

**The status of an inversely coupled oxic/sulphidic oscillator (OSO) in the whole body chemocline will determine clinical unfolding in Systemic Inflammatory Response Syndrome (SIRS)\* of any aetiology.**

Paul Ramesh Thangaraja<sup>a,b</sup>

FRCS (Cardiothoracic Surgery)

**Abstract**

Life evolved in an euxinic world with subsequent oxic 'invasion' leading to two parallel but interconnected biospheres, hydrogen sulphide (H<sub>2</sub>S) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) exemplify these worlds respectively. Their concentration gradients have informational value in meromictic lakes. Similarly, it is posited, there exists a whole body chemocline in humans in which the two molecules form an inversely coupled oxic/sulphidic oscillator (OSO). The OSO is hormetic and characterised by a range of amplitudes and frequencies in health. Deviations from its baseline profile heralds the onset of SIRS before the appearance of clinical signs. Loss of oscillator status and transition to a steady state causes widespread intercellular and inter-organ communication failure presaging multi-organ dysfunction. The salient clinico-pathophysiological features of SIRS of any aetiology are emergent phenomena related to the OSO profile. Extent of recovery of organ function will mirror the recovery of the OSO profile thereby providing a tool to predict outcomes in SIRS.

**Keywords:** Sepsis, SIRS, oxic sulphidic oscillator, risk prediction, multiorgan failure, chemocline.

**Address for correspondence**

Dr. Paul Ramesh T,

Apollo Hospitals, 21, Greaves Lane, Greaves Road, Chennai - 600006, India.

Tel. No. +91-44-9840070824. Fax. No. +91-44-28290200

paulramesh@gmail.com

Affiliations:

<sup>a</sup>Dept. of Cardiothoracic Surgery, Apollo Hospital, Greams Road, Chennai - 600006, India.

<sup>b</sup>Dept. of Mechanical Engineering, IIT-Madras. Guindy, Chennai - 600036, India.

**The status of an inversely coupled oxic/sulphidic oscillator (OSO) in the whole body chemocline will determine clinical unfolding in Systemic Inflammatory Response Syndrome (SIRS)\* of any aetiology.**

*\*For the purposes of this article SIRS is the term used to describe the composite clinico-pathological entity that encompasses Systemic Inflammatory Response Syndrome (SIRS), Compensatory Anti-inflammatory Response Syndrome (CARES), and Mixed Antagonist Response Syndrome (MARS) of any aetiology infective (sepsis) or otherwise (SIRS) as the hypothesis proposed, in a sense, seeks to unify them.*

## INTRODUCTION

The prediction of organ dysfunction in patients with sepsis or SIRS is not possible with current risk score profiles or biomarkers. Hence they have little value in timing interventions especially ones with narrow safety margins such as initiation of steroids and extracorporeal membrane oxygenation (ECMO) therapy. There has been significant advances in the understanding of oxidant injury and sulphidic responses to it. However this information has not been harnessed to the bedside. A hypothesis that is anchored in ancient biochemical connections but providing a clinical context may help improve understanding of a complex clinical problem.

## THE HYPOTHESIS

It is posited that there exists, in humans, a whole body (corporeal) chemocline. The chemocline is a composite of reactive oxygen species (an oxycline) and reactive sulphur species (a sulphidocline) exemplified by hydrogen peroxide and hydrogen sulphide respectively.  $H_2O_2$  and  $H_2S$  form an inversely coupled oscillator. The maximum and minimum values of their concentrations represents the amplitude, the rate of change of concentrations represents the frequency of the oscillator. This oxic/sulphidic oscillator (OSO) on account of its relatively higher permeability across membranes is a fundamental signaller of the redox status of the whole body chemocline. The oscillator occupies

the bottom of an inverted pyramid of an informational cascade and has an intrinsic range of values in health. The status thus communicated is perceived intracellularly, inter-cellularly and between organs being vital to the integrative function at both organ and organismal level. The superposition of individual cellular OSO status will determine the organ level status. The superposition of the OSO status of individual organ systems will determine whole body status. The characteristic of the OSO is hormetic in nature. Lower amplitude and frequencies will signal health, higher amplitude and frequencies will signal transition to an acute disease state such as SIRS. Loss of variability (decomplexification) of the OSO meaning loss of ability to oscillate and reaching a steady state is associated with intercellular and inter-organ communication breakdown and portends organ failure. Degree of return of intrinsic variability will determine extent of recovery of organ function.

### **BASIS OF THE HYPOTHESIS**

The hypothesis is based on the contention that the energetics of the biosphere evolved initially in an euxinic world and that subsequent oxic 'invasion' set the stage for competing electron acceptors. This ancient relationship between the two molecules, while modified by eons of evolutionary adaptations, has provided an inversely coupled oscillator that functions as a primordial informational unit in humans. The logical basis of the assertion can be distilled into the composite question — is there currently any indication of a corporeal chemocline, if so does it oscillate, if it does oscillate does it convey information and could it predict outcomes in SIRS?

#### **Is there an indication for the existence of an oscillating inversely coupled chemocline?**

The entire biosphere inhabits an inversely coupled oxic/sulphidic vertical chemocline extending from the atmosphere to deep hydrothermal vents. Present day meromictic lakes[1] like Proterozoic oceans[2] are characterised and stratified by similar chemoclines wherein both molecules through their role as terminal electron acceptors of the electron transport chain (ETC) fuel life. The corporeal chemocline could be conceptualised as mirroring the ones found in meromictic lakes and extending from the intravascular compartment through the extracellular fluid (ECF) space to the inside of

the cell. The resistance of cell membranes to  $H_2O_2$  permeability[3] and differential distribution of the sulphane sulphur pool help maintain concentration gradients. The current best estimates of the concentrations of the constituents [4–8] of the corporeal chemocline are given in the **tabular column**. The interaction of the lake with its environment results in seasonal positional changes of the oxic/sulphidic transition zone as a result of nutrient trapping, down-swelling of oxygen and up-swelling of  $H_2S$ [9]. Similarly the corporeal chemocline bounded by an environment that extends from the oxygen rich lungs to the sulphide rich colon communicates to the outside via the aerodigestive tract. In general there is a net movement of  $H_2O_2$  from plasma towards the cell and  $H_2S$  from cell towards plasma[5,10]. Fluctuations in the established rhythm could be altered by external changes in alveolar gas (eg.  $H_2S$  inhalation), breakdown of the colonic barrier or increased production of  $H_2O_2$  intravascularly as during an oxidative burst.

#### **Is it a primordial informational unit?**

The presence of unicellular organisms like *E.proximas* in meromictic lakes corresponds to  $H_2S$  levels during seasonal oscillation of the transition zone[1]. Cable bacteria with exceptionally long ETCs[11] and *E.coli* with variable specificity ETCs[12] make the choice of electron acceptor based on availability of oxygen or sulphide. The fundamental nature of the biochemical interconnectedness of the two molecules [13] are shown in the **figure**.

#### **Is the oscillator hormetic in nature and could it signal health and disease ?**

The basis of this contention could be made by examining the data from various positions of the imagined chemocline — the two extreme locations, the colon and the lung and the major organ system that lies between and interacts with them both — the vascular system.

$H_2O_2$  at a lower concentration protects against colitis but facilitates bacterial invasion at high concentrations.[14] Serum  $H_2S$  levels remained constant in healthy controls across age groups but was inversely related to severity of COPD[15].

Both molecules display clear hormesis with regard to vasodilatation and constriction in resistance arteries[16,17].

A recent interesting study highlights an important aspect of the hypothesis. Lung function from rejected lung organ donors improved in function after inserting them in pigs[18] Here it is submitted that the donor lung in an OSO that has lost its variability (the brain dead donor) was dysfunctional and its reintroduction into the normal chemocline of the pig improved function. The above interactions suggest a role for the OSO as an indicator of health and disease.

### **PREDICTIONS MADE BY THE HYPOTHESIS**

#### **What would determine an individual patients OSO profile?**

The oscillator profile of a given patient would be measured by the rate of change of  $H_2O_2$  concentration (slope of incline), peak concentration, plateau concentration, time at plateau, rate of decrease of concentration, ability to reach baseline, difference of final concentration from baseline concentration. A similar  $H_2S$  profile will also be constructed and the time lag between the two ascertained.

The OSO trajectory will need to be defined in two time frames — the immediate OSO status (minutes to less than 1 hour of the stimulus) and the delayed OSO status (probably 6-24 hours but before onset of clinical organ dysfunction). Thus the composite OSO profile would consist of three components — baseline, immediate and delayed status. A hyper and hypo-oxidant profile each with a corresponding, hyper, normal and hypo-sulphidic response implies at least six basic profiles.

#### **Can it predict the clinical unfolding of SIRS?**

Transition from physiological to a pathophysiological state has been described as a loss of intrinsic variability and progression to a steady state (decomplexification)[19]. This decomplexification, of hitherto unidentified biological oscillators, was postulated as the reason for development of multi-organ dysfunction syndrome (MODS) in sepsis[20].  $H_2O_2$  and  $H_2S$  are prime candidates for the oscillator in SIRS as they reflect vigour of the oxidative burst, the rapidity of its communication through the chemocline and the sulphidic response. Baseline differences in the profile and speed of

subsequent loss of variability of the signal should generate distinct OSO profiles. These distinctive profiles should correlate with extant clinical and pathophysiological features of SIRS. This implies that children, adults with and without comorbidities, immunosuppressed patients would have different OSO profiles.

In addition certain striking clinical features — the increased incidence of neurological dysfunction in non infective vs infective SIRS inspite of the same crude mortality rate, the greater incidence of SIRS with head injury than other forms of trauma, patients who develop neurological complications during sepsis having poorer short and long term outcomes, the relative protection afforded to SIRS by BMI of a particular range, the wide range in mortality (15-71%) from cardiac arrest in sepsis — should correlate with distinctive OSO profiles.

### **TESTING THE HYPOTHESIS**

Given the questions raised about sensitivity and specificity of measurements of both molecules[5,7,21], the greatest challenge in testing the hypothesis would be the development of specific, accurate point of care devices.

#### **Clinical studies**

The profiles generated from blood levels of  $H_2O_2$  and  $H_2S$  in elective cardiac surgical patients would provide a practical clinical model to test the hypothesis for the following reasons:

1. Cardiopulmonary bypass (CPB) is associated with a substantial oxidative stress.
2. The baseline (pre-insult) status of the OSO and the time of the insult ie. surgery and CPB are determinable. This enables fold change measurement at different time points which if indexed to body mass, plasma protein level, free haemoglobin, pH, temperature, salinity could provide measurements that are comparable in other clinical situations.
3. Comparison of profiles with septic patients presenting at different points of the clinical syndrome will help determine equivalency of sepsis with the surgical SIRS.

**Testing convergence with pathophysiological features such as immunological, vascular and bioenergetic dysfunction.**

### **Immunological dysfunction**

The dysregulated immune response in SIRS could be described as an oscillator from SIRS to CARES through a variable MARS landscape[22]. H<sub>2</sub>O<sub>2</sub> has been shown to have both positive and negative feedback regulation of the inflammatory response[23]. IL-6 concentrations are elevated in patients who develop MODS from trauma induced SIRS[24] and H<sub>2</sub>S has been described as modifying the IL6 response in experimental studies[25].

Concomitant measurements of inflammatory biomarkers could ascertain correlation with OSO profiles.

### **Endothelial dysfunction (Vasoplegia and microcirculatory failure)**

The endothelial response is intimately connected to the immune response with two striking features, vasoplegia and microcirculatory failure. The latter expressed as both increased microvascular permeability and functional shunting.

A familiar clinical picture is the transition from normal blood pressure to hypotension to death producing a therapeutic response of volume resuscitation, low dose pressor, high dose pressor with corticosteroids, inotropes and finally an inability to maintain pressures in spite of maximal treatment. The ability of the profiles to discern between normal, responsive and unresponsive vasoplegic patients could be studied.

The maintenance of normal range ECF profile in a given organ in the face of a changing intravascular profile indicates the robustness of the endothelial barrier. The blood brain barrier[26] and the sinusoids of the liver represent contrasting endothelial permeability under normal conditions. Microcirculation imaging and organ specific capillary transit time could be valuable adjuncts to test the association of distinctive intravascular to ECF profiles with worsening of microvascular permeability and functional shunting.



### **Bioenergetic dysfunction**

Hyperlactemia, a feature of both post surgical SIRS[27] and sepsis[28] could be indicative of either inadequate oxygen delivery or an inability to use oxygen.  $H_2O_2$  may favour aerobic glycolysis as a less energetic but less catabolic pathway designed to aid recovery[29].

$H_2S$ , the first identified inorganic substrate, can at low concentrations be an adjunct to the Krebs cycle but at high concentrations produces profound bioenergetic failure by direct inhibition of cytochrome c oxidase[30].

An inability to trigger aerobic glycolysis resulting in a blunted lactate response alongside a histopathological correlation with less recoverable forms of cell death should have distinct OSO profiles[31].

### **Testing predictive accuracy compared to extant risk scores**

Current risk scores predict mortality based on evolving organ dysfunction and as such measure criticality. An ability to predict the onset of organ dysfunction would have value in timing therapeutic interventions (eg. steroids and mechanical support) for an optimal risk-benefit outcome. If the hypothesis holds, it should at best out perform current risk score prediction, in particular among low risk patients who die and high risk patients who survive, or at the least significantly enhance current risk score prediction.

### **Animal studies**

The hypothesis asserts that the oscillator is a network communicator, hence it is obligatory to test it in the intact animal or at the least an intact perfused organ system that is ensconced in a setup that can simulate the potential range of the oscillator.

## **CONCLUSION**

The hypothesis is mechanistic and reductionist in its attempt to explain a complex syndrome on the basis of a possible relationship between two simple molecules. It disregards the role of nitric oxide (NO) in the relationship. The envisaged chemocline in the intracellular region is over simplistic in

its lumping together of all intracellular organelles, except the mitochondria, into the term 'cytoplasm'. NO executes many of its vasomotor actions through interactions with  $H_2O_2$  and  $H_2S$ [32,33]. The simplistic intracellular portion of the chemocline was influenced by Searcy's sulphur hypothesis[34] which toggles the sulphur between oxidised and reduced forms between these two locales. Current risk scores and biomarkers are suboptimal for timing therapy. The expectation is that insight gained while testing the hypothesis could be useful in the development of a practical bedside test to time high risk interventions. Meanwhile 'that more intimate beauty' of investigating the possibility of an informational signal that extends back to the origins of life should be a reward in itself.

## **Acknowledgements**

The author wishes to thank - Jayashree Gopal, Sunder T, Madhankumar, Suresh, Ashis Sen, Muraleedharan, Madhulika Dixit, Uma Rani, Baskaran, Subrahmanyam , Rahul Siddharthan and Gautham Menon - for the conversations that helped evolve the idea over many years.

## **References**

- 1 Smayda JT, Thorne-Miller B, Tomas C. Annual phytoplankton cycle in a meromictic anoxic basin of a Rhode Island (USA) estuarine river. *Hydrobiologia* 2020; **847**: 501–518.
- 2 Canfield DE. A new model for Proterozoic ocean chemistry. *Nature* 1998; **396**: 450–53.
- 3 Folmer V, Pedroso N, Matias AC, Lopes S, Antunes F, Cyrne L, Marinho SH. H<sub>2</sub>O<sub>2</sub> induces rapid biophysical and permeability changes in the plasma membrane of *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta* 2008; **1778**: 1141–1147.
- 4 Peters S, Kronseder A, Karrasch S et al. Hydrogen peroxide in exhaled air: a source of error, a paradox and its resolution. *ERJ Open Res* 2016; **2**: 00052-2015.
- 5 Forman HJ, Bernardo A, Davies K. What is the concentration of hydrogen peroxide in blood and plasma? *Archives of Biochemistry and Biophysics* 2016; **603**: 48e53.
- 6 Knaus UG, Hertzberger R, Pircalabioruc G, Yousefib SP, Santos F. Pathogen control at the intestinal mucosa – H<sub>2</sub>O<sub>2</sub> to the rescue. *Gut Microbes* 2017; **8 (1)**: 67-74.
- 7 Furne J, Saeed A, Levitt MD. Whole tissue hydrogen sulfide concentrations are orders of magnitude lower than presently accepted values. *Am J Physiol Regul Integr Comp Physiol* 2008; **295**: R1479–R1485.
- 8 Jimenez M, Gil V, Martinez-Cutillas M, N Mañé N, Gallego D. Hydrogen sulphide as a signalling molecule regulating physiopathological processes in gastrointestinal motility. *British Journal of Pharmacology* 2017; **174**: 2805–2817.
- 9 Meyer KM, Kump LR. Oceanic Euxinia in Earth History: Causes and Consequences. *Annu. Rev. Earth Planet. Sci.* 2008; **36**: 251–88.
- 10 Wang R. Physiological implications of hydrogen sulfide: A whiff exploration that blossomed. *Physiol Rev* 2012; **92**: 791–896.
- 11 Bjerga JT, Boschker HT, Larsen S et al. Long-distance electron transport in individual, living

cable bacteria. *PNAS* 2018; **115 (22)**: 5786–5791.

12 Forte E, Giuffrè A. How bacteria breathe in hydrogen sulfide-rich environments. *Biochem (Lond)* 2016; **38 (5)**: 8–11.

13 Olson KR. Hydrogen sulfide, reactive sulfur species and coping with reactive oxygen species. *Free Radical Biology and Medicine* 2019; 140: 74–83.

14 Singha AK, Hertzbergerb RY, Knaus UG. Hydrogen peroxide production by lactobacilli promotes epithelial restitution during colitis. *Redox Biology* 2018;**16**: 11–20.

15 Chen Y, Yao W, Geng B et al. Endogenous Hydrogen Sulfide in Patients With COPD. *Chest* 2005; **128(5)**: 3205–3211.

16 Shimokawa H, Godo S. Nitric oxide and endothelium-dependent hyperpolarization mediated by hydrogen peroxide in health and disease.

*Basic Clin Pharmacol Toxicol.* 2020; **127**: 92–101.

17 Kanagy NL, Szabo C, Papapetropoulos A. Vascular biology of hydrogen sulfide. *Am J Physiol Cell Physiol.* 2017; **312(5)**: C537–C549.

18 Hozain AE, O'Neill JD, Pinezich MR. Xenogeneic cross-circulation for extracorporeal recovery of injured human lungs. *Nat Med* 2020;**26**: 1102–1113.

19 Lipsitz AL, Goldberger AL. Loss of 'Complexity' and Aging. Potential Applications of Fractals and Chaos Theory to Senescence. *JAMA* 1992; **267**: 1806–1809.

20 Godin PJ, Buchman TG. Uncoupling of biological oscillators: A complementary hypothesis concerning the pathogenesis of multiple organ dysfunction syndrome. *Critical Care Medicine* 1996; **24(7)**: 1107–1116.

21 DeLeon ER, Gao Y, Huang E, Arif M, Arora N, Divietro A, Patel S, Olson KR. A case of mistaken identity: are reactive oxygen species actually reactive sulfide species? *Am J Physiol Regul Integr Comp Physiol* 2016; **310**: R549–R560.

22 Osuchowski, MF, Craciun F, Weixelbaumer KM, Duffy ER, Remick DG. Sepsis Chronically in

MARS: Systemic Cytokine Responses Are Always Mixed Regardless of the Outcome, Magnitude, or Phase of Sepsis. *The Journal of Immunology* 2012; **189**: 000–000.

23 Oliveira S, Lopez-Munoz A, Candel S, Pelegrin P, Calado A, Mulero V.

ATP Modulates Acute Inflammation In Vivo through Dual Oxidase 1–Derived H<sub>2</sub>O<sub>2</sub> Production and NF-κB Activation. *The Journal of Immunology* 2014; **192**: 5710–5719.

24 Qiao Z, Wang W, Yin L et al. Using IL-6 concentrations in the first 24 h following trauma to predict immunological complications and mortality in trauma patients: a meta-analysis. *European Journal of Trauma and Emergency Surgery* 2018; **44**: 679–687.

25 Fouad AA, Hafez HM, Hamouda AAH. Hydrogen sulfide modulates IL-6/STAT3 pathway and inhibits oxidative stress, inflammation, and apoptosis in rat model of methotrexate hepatotoxicity. *Human and Experimental Toxicology* 2020; **39(1)**: 77–85.

26 Shaji CA, Robinson BD, Yeager A et al. The Tri-phasic Role of Hydrogen Peroxide in Blood-Brain Barrier Endothelial cells. *Sci Rep* 2019; **9**:133.

27 Stephens HE, Epting CL, Backer CL, Wald EL. Hyperlactatemia: An Update on Postoperative Lactate. *World Journal for Pediatric and Congenital Heart Surgery* 2020; **11(3)**: 316–324.

28 Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. *Critical Care* 2014; **18**: 503.

29 Molavian HR, Kohandel M, Sivaloganathan S. High Concentrations of H<sub>2</sub>O<sub>2</sub> Make Aerobic Glycolysis Energetically More Favorable for Cellular Respiration. *Frontiers in Physiology* 2016; **7**: 362.

30 Módis K, Coletta C, Erdélyi K, Papapetropoulos A, Szabo C. Intramitochondrial hydrogen sulfide production by 3-mercaptopyruvate sulfurtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. *FASEB J* 2013; **27**: 601–611.

31 Xiang J, Wan C, Guo R, Guo D. Is Hydrogen Peroxide a Suitable Apoptosis Inducer for All Cell Types? *BioMed Research International* 2016; **7343965**: 6.

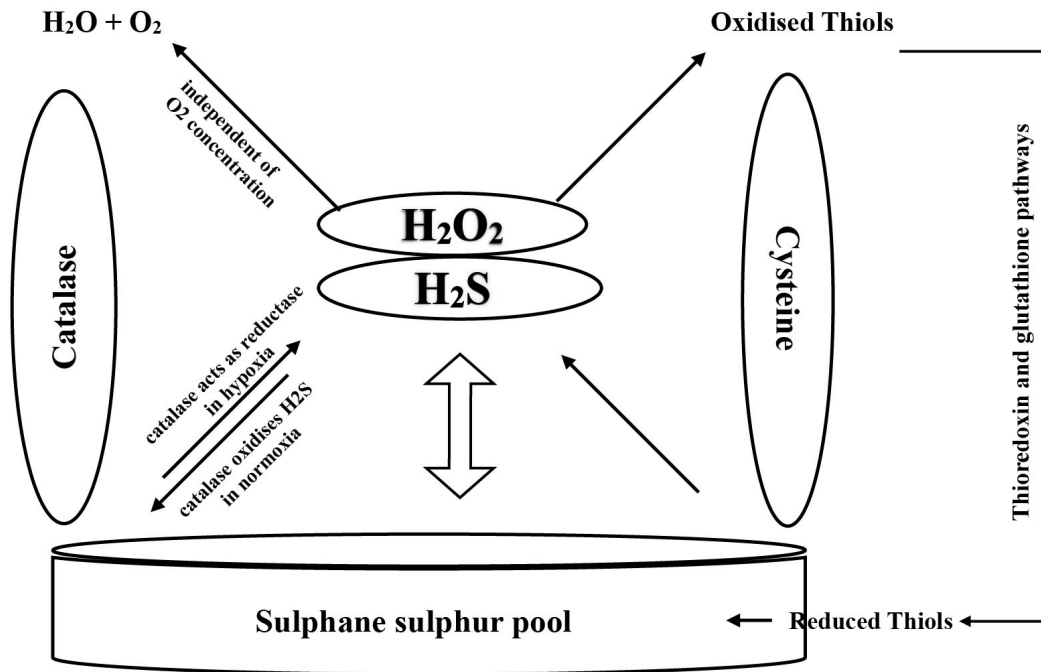
32 Obradovic M, EssackM, Zafirovic S. Redox control of vascular biology. *BioFactors*. 2020; **46**: 246–262.

33 Wu D, Hu Q, Zhu D. An Update on Hydrogen Sulfide and Nitric Oxide Interactions in the Cardiovascular System. *Oxidative Medicine and Cellular Longevity Volume* 2018; **4579140**:16.

34 Searcy GD. Metabolic integration during the evolutionary origin of mitochondria. *Cell Research* 2003; **13(4)**:229–238.

Tables and figures.

Figure - 1 Simplified biochemical relationships between the constituents of the chemocline.





**Tabular column - 1** Best estimates of the concentrations of the main constituents of the corporeal chemocline.

<b>Best estimates of the concentration of the main constituents of the corporeal chemocline</b>		
	$H_2O_2$ [4-6]	$H_2S$ [7,8]
Alveolar space	4 microM	15 picoM
Arterial blood	1-5 microM	100 picoM - 1 microM
Intracellular	10 nanoM	15 nanoM
Colonic lumen	?nanoM - low microM	40 microM