



# Mechanisms Of Post-Operative Sepsis And Renal Impairment In Obstructive Jaundice

by Comus John Whalan

Department of Surgery,  
the University of Adelaide

A thesis submitted in compliance with the  
requirements of the University of Adelaide  
for the degree of Doctorate of Medicine  
(M. D.)

March 1998

# Abstract

After open operations to relieve obstructive jaundice, several complications are excessively common. These include renal impairment, infections and sepsis. Under some circumstances, bile components can injure the bowel wall.

The hypothesis underlying this thesis was that after such operations, bile returning to the intestinal lumen increases bowel-wall permeability, allowing potentially harmful bowel contents to enter the tissues. These contents may include agents known to be harmful, such as bacteria and endotoxin, as well as agents whose toxicity is unknown, such as urobilinogen. A second hypothesis was that urobilinogen is nephrotoxic.

The aims were firstly, to develop a method of reversible obstructive jaundice in the rat. Secondly, to use the model to measure alterations in bowel-wall permeability to labelled bacteria, endotoxin and ethylenediaminetetraacetic acid (EDTA) after the relief of obstructive jaundice. Thirdly, to assess the toxicity of urobilinogen to renal cells cultured in vitro. Finally, to measure urobilinogen in various body fluids (including serum and urine) in rats and in human patients undergoing operations to relieve obstructive jaundice.

Various difficulties were encountered with the model of reversible obstructive jaundice, which limited its usefulness in measuring bacterial translocation from the bowel to the tissues. However, modification of the model allowed it to be used to assess alterations in bowel-wall permeability after bile was returned to the intestinal lumen. No such alterations were seen with normal bile or bile from subjects with obstructive jaundice, even when the bile was infected.

Urobilinogen was found to be toxic to cultured renal cells in concentrations which may reasonably be expected in disease, although caveats apply to this conclusion.

A urobilinogen assay, previously described by other workers, was found to be unsuitable for assay of specimens from jaundiced subjects, because interference from an unknown substance or substances occurred.

In summary, bile returning to the intestinal lumen of rats with obstructive jaundice did not alter bowel-wall permeability to endotoxin or EDTA. Urobilinogen was toxic to in vitro cultured renal cells in concentrations that may be expected to occur in disease. A previously-described assay for urobilinogen was found not to be useful for assaying specimens from jaundiced subjects.

# INDEX

<b><u>Abstract</u></b> .....	1
<b><u>Declaration</u></b> .....	3
<b><u>Acknowledgments</u></b> .....	4
<b><u>Index</u></b> .....	5
<b><u>1. Introduction and literature review</u></b> .....	9
<b><u>1.1. Note on definitions</u></b> .....	9
<b><u>1.2. Overview of the clinical problem</u></b> .....	11
<b><u>1.3. Complications of jaundice</u></b> .....	13
<b>1.3.1. Complications of non-obstructive jaundice</b> .....	13
1.3.1.1. Sepsis and infection in non-obstructive jaundice.....	13
1.3.1.2. Renal impairment in non-obstructive jaundice.....	14
<b>1.3.2. Complications of obstructive jaundice</b> .....	19
1.3.2.1. Complications of unrelieved obstructive jaundice.....	19
1.3.2.1.1. Renal impairment in unrelieved obstructive jaundice.....	19
1.3.2.1.2. Sepsis and infection in unrelieved obstructive jaundice.....	29
1.3.2.2. Complications of operations to relieve obstructive jaundice.....	29
1.3.2.2.1. Historical overview.....	29
1.3.2.2.2. Renal impairment after operations to relieve obstructive jaundice.....	31
1.3.2.2.3. Sepsis and infection after operations to relieve obstructive jaundice.....	44
1.3.2.3. Complications following non-operative treatment of obstructive jaundice..	46
1.3.2.3.1. Introduction.....	46
1.3.2.3.2. Percutaneous drainage of obstructive jaundice.....	47
1.3.2.3.3. Endoscopic drainage of obstructive jaundice.....	49
<b><u>1.4. Proposed mechanisms of complications after operations to relieve obstructive jaundice</u></b> .....	51
<b>1.4.1. Possible increased bowel-wall permeability after operations to relieve obstructive jaundice</b> .....	51
1.4.1.1. Introduction.....	51
1.4.1.2. Alterations in gut flora in obstructive jaundice.....	52
1.4.1.3 Increased bowel-wall permeability in obstructive jaundice.....	54
1.4.1.4. Impaired ability of the liver to clear portal blood in obstructive jaundice.....	55
1.4.1.5. Summary.....	56

<b>1.4.2. Possible role of urobilinogen in acute renal failure after operations to relieve obstructive jaundice.....</b>	<b>57</b>
1.4.2.1. Overview of the physiology of urobilinogen.....	57
1.4.2.2. Evidence to support the 'urobilinogen theory'.....	60
1.4.2.2.1. Evidence to suggest increased renal exposure to urobilinogen occurs after operations to relieve obstructive jaundice.....	60
1.4.2.2.2. Evidence to suggest that urobilinogen may be nephrotoxic.....	63
1.4.2.3. Note on the chemistry of the urobilinoid compounds.....	65
<b><u>1.5. Experimental models of reversible obstructive jaundice.....</u></b>	<b>67</b>
<b><u>2. Aims.....</u></b>	<b>71</b>
<b><u>3. Materials &amp; methods.....</u></b>	<b>73</b>
<b><u>3.1. Sepsis and infection.....</u></b>	<b>73</b>
<b>3.1.1. Effect on bacterial translocation of the return of bile to the intestinal lumen.....</b>	<b>73</b>
3.1.1.2. Experimental techniques.....	76
3.1.1.2.1. Anaesthesia.....	76
3.1.1.2.2. Thiry-Vella loop.....	76
3.1.1.2.3. Bile duct ligation.....	77
3.1.1.2.4. Biliary diversion method.....	77
3.1.1.2.5. Preparation of standard Escherichia coli suspension.....	80
3.1.1.2.6. Harvest procedures.....	80
3.1.1.2.7. Assessment of bacterial translocation.....	91
3.1.1.2.8. Comparative histopathology.....	82
<b>3.1.2. Effect on bowel-wall permeability of the return of bile to the intestinal lumen.....</b>	<b>82</b>
3.1.2.1. Experimental methods.....	83
3.1.2.1.1. Method of labelling endotoxin with Iodine-125.....	83
3.1.2.1.2. Method of checking proportion of bound to unbound I-125.....	88
3.1.2.1.3. Preparation of <sup>125</sup> I-labelled endotoxin solution.....	90
3.1.2.1.4. Preparation of <sup>51</sup> Cr-EDTA solution.....	90
3.1.2.1.5. Technique for measuring permeability of the bowel wall.....	90
<b><u>3.2. Renal impairment.....</u></b>	<b>92</b>
<b>3.2.1. Effect of urobilinogen on in vitro cell cultures.....</b>	<b>92</b>
3.2.1.1. Experimental techniques.....	92
3.2.1.1.1. Synthesis of urobilinogen.....	92
3.2.1.1.2. Synthesis of control or 'blank' solution.....	93

3.2.1.1.3. Urobilinogen assay.....	94
3.2.1.1.4. Cell culture methods.....	95
3.2.1.1.5. Neutral Red assay.....	97
3.2.1.1.6. Effect of urobilinogen on Vero cells.....	98
3.2.1.1.7. Effect of urobilinogen on T47D cells.....	99
3.2.1.1.8. Control experiments.....	100
3.2.1.1.8.1. Role of urobilin in toxicity.....	101
3.2.1.1.8.2. Role of osmolarity in toxicity.....	101
3.2.1.1.8.3. Role of Fe <sup>2+</sup> versus Fe <sup>3+</sup> in toxicity.....	102
<b>3.2.2. Urobilinogen levels after relief of obstructive jaundice in rats.</b>	<b>103</b>
<b>3.2.3. Urobilinogen levels after relief of obstructive jaundice in human patients.....</b>	<b>104</b>
<b><u>4. Results</u>.....</b>	<b>106</b>
<b><u>4.1. Sepsis and infection</u>.....</b>	<b>106</b>
<b>4.1.1. Bacterial translocation.....</b>	<b>106</b>
4.1.1.1. Verification of experimental models.....	106
<b><u>4.2. Bowel-wall permeability</u>.....</b>	<b>114</b>
<b>4.2.1. Rat bile.....</b>	<b>114</b>
4.2.1.1. Rat bile: jaundiced, non-infected.....	114
4.2.1.2. Rat bile: jaundiced and infected.....	126
<b>4.2.2. Human bile.....</b>	<b>137</b>
4.2.2.1. Human bile: non-jaundiced, non-infected.....	137
4.2.2.2. Human bile: jaundiced, non-infected.....	149
<b><u>4.3. Renal impairment</u>.....</b>	<b>162</b>
<b>4.3.1. Creation of a standard curve of urobilinogen concentration....</b>	<b>162</b>
<b>4.3.2. Urobilinogen assay.....</b>	<b>166</b>
<b>4.3.3. Cell culture methods: Neutral Red assay.....</b>	<b>169</b>
<b>4.3.4. Effect of urobilinogen on Vero cells.....</b>	<b>173</b>
<b>4.3.5. Control experiments.....</b>	<b>182</b>
4.3.5.1. Fe <sup>2+</sup> /Fe <sup>3+</sup> controls.....	183
4.3.5.2. Urobilin controls.....	185
4.3.5.3. Osmolar controls.....	187
<b>4.3.6. Miscellaneous experiments.....</b>	<b>189</b>
4.3.6.1. Attempts to detoxify 'blank' solution.....	189
4.3.6.2. Attempts with other cell lines.....	192

<b>4.3.7. Urobilinogen levels after relief of obstructive jaundice</b>	
<b>in a rat model and in human patients.....</b>	193
<b><u>5. Discussion and conclusions</u></b> .....	196
<b><u>5.1. Relief of obstructive jaundice</u></b> .....	196
<b>5.1.1. The effect on bacterial translocation, of relieving obstructive</b>	
<b>jaundice.....</b>	196
5.1.1.1. Problems with the Thiry-Vella loop.....	196
5.1.1.2. Problems with the 'reversible' model of obstructive jaundice.....	198
<b>5.1.2. The effect of relief of obstructive jaundice on bowel-wall</b>	
<b>permeability.....</b>	198
5.1.2.1. Implications of the results.....	199
5.1.2.1.1. Implications of the results in comparison to the work of others.....	199
5.1.2.1.1.1. Absence of a harmful effect of bile.....	199
5.1.2.1.1.2. Absence of a beneficial effect of bile.....	200
5.1.2.1.2. Implications of the results, for patient treatment.....	201
5.1.2.2. Caveats.....	201
<b><u>5.2. Renal impairment</u></b> .....	203
<b>5.2.1. Limitations of experimental techniques</b> .....	203
5.2.1.1. Limitations of the urobilinogen assay.....	203
5.2.1.2. Limitations of cell culture techniques.....	204
<b>5.2.2. Effect of urobilinogen on cultured renal cells</b> .....	205
5.2.2.1. Implications of the results.....	205
5.2.2.1.1. Implications of the results in comparison to the work of others.....	205
5.2.2.1.2. Implications of the results for treatment of human patients.....	206
<b>5.2.3. Caveats</b> .....	207
<b>5.2.4. Arguments against the 'urobilinogen theory'</b> .....	209
5.2.4.1. Other current theories explaining post-operative acute renal failure.....	209
5.2.4.2. Specific arguments against urobilinogen causing acute renal failure.....	212
<b><u>5.3. Summary</u></b> .....	215
<b><u>6. Bibliography</u></b> .....	216

# 1. INTRODUCTION AND LITERATURE REVIEW

## 1.1 Note on Definitions

Sepsis and renal failure have long been recognised as being excessively common after operations on patients with obstructive jaundice (OJ). However, the terms 'renal failure', 'renal impairment' and especially 'sepsis' have been used rather loosely in the past, although sepsis has recently been more precisely defined (as discussed in more detail on p. 45). This often means that when comparing papers that report these complications, only limited conclusions can be drawn. With these limitations in mind, the definitions used by previous workers have been accepted, but where necessary, comment is made on whether those definitions may have affected either the interpretation of the author's own results, or their comparison to other work.

The aim of this thesis is to investigate some of the possible mechanisms underlying these common complications of operations to relieve obstructive jaundice. It refers specifically to conventional 'open' operations, i.e. those involving laparotomy, because it is after these procedures that these problems have been most frequently reported by previous workers. However, other means of treating obstructive jaundice, such as endoscopic and percutaneous approaches, are also discussed where relevant.

Many earlier papers using the term 'jaundice' do not precisely define it (e.g. Robson 1903, Heuer 1934, Thompson et al 1940, Aird 1953, Williams et al 1960, Hadjis et al 1986). Presumably, this is at least partly because of the limited laboratory tests available to the authors of many of these papers.



To some extent it is a subjective term, for the simple reason that it describes the discolouration of the skin occurring in hyperbilirubinaemia, and the ease with which this discolouration can be seen varies between individuals. Jaundice is not usually visible until the serum bilirubin is about three times the normal level, or about 50  $\mu\text{mol/L}$  (Gollan and Schmid 1979).

This problem of subjectivity is seldom encountered in more recent papers dealing with the subject, because most such papers use a biochemical definition based on serum bilirubin levels. However, it is replaced by a lesser problem, namely a lack of uniformity in this definition. Commonly, the (arbitrary) level of a total serum bilirubin of more than 100 micromoles per litre ( $\mu\text{mol/L}$ ) is used (e.g. Armstrong et al 1984a, Thompson et al 1986 and 1989, Pain et al 1991, Parks et al 1994), but others have used different definitions, such as a total serum bilirubin of more than 50  $\mu\text{mol/L}$  (Gillen and Peel 1986), more than 136  $\mu\text{mol/L}$  (Semeraro et al 1989), or more than 200  $\mu\text{mol/L}$  (Smith et al 1985). This lack of uniformity is clearly important when comparing results from different studies, because patients with higher pre-operative serum bilirubin levels may be at greater risk of post-operative complications (Hunt et al 1980, Greig et al 1988).