THE PROTEIN BALANCE OF NORMAL, LYMPHOEDEMATOUS AND INJURED TISSUES AND THE ACTION OF A BENZOPYRONE, COUMARIN

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## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUMMARY</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>DECLARATION</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>BIBLIOGRAPHY OF PUBLICATIONS FROM THIS THESIS</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>SECTION 1</td>
<td>GENERAL INTRODUCTION</td>
<td>9</td>
</tr>
<tr>
<td>1.1</td>
<td><strong>Structure, Function and Permeability of</strong></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><strong>the Blood-Tissue-Lymph System</strong></td>
<td></td>
</tr>
<tr>
<td>1.1.1</td>
<td>Continuous Capillaries</td>
<td>10</td>
</tr>
<tr>
<td>1.1.2</td>
<td>Fenestrated Capillaries</td>
<td>12</td>
</tr>
<tr>
<td>1.1.3</td>
<td>Interstitial Tissue</td>
<td>14</td>
</tr>
<tr>
<td>1.1.4</td>
<td>Initial Lymphatics</td>
<td>15</td>
</tr>
<tr>
<td>1.1.5</td>
<td>Collecting Lymphatics</td>
<td>18</td>
</tr>
<tr>
<td>1.2</td>
<td><strong>Oedema</strong></td>
<td>18</td>
</tr>
<tr>
<td>1.2.1</td>
<td>Low-Protein Oedema</td>
<td>20</td>
</tr>
<tr>
<td>1.2.2</td>
<td>High-Protein High-Flow Oedema</td>
<td>21</td>
</tr>
<tr>
<td>1.2.3</td>
<td>Chronic Inflammation</td>
<td>22</td>
</tr>
<tr>
<td>1.2.4</td>
<td>High-Protein Low-flow Oedema; Lymphoedema</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>The Acute Phase</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>The Latent Period</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>The Chronic Phase</td>
<td>26</td>
</tr>
</tbody>
</table>
SECTION 1  GENERAL INTRODUCTION (continued)

1.3 Therapy  

1.3.1 Low-protein Oedema  
1.3.2 High-Protein Oedema  
1.3.3 The Benzopyrones  

SECTION 2  PROTEIN FLUX IN A BLOOD-TISSUE-LYMPH SYSTEM  

2.1 Introduction  
2.2 Materials and Methods  
2.3 Compartmental Analysis  
2.4 The Calculated Rates of Protein Flux  
2.5 Protein Flux in the Blood-Tissue-Lymph System  
2.6 Protein Flux Across the Vascular Endothelium  
2.7 Protein Flux Against its Concentration Gradient  
2.8 The Direction of Net Protein Flux  

27  
27  
27  
27  

30  

30  
32  
33  
45  
46  
47  
51  
53
SECTION 3 OEDEMA AND BENZOPYRONE THERAPY

3.1 With Normal Lymphatics and Proteolysis

3.1.1 Introduction

3.1.2 Oedema Production and Coumarin Treatment

3.1.3 Measurement and Analysis

3.1.4 The Rates of Resolution of Oedema

3.1.5 Discussion

  Low-Protein Oedema

  High-Protein High-Flow Oedema

3.1.6 Conclusions

3.2 Further Investigations of an Experimental Model of Acute Lymphoedema

3.2.1 Introduction

3.2.2 Creation of Lymphostasis

3.2.3 Plethysmography

3.2.4 Percentage Change in Limb Volume

3.2.5 Regression Analysis

3.2.6 Lymphoedema

3.2.7 The Four Phases of Acute Lymphoedema
SECTION 3 OEDEMA AND BENZOPYRONE THERAPY (continued)

3.3 The Effect of Lymphostasis on Oedema
   Resolution 87
   3.3.1 Introduction 87
   3.3.2 Injection and Measurement Procedures 87
   3.3.3 The Rates of Resolution of Oedema 89
   3.3.4 Discussion 96
      The Effect of Coumarin 96
      The Effect of Lymphostasis 97

3.4 The Effect of the Macrophages on Oedema
   Resolution 99
   3.4.1 Introduction 99
   3.4.2 Destruction of Macrophages 100
   3.4.3 Injection and Measurement Procedures 100
   3.4.4 The Rates of Resolution of Oedema 101
   3.4.5 Discussion 108
      The Effect of Coumarin 108
      The Effect of Macrophages 109
SECTION 4  EXCESS PLASMA PROTEINS AS A CAUSE OF CHRONIC INFLAMMATION AND LYMPHOEDEMA  111

4.1  Introduction  111
4.2  Materials and Methods  113
4.3  Method of Analysis  116
   The Ineffectiveness of Analysis by Biopsy Alone  118
4.4  Results  120
   Visual Observations and Injection Site Thickness  121
   Wet Weight  124
   Water Content  127
   Fat Content  129
   Collagen Content  132
4.5  Chronic Inflammation  136
4.6  Excess Plasma Proteins as the Cause  137
4.7  Chronic Lymphoedema  138
4.8  The Effect of Coumarin  140
4.9  Conclusions  143
SUMMARY

The protein balance of the blood-tissue-lymph system was investigated under normal conditions and during various forms of oedema, in an attempt to obtain a clearer picture of the changes which occur during inflammation. The ability of the system to resolve the oedema and to return to normal was studied, together with the effects of the benzopyrene, coumarin.

Various injuries increase vascular permeability and result in local oedema in the tissues. The increased transport of macromolecules from the blood to the tissues has been widely investigated, particularly as the result of moderate histamine injury. However, the possibility of backflux from the tissues to the blood has not previously been considered. A compartmental model of all available pathways for protein within a blood-tissue-lymph system (using Guyton's capsules) was developed to allow all the rates of flux to be calculated. The effect of histamine injury, and hence acute inflammation (local oedema), was also studied. Protein flux against its concentration gradient was detected under normal conditions and the rate was increased by histamine injury. Much of this flux was across close and open junctions, although it seems likely that vesicles also contributed. More protein returned to the blood directly from the tissues than passed to the
lymphatics under both normal and histamine-injured conditions. As expected histamine also caused significant increases in the rates of protein flux from the blood to the tissues and from the tissues to the lymphatics. In both normal and histamine-injured conditions the net protein flux between the blood and the tissues was directed from the blood.

Local oedema was produced by injecting various substances into the subcutaneous tissues. The rates at which these oedemas were resolved were calculated and compared. The resolution of high-protein oedema was biphasic. The first phase was more rapid and probably dependent on optimal lymphatic function. The second phase was slower and continued until the tissues returned to normal. It was probably dependent on proteolysis and began when the more central lymphatic collectors became filled, causing local lymph flow to be progressively reduced. The benzopyrone, coumarin, increases the proteolysis of accumulated abnormal protein. It also significantly reduces high-protein oedemas. One purpose of this study was to link these effects of the drug directly by showing that the products of proteolysis, such as amino acids, are removed from the site of oedema more rapidly than the proteins themselves. This was proven by the fact that oedema caused by the injection of amino acids was resolved more rapidly than that caused by
plasma. The effect of coumarin on the resolution of these oedemas was investigated. It had no effect in the non-protein oedemas, nor did it affect the resolution of protein-induced oedema in the first four hours. It did increase the rate of resolution in the second (or proteolysis) phase of plasma removal. This further confirms that coumarin enhances proteolysis and reduces high-protein oedema.

During the course of this study, it became necessary to obtain more detailed information regarding an often-used experimental procedure for producing lymphostasis. Of particular interest was the time course of the development of lymphoedema. This information had not been obtained previously. Therefore, a complete study of this experimental procedure was undertaken. Lymphostasis led to four separate, quite distinct phases in the development of lymphoedema. An initial rapid rate of increase in limb volume (approximately 1.5 percent/hour) followed by a plateau and a slight reduction in the swelling. Then followed a longer period at a slower rate of swelling (0.32-0.38 percent/hour) until a maximum of a 46-48 percent increase in limb volume was reached. The limb volume then returned to normal (0.34-0.45 percent/hour). The lengths of the various stages appear to be dependent on the tightness of the ligatures. Lymphostasis reduced the rate of resolution of
high-protein oedema by approximately 40 percent (in the first four hours) indicating that the lymphatics do play a very vital part in those initial stages of oedema resolution. The macrophages are very important in the resolution of high-protein oedema only after four hours but their destruction did not reduce the effectiveness of coumarin. It appears that coumarin is able to enhance proteolysis by other cells.

Willoughby and Di Rosa (1970) hypothesized that altered proteins in the tissues were a cause of chronic inflammation. It has also been suggested that lymphoedema is a form of inflammation. Therefore, it is possible that the accumulation of plasma proteins in the tissues as a result of lymphostasis could be responsible for all the pathological changes found. Repeated doses of plasma were injected into the subcutaneous tissues of immunologically-tolerant rats for up to 64 days and, effectively, "lymphoedema was produced without lymphostasis". Changes typical of chronic inflammation were found in the skin, and to a much greater extent, in the fascia. Coumarin significantly reduced the extent of this inflammatory reaction in the skin but not in the fascia. The accumulation of excess proteins in the tissues did cause chronic inflammation. Thus chronic lymphoedema can be regarded as a form of chronic inflammation. Evidence also indicates that, in the
presence of excess plasma proteins, the benzopyrone, coumarin, is capable of stimulating cells other than macrophages in the skin.