHg per 4 grams wet weight of loop, giving 9.4 ul/hr/mmHg/g in the rat jejunum.

In the cat ileum [64] the capillary filtration coefficient was calculated to be 62.5 ul/min/mmHg in 30 cm long loops or approximately 125 ul/hr/mmHg/g. The filtration coefficient for the intestinal membrane is therefore about 7.5% of the capillary membrane.

If flow through small pores depends on the fourth power of the radius, the ratio of 13.4 in the calculated coefficients can be attributed to an approximate doubling of pore size in the capillary compared to the intestine. The intestinal capillary pore radius is assumed to be approximately 40 Å [5] meaning that the intestinal pore radius would be about 20 Å.

These figures are consistent with estimates of the filtration coefficient for the mucosal membrane when mannitol was used to draw fluid into the lumen in normal and cholera treated rabbit ileum [45,46]. In the cholera treated loops, pore size doubled from 6 Å to about 12 Å. It seems likely that the vasodilatation that occurs on perfusion with low sodium ion solutions [41] is reflected in an increased pore size and explains why low rates of fluid secretion can occur when the gut is perfused with low sodium ion containing perfusates.

With the intestinal filtration coefficient being very much lower than that of the capillary, interstitial pressure is likely to be close to and determined by capillary pressure. This means that vasodilatation and intestinal tight junction hydraulic permeability are the two most important variables that determine fluid secretion. Further, that fluid flows into the lumen are rate limited by the filtration coefficient of the mucosal membrane rather than that of the capillary endothelium.

This simplified vasodilatation model of secretion indicates that fluid secretion pathology in cholera and other disease causing vasodilatation would therefore be severe given an increase in capillary pressure but devastating when accompanied by alterations in the intestinal hydraulic conductivity coefficient.

The blood pressure argument points to filtration being the cause of secretion into the lumen in several disease states where the presence of enterotoxin in the intestine lowers the blood pressure. This is undoubtedly the case with exposure to cholera toxin [65] but is not the case with *E. coli* STa enterotoxin, since this does not reduce arterial blood pressure or cause net secretion. In addition, the requirement that there should be an intact vasculature in order for the capillary pressure effect to manifest itself, also is

supported by the very fundamental but frequently overlooked finding that the actions of cholera toxin [42] and VIP can be detected *in vivo* when there is an intact blood supply but cannot be reproduced in various *in vitro* preparations when net transfer of fluid is measured in the absence of any capillary pressure.

One might infer secretion from misleading short-circuit current and 'unidirectional' flux measurement experiments but the fundamental basis of secretion, net mass movement of fluid, cannot be demonstrated when this is sought in various *in vitro* preparations that necessarily lack vasculature with normal capillary pressures within them. Indeed, it is impossible to demonstrate the anti-absorptive effect of *E. coli* STa *in vitro* since the maintenance of tissue integrity requires the presence of glucose in the incubation fluid and this overcomes any effects of STa on NHE:3 driven fluid absorption.

Most if not all *in vitro* experiments have therefore of necessity to be done in an incubation solution that resembles a dilute oral rehydration solution. In contrast, when the blood supply is intact by using *in vivo* preparations, secretion can be shown to arise when there is vasodilatation, leading to increased capillary pressure and also possibly an increase in the capillary filtration coefficient, although the increased pressure gradient directed towards the lumen is the decisive variable that requires to change in diarrhoeal disease states, where the epithelial layer is still intact. Loss of epithelial integrity would lead to diarrhoea but this would still be a phenomenon driven by capillary pressure considerations.

10.3 Metabolic Considerations

The rate of ionic secretion from enterocytes assuming that fluid secretion was close to isotonic can be calculated in order to estimate the associated metabolic cost. Rates of secretion in cholera would require rates of oxidative metabolism within the enterocytes that seem unlikely to be achieved. An estimate of secretory capacity in man can be inferred from studies on glucose absorption in human subjects. When a 140 mM solution of glucose is perfused through a 30 cm jejunal loop, fluid absorption occurs at a rate of 300 mls/30 cm loop/hr [66]. The rate of delivery of substrate is likely to be 28 times higher than from blood and in proportion would restrict substrate access to a secreting cell by this amount. Assuming an enterocyte can work in reverse, this could achieve 2.5 litres per day per 3 metres of small intestine. While this is a severe loss, it is some way off still from very large rates of 10 or more litres seen at the height of a cholera episode. In some cases, there has been an hourly rate that translated into 19 litres per day (Carpenter, [67] Fig. 3, Group 1, chapter 7).

In addition, no differences have been found between rates of oxidative metabolism in cholera treated and in normal tissue from everted rabbit jejunum [51] regardless of whether there was *in vivo* or *in vitro* exposure to toxin [68]. There are however increased rates of glycolysis of the order of 30% in cholera treated animals *in vivo* but not when enterotoxin was added *in vitro* [69] Evidently, any increase in short-circuit current will also not be due to increased rates of metabolism since short-circuit current too is an *in vitro* measured variable and does not seem to assume very high values in cholera treated tissues.

The oxidative burden associated with tissues exposed to cholera of about a 30% increase in metabolic rate is more consistent with the switch to increased rates of absorption in the face of secretion or a change to a more energy costly type of ion transport. An energy cost after a switch to energetically costlier transport systems does occur in the kidney [70].

The difficulty with interpretation of *in vivo* work is that while there may be a primary action of an agent on the system under investigation, what may subsequently be measured is the physiological response to the original stimulus. The increase in lactic acid production in cholera *in vivo* but not *in vitro* may reflect the vasomotor responses to cholera challenge. It is known that cholera toxin increases the production of VIP which then causes vasodilatation and the possibility of fluid secretion by the mechanism proposed by the filtration model.

A compensatory response to imposed vasodilatation is likely to be vasoconstriction through local nor-adrenaline production rather than direct interference with VIP production, although this is also possible. It is the case that adrenaline and nor-adrenaline cause a tenfold increase in lactic acid production *in vitro* in the mouse intestine [71]. It is likely therefore that the effects of cholera enterotoxin on lactic acid production are only seen *in vivo* [69] because this is the only circumstance where catecholamine mediated vasoconstriction would

arise – this effect would not be seen *in vitro* because the vasodilatatory effect of VIP would not occur or would not be evident even if it did occur, nor would compensation via vasoconstriction. Hence, it is unlikely *in vitro* that there would be localised production of hormones that increase lactate production, if the effect of vasodilatation that occurs *in vivo* had not occurred.

11. CONCLUSION

In conclusion, there are increases in metabolism associated with cholera mediated secretion of fluid but these do not seem to account fully for energy demand that the rates of fluid secretion that can arise might impose on the enterocytes. In support of the vasodilatation model is the conjecture that fluid loss through increased capillary pressure imposes no additional energy cost on the mucosa. In contrast, compensatory mechanisms such as enhanced fluid absorption could increase the metabolic requirements of cholera challenged mucosa.

At present, a widely accepted paradigm for secretory diarrhoeal disease is the model that requires secretion of chloride ion towards the lumen by the enterocyte. In this model, there will be an osmotic imbalance at the brush border of the enterocyte and therefore an osmotic force that will pull fluid into the lumen. In this review, a second model of arteriolar and therefore capillary vasodilatation is presented that sets out the rival case that it is the arteriolar smooth muscle that is the ultimate target of *Vibrio cholerae*. This review indicates crucial historical moments at which time, critical errors of logic were made that wrongly identified the epithelial cell as the main point at which Vibrio enterotoxin acts. The theme in this review is that faulty arguments were made concerning the fundamental incorrectness of the vasodilatation model for secretion. Only when these arguments are rebutted can the strength of the vasodilatation argument be appreciated. Necessarily, therefore, resort has to be made to the historical literature as well as that of the recent past.

The conflation of fluid secretion because of increased hydraulic permeability with increased solute permeability can be seen to be a fundamental flaw that reoccurred many times in arguments against the vasodilatation model for secretion. Similarly, the focus on a biochemical model which requires that all can be explained by reference to events within the enterocyte can also be seen to exclude *a priori* any involvement of physical forces in the secretory process.

This review is not balanced in the usual sense that a current review attempts to summarise a present state of knowledge at the time of writing. Instead, it advocates the vasodilatation hypothesis, assembles evidence for it and challenges the historical evidence proffered against it. In consequence, supporting evidence in favour of the currently preferred enterocyte secretion model is necessarily also challenged. This review does so to draw attention to alternatives to the enterocyte chloride ion secretion model which is now a failing hypothesis that is not supported by evidence that rises to the level of proof. It has been remarkably unproductive given that it still has not led to diarrhoeal disease treatments based on the central tenet of suppression of excessive chloride ion secretion. It is likely that the reason for this is that enterocyte secretion is an incorrect hypothesis; pathological vasodilatation is likely to be how large volumes of fluid are secreted into the small intestine.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Lucas ML. Diarrhoeal disease through enterocyte secretion: A doctrine untroubled by proof. Exp Physiol. 2010;95:479-484.
2. Lucas ML, Thom MMM, Bradley JM, O'Reilly NF, McIlvenny TJ, Nelson YB. *Escherichia coli* heat stable (STa) enterotoxin and the upper small intestine: Lack of evidence *in vivo* for net fluid secretion. J. Mem. Biol. 2005;206:29–42.
3. Starling EH. On the absorption of fluid from the connective tissue spaces. J. Physiology. 1896;19:312-326.
4. Starling EH. Recent advances in the physiology of digestion. 2nd ed., Lecture 9, Constable, London; 1911.
5. Levick JR. Cardiovascular physiology. Edward Arnold, London. 2003;6.
6. Levick JR. Cardiovascular Physiology, Edward Arnold, London. 2009;258-275.
7. Guyton AC, Hall JE. Text book of medical physiology. 9th Edn. Saunders Press, New York, USA; 1966.
8. Vander AJ, Sherman JH, Luciano DS. Human physiology. Chapter 14. Figure 14-

50. Effect of arteriolar vasodilation. McGraw-Hill, Boston, Mass, USA; 1994.
9. Berne RM, Levy MN, Koeppen BM, Stanton BA. Physiology. Chap 27. The Microcirculation & Lymphatics. Mosby, St. Louis, U.S.A.; 1998.
10. Von Haller A. Elementa Physiologiae corporis humani. Tome, Berne, Switzerland. 1765;7:73-74.
11. Lieberkühn, J De Fabrica et actione villorum intestinorum tenuium Hominis (in translation). J Physiol Biochem. 2008;64(4):393-414.
12. Hendrix TR, Bayless TM. Intestinal secretion. Annual Rev Physiol. 1970;32: 139-164.
13. Becquerel A. Note relative à quelques analyses du sang, des vomissements, des évacuation alvines et des urines des cholériques. Arch gen Médicine. 1849;21: 192–206.
14. Cohnheim J. Vorlesungen über allgemeine Pathologie. August Hirschwald Press, Berlin, Germany. 2nd Edition. 1880;128-129.
15. Cohnheim J. Lectures on general pathology; Section III-IV Volume CXXXIII. The New Sydenham Society, London. 1890;949-960.
16. Wendt EA. Treatise on Asiatic cholera. William Wood & Co. New York, USA. 1885;226.
17. Störk O. Über cholera. Beiträge zur Pathologische Anatomie. 1916;62:9–174.
18. Pollitzer R. Cholera Monograph No: 43, Geneva, World Health Authority; 1959.
19. Koch R. An address on cholera and its bacillus. Brit Med J (ii). 1884;403-407.
20. De SN. Enterotoxicity of bacteria-free culture-filtrate of *Vibrio cholerae*. Nature. 1959;183:1553-1554.
21. Finkelstein RA, Norris HT, Dutta NK. Pathogenesis of experimental cholera in infant rabbits. I. Observations on the intraintestinal infection and experimental cholera produced with cell-free products. J Infect Dis. 1964;114:203–216.
22. Mallik KCB, Ganguli NC. Some observations on pathogenesis of cholera. Indian J Med Res. 1964;52:894-901.
23. Craig JP. A permeability factor (toxin) found in cholera stools and culture filtrates and its neutralization by convalescent sera. Nature. 1965;207:614–616.
24. Bork K. Physical forces in blister formation. The role of colloid osmotic pressure and of total osmolarity in fluid migration into the rising blister. J Invest Dermatol. 1978;71: 209-212.
25. Kvietys PR, Granger DN. Role of intestinal lymphatics in interstitial volume regulation and transmucosal water transport. Ann NY Acad. Sci. 2010;1207(S1):E29-E43.
26. Johnston MEA, Kennedy TG. Estrogen and uterine sensitization for the decidual cell reaction in the rat: Role of prostaglandin E2 and adenosine 3':5'-cyclic monophosphate. Biology of Reproduction. 1984;31:959–966.
27. Keusch GT, Atthasampunna P, Finkelstein RA. A vascular permeability defect in experimental cholera. Proc Soc Exp Biol Med. 1967;124:822–825.
28. Dalldorf FG, Keusch GT, Livingston HL. Transcellular permeability of capillaries in experimental cholera. Amer J Pathol. 1969;57:153–160.
29. Elliott HL, Carpenter CCJ, Sack RB, Yardley JH. Small bowel morphology in experimental canine cholera. Lab Invest. 1970;22:112–120.
30. Dammin GJ, Benenson AS, Feldman D, Formal SB, Goldstein HB, Merrill TG, Sprinz H. Clinical and histopathologic correlations in acute diarrheal disease. Proceedings of the cholera research symposium. SEATO, NIH and the University of Hawaii, US Government Printing Office. Washington, D. C, USA. 1965;205-211.
31. Asakura H, Tsuchiya M, Watanabe Y, Enomoto Y, Morita A, Morishita T, Fukumi H, Ohashi M, Castro A, Uylangco C. Electron microscopic study on the jejunal mucosa in cholera. Gut. 1974;15:531-544.
32. Mathan MM, Chandy G, Mathan VI. Ultrastructural changes in the upper small intestinal mucosa in patients with cholera. Gastroenterology. 1959;109:422–430.
33. Horstmann E. Über das endothel der zottenkapillaren im dünndarm des meerschweinchens und des menschen. Z. Zellforsch. Mikrosk. Anat. 1966;72:364-369.
34. Clementi F, Palade GE. Intestinal capillaries. I. Permeability to peroxidase and ferritin. J. Cell Biol. 1969;41:33-58.
35. Yardley JH, Brown GD. Horseradish peroxidase studies in the intestine in experimental cholera. Lab Invest. 1973;28: 482–493.
36. Lange S, Delbro DS, Jennische E, Johansson E, Lönnroth I. Recombinant or plasma-derived antisecretory factor inhibits

cholera toxin-induced increase in Evans blue permeation of rat intestinal capillaries. Dig. Dis. Sci. 1998;43:2061-2070.

37. Magnusson KE, Kihlström E, Sundqvist T. Effect of cholera toxin on rat intestinal permeability assessed with fluorescent dextran 3000. FEMS Microbiology Letters. 1985;29:15–18.
38. Lange S, Delbro DS. Adrenoceptor-mediated modulation of Evans blue dye permeation of rat small intestine. Dig. Dis. Sc. 1995;40:2623-2629.
39. Hemlin M, Sjövall H. Evidence for sympathetic regulation of the hydraulic conductance of rat jejunal mucosa *in vivo*. Acta Physiol Scand. 1989;135:459-467.
40. Tantisira MH, Sjövall H, Jodal M, Lundgren O. Intestinal fluid transport in the small intestine of the normotensive and spontaneously hypertensive rats: the importance of enteric nerves, chloride and bicarbonate secretion. Acta Physiol Scand. 1990;138:213-219.
41. Lee JS. Effects of pressures on water absorption and secretion in rat jejunum. Am. J. Physiol. 1973;224:1338–1344.
42. Strombeck DR. The production of intestinal fluid by cholera toxin in the rat. Proc Soc exp Biol Med. 1972;140:297–303.
43. Fromm, D. Intestinal absorption during hypovolemic shock. Ann. Surg. 1973;177: 448-453.
44. Bacalzo LV, Jr, Parkins FM, Miller LD, Parkins WM. Effect of prolonged hypovolemic shock on jejunal fluid and sodium transport. Surg Gyn Obstet. 1972; 134:399–417.
45. Love AHG. Permeability characteristics of the cholera infected small intestine. Gut. 1969a;10:105-107.
46. Love, AHG. Water and sodium absorption by the intestine in cholera. Gut. 1969b;10: 63-67.
47. Nalin, DR, Ally, K, Hare, R, Hare, K. Effect of cholera toxin on jejunal osmoregulation of mannitol solutions in dogs. J Infect Diseases. 1972;125:528-532.
48. Carpenter, CCJ, Greenough III, WB, Sack, RB. The relationship of superior mesenteric blood flow to gut electrolyte loss in experimental cholera. J. Infect. Dis. 119. 1969;182–193.
49. Field M. Intestinal secretion: Effect of cyclic AMP and its role in cholera. New Engl J Med. 1971;284:1137-1143.
50. Di Bona DR, Chen LC, Sharp GWG. A study of intercellular spaces in the rabbit jejunum during acute volume expansion and after treatment with cholera toxin. J Clin Invest. 1974;53:1300–1307.
51. Norris, HT, Majno G. On the role of the ileal epithelium in the pathogenesis of experimental cholera. Amer. J. Pathol. 1968;53:263-279.
52. Fasano A, Uzzau S, Fiore C, Margaretten K. The enterotoxic effect of zona occludens toxin on rabbit small intestine involves the paracellular pathway. Gastroenterology. 1997;112:839-846.
53. Gordon RS. Jr, Gardener JD, Kinzie JL. Low mannitol clearance into cholera stool against filtration as the source of stool fluid. Gastroenterology. 1972;63:407-412.
54. Fordtran, JS, Rector, FC. Jr, Ewton, MF, Soter, N., Kinney, J. Permeability characteristics of the human small intestine. J Clin Invest. 1965;44:1935 - 1944.
55. Petritsch W, Eherer AJ, Holzer-Petsche U, Hinterleitner T, Beubler E, Krejs GJ. Effect of cholera toxin on the human jejunum. Gut. 1992;33:1174-1178.
56. Rohde JE, Chen, LC. Permeability and selectivity of canine and human jejunum during cholera. Gut. 1972;13:191-196.
57. Scherer RW, Harper DT, Banwell JG, Hendrix TR. Absence of concurrent permeability changes in intestinal mucosa with secretion. Johns Hopkins Med J. 1974;134:156–167.
58. Lifson N, Hakim AA, Lender EJ. Effects of cholera toxin on intestinal permeability and transport interactions. Amer J Physiol. 1972; 223:1479–1487.
59. Hendrix TR. Cholera toxin and intestinal transport in “Intestinal Absorption and Malabsorption”. T. Z. Csaky, Editor. Raven Press, New York, USA. 1975;253-271.
60. Hendrix TR. The effect of cholera enterotoxin on intestinal permeability. Chapter 26 in “Pharmacology of Intestinal Permeation. Springer Press, Berlin, Germany. 1984;70/II.
61. Darcy, H. Les Fontaines Publiques de la Ville de Dijon. Dalmont, Paris, France; 1856.
62. Cedgård S, Hallbäck DA, Jodal M, Lundgren O, Redfors S. The effects of cholera toxin on intramural blood flow distribution and capillary hydraulic conductivity in the cat small intestine. Acta Physiol Scand. 1978;102:148-158.
63. Lucas ML, Morrison JD. An investigation into the relationship between small

intestinal fluid secretion and systemic arterial blood pressure in the anaesthetized rat. Physiological Reports. 2015;3:e12407:1-14.

64. Granger DN, Granger JP, Brace RA, Parker RE, Taylor AE. Analysis of the permeability characteristics of cat intestinal capillaries. Circ. Res. 1979;44:335–344.
65. Barrua D. A history of cholera. Chapter 1 in Cholera Eds: Barua, D, Greenough, W. B. III. Plenum Medical Book Co. New York & London; 1992.
66. Sladen GE. Conservation of fluid and electrolytes in the human gut. In Intestinal Absorption and its Derangements. J Clin. Pathology. Ed AM Dawson. BMA, London, UK. 1971;99-107.
67. Carpenter CCJ. Clinical and pathophysiologic features of diarrhea caused by *Vibrio cholerae* and Escherichia coli studies. Chapter 7 of Secretory Diarrhea. Eds Field, M, Fordtran, JS, Schultz, SG. Amer Physiol Soc, Bethesda, Maryland, USA; 1980.
68. Keusch GT, Rahal JJ, Weinstein L, Grady GF. Biochemical effects of cholera enterotoxin: Oxidative metabolism in the infant rabbit. Am. J. Physiol. 1970;218: 703–707.
69. Keusch GT, Weinstein L, Grady G. Biochemical effects of cholera enterotoxin. II. Glucose Metabolism in the Intestine of the Infant Rabbit. J Infect Dis. 1971;124: 188-193.
70. Deng A, Miracle CM, Lortie M, Satriano J, Gabbai FB, Munger KA, Thomson SC, Blantz RC. Kidney oxygen consumption, carbonic anhydrase and proton secretion. Amer J Physiol. 2006;290:F1009 – 1015.
71. Mary, PL, Rao, JP. Metabolic basis of catecholamine induced water transport in everted gut sacs of mouse intestine. Clin Exp Pharm Physiol. 1991;18:483-488.

Appendix: The relationship between arteriolar diameter and capillary pressure

The extent to which formation of interstitial fluid occurs depends on a minute to minute basis on the intracapillary pressure which is controlled by the extent of arteriolar vasodilatation in the arteriole preceding the capillary. The extent of interstitial volume is determined by production of fluid and its removal by the lymphatic system. In general, noradrenergic nerves cause vasoconstriction which will result in a reduction of intracapillary pressure and absorption of interstitial fluid. There are many other neurotransmitters that cause vasoconstriction or vasomotion because many competing neural systems control mean arterial blood pressure by altering peripheral resistance. A useful summary of the present state of knowledge of extrinsic neural control of arteriolar diameter can be found in Levick (2009).

Arteriolar vasomotion and also venular constriction can alter the balance between intracapillary pressure and interstitial pressure and hence whether fluid absorption or filtration occurs (Fig. 8). Also evident is the fact that longer term changes in capillary blood protein content such as the lowering in starvation will lead to oedema and possibly moderate fluid secretion. In the short term, it is the extent of arteriolar smooth muscle tone that determines the pressure inside the capillaries on a minute by minute basis. This can be seen easily by reference to a simplified form of the Bernoulli equation.

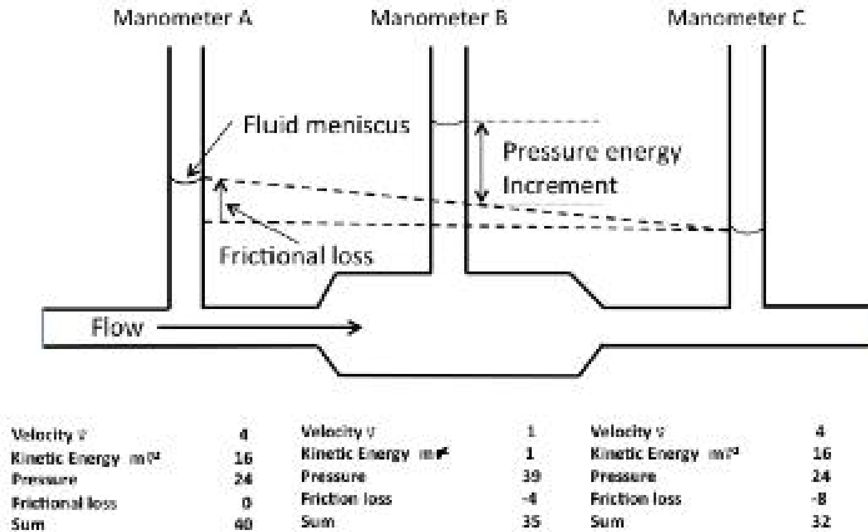

Fig. 8. Changes in pressure as fluid flows through a section of tubing of doubled radius

In a frictionless system i.e. where there are no losses in energy as fluid moves along a horizontally placed tube, the fluid has pressure and also kinetic energy, while gravity energy can be neglected. Total energy does not change since pressure and kinetic energy sum to a constant. Reductions in kinetic energy cause increases in pressure. The modified Bernoulli equation used here is:-

$$P_1 + mV_1^2 = P_2 + mV_2^2 \qquad (5)$$

In any system of tubing where a thin section expands to a wider section and then reverts back to a thin section again (Fig. 8), flow is equal in all sections but the velocity is not. For flow to be equal, as fluid enters a wider section, the velocity will be slower, given the wider cross section volume that the flow fills. In equation (6) it is clear that if the velocity V_2 in the wider section is slower than V_1 in the thinner section, the kinetic energy term is less and the pressure term P_2 must become larger than P_1. In the worked example, if input velocity falls from 4 to 1 arbitrary units, the kinetic energy falls to one sixteenth of the value it previously had. Since both forms of energy are required in this example to sum to 40 mmHg, the pressure in the wider section rises from 24 to 39 mmHg. A manometer placed

at site A would record 24 mmHg, manometer B would show 39 mmHg and at manometer C, the pressure would revert to the input pressure, given no frictional losses.

In a system with friction there would be a gradient in pressure between manometer A and manometer C such that the meniscus in manometer C would be lower. The pressure drop at manometer B would be halfway and the hydrostatic pressure would be added on to that. The diagram therefore shows the real case and the ideal case but it is evident that hydrostatic pressure rises when there is widening of the tubing. This means that vasodilatation will increase capillary pressure and also the formation of interstitial fluid while vasoconstriction will reduce capillary pressure and hence the rate of formation of interstitial fluid. Vasoconstriction of the arteriole through adrenergic vasoconstriction will counteract fluid loss into the interstitial space and hence into the intestinal lumen. In contrast vasodilatation caused by dilators such as acetylcholine or vasoactive intestinal polypeptide (VIP) will increase the formation of interstitial fluid.

In addition, local paracrine or autocrine factors (e.g. endothelins, adenosine, somatostatin and histamine) and indicators of the state of metabolism, (local partial pressure of oxygen and of carbon dioxide) also change arteriolar resistance, capillary pressure and therefore the rate of interstitial fluid formation. Venoconstriction will raise capillary pressure because of restricted venous outflow. Venoconstrictors such as vasopressin may therefore also increase interstitial fluid production. This action on capillary pressure would be independent of any action of these agents on the capillary filtration coefficient.

© 2018 Lucas; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23070