A HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE OVARY
AND VITELLOGENESIS IN THE DOGFISH SQUALUS ACANTHIAS

by

JAMES ALLEN REID

B.Sc., University of British Columbia, 1970

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department
of
ZOOLOGY

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
January, 1975
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology

The University of British Columbia
Vancouver 8, Canada

Date March 10, 1975
ABSTRACT

Protein yolk formation is being studied in an ever increasing number of organisms and in each the method of formation (i.e. intraoocytic vs extraoocytic) and the organelles involved (Golgi, mitochondria etc.) differ even though the final product is markedly similar. Previous emphasis on elasmobranchs in this laboratory and the availability of the specimen favoured the selection of *Squalus acanthias* as the experimental animal as well as the lack of any pertinent studies on its ovary or vitellogenic processes.

While examining the problem of yolk formation with both histochemical and ultrastructural techniques, several other critical areas of investigation were pursued. As a result, the composition of the follicular envelope and the formation of its associated membranes, the surface epithelium, the tunica albuginea, the ovarian cortex and the lacunae border cells were also examined.

An examination of representative oocytes from 0.1 to 4.0 mm in diameter revealed many important facts. First, these oocytes are in a prolonged diplotene with up to 100 nucleoli present, each rich in rRNA represented ultrastructurally by their granular nature. Secondly, a complex Balbiani body rich in protein and RNA and composed of mitochondria and SER is present. Here, this mitochondrial mass is the center for mitochondrial multiplication. Thirdly, lipid yolk is formed before proteinaceous yolk and it arises *de novo* in the ooplasm from extraoocytically derived materials. Finally, proteid yolk arises from two sources. Initially, multigranular (MGB) and multivesicular (MVB) bodies form from Golgi, SER, micropinocytotic vesicles and vesicles derived from the nuclear membrane which fuse together forming granular-vesicular
bodies (GVB). In oocytes larger than 0.4 mm, distended Golgi lamellae, SER cisternae and loops of SER vesicles form limiting membranes and take up MGBs, MVBs and small GVBs. The contents of these large GVBs then dissolve, reaggregate and crystallize forming a mature yolk platelet.

Of the other areas examined, the basal lamina, the vitelline membrane and the follicle cells were the most relevant to yolk formation. The follicle cells were implicated in the formation of both the lamina and the vitelline membrane. The former are predominantly acid and sulphated mucopolysaccharides while the latter is a protein-polysaccharide complex. Both membranes provide support and chemical selectivity. The evidence for steroid activity in the follicle cells of these stages was inconclusive.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td></td>
</tr>
<tr>
<td>Experimental Animals</td>
<td>4</td>
</tr>
<tr>
<td>Preparation for Histochemical Analysis</td>
<td>4</td>
</tr>
<tr>
<td>Histochemical Procedures</td>
<td>5</td>
</tr>
<tr>
<td>Preparation for Electron Microscopy</td>
<td>7</td>
</tr>
<tr>
<td>RESULTS</td>
<td></td>
</tr>
<tr>
<td>The Ovary</td>
<td>9</td>
</tr>
<tr>
<td>Histochemical Analysis</td>
<td>9</td>
</tr>
<tr>
<td>Protein</td>
<td>9</td>
</tr>
<tr>
<td>Mucoproteins and Neutral Mucopolysaccharides</td>
<td>11</td>
</tr>
<tr>
<td>Ribonucleic Acid</td>
<td>14</td>
</tr>
<tr>
<td>Acidic and Sulphated Mucopolysaccharides</td>
<td>18</td>
</tr>
<tr>
<td>Phospholipids and Lipids</td>
<td>24</td>
</tr>
<tr>
<td>Electron Microscopical Observations</td>
<td>30</td>
</tr>
<tr>
<td>The Ovarian Epithelium</td>
<td>30</td>
</tr>
<tr>
<td>The Tunica Albuginea</td>
<td>37</td>
</tr>
<tr>
<td>The Cortex</td>
<td>37</td>
</tr>
<tr>
<td>The Intercellular Space</td>
<td>43</td>
</tr>
<tr>
<td>The Lacunae Border Cells</td>
<td>43</td>
</tr>
<tr>
<td>The Red Blood Cells</td>
<td>46</td>
</tr>
<tr>
<td>The Thecal Cells</td>
<td>48</td>
</tr>
<tr>
<td>The Basal Lamina-Lamella Complex</td>
<td>53</td>
</tr>
<tr>
<td>The Follicle Cells</td>
<td>62</td>
</tr>
<tr>
<td>The Vitelline Membrane</td>
<td>71</td>
</tr>
<tr>
<td>Topic</td>
<td>Page</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>The Oocyte Nucleus</td>
<td>77</td>
</tr>
<tr>
<td>The Mitochondrial Mass</td>
<td>85</td>
</tr>
<tr>
<td>Lipid Yolk</td>
<td>86</td>
</tr>
<tr>
<td>Protein Yolk</td>
<td>88</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td></td>
</tr>
<tr>
<td>Histochemistry</td>
<td>114</td>
</tr>
<tr>
<td>Oocyte</td>
<td>114</td>
</tr>
<tr>
<td>Vitelline Membrane</td>
<td>115</td>
</tr>
<tr>
<td>Follicle Cells</td>
<td>115</td>
</tr>
<tr>
<td>Basement Lamina-Lamella Complex</td>
<td>117</td>
</tr>
<tr>
<td>Theca</td>
<td>119</td>
</tr>
<tr>
<td>Stroma</td>
<td>120</td>
</tr>
<tr>
<td>Tunica Albuginea</td>
<td>121</td>
</tr>
<tr>
<td>Ultrastructure</td>
<td></td>
</tr>
<tr>
<td>Ovarian Epithelium</td>
<td>123</td>
</tr>
<tr>
<td>Tunica Albuginea</td>
<td>125</td>
</tr>
<tr>
<td>Cortex</td>
<td>126</td>
</tr>
<tr>
<td>The Intercellular Space</td>
<td>127</td>
</tr>
<tr>
<td>Lacunae Border Cells</td>
<td>128</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>129</td>
</tr>
<tr>
<td>Thecal Cells</td>
<td>129</td>
</tr>
<tr>
<td>Basement Lamina-Lamella Complex</td>
<td>130</td>
</tr>
<tr>
<td>Follicle Cells and the Vitelline Membrane</td>
<td>134</td>
</tr>
<tr>
<td>Oocyte Nucleus</td>
<td></td>
</tr>
<tr>
<td>A. Nucleoli</td>
<td>141</td>
</tr>
<tr>
<td>B. Nuclear Pores</td>
<td>143</td>
</tr>
<tr>
<td>C. Nuclear Membrane</td>
<td>143</td>
</tr>
</tbody>
</table>
Mitochondrial Mass 144
Lipid Yolk 147
Protein Yolk 150
SUMMARY 157
BIBLIOGRAPHY 161
APPENDICES

Histochemical Techniques Employed 174
   Staining 174
   Enzyme Treatment 175
   Selective Blocking and Unblocking 176
LIST OF TABLES

TABLE                                Page
IA, B, C. Combined Histochemical Results 29
LIST OF ILLUSTRATIONS

FIGURE Page

1 to 2 DNFB staining 10
3 to 4 PAS-trichrome staining 12
5 PAS-trichrome staining 13
5 PAS-dichrome, control 13
7 PAS-dichrome, diastase treated 13
8 to 9 Methyl Green-Pyronin staining 15
10 to 11 Toluidine blue for nucleic acids and acid mucopolysaccharides 17
12 Toluidine blue, RNase treated 17
13 Alcian blue staining 19
14 Hale's colloidal iron reaction 21
15a, 16a Toluidine blue control for hyaluronidase. Water mounted. 22
15b, 16b Toluidine blue hyaluronidase treated. Water mounted 22
17 to 18 Aldehyde Fuchsin staining 23
19 AF following Methylation-Demethylation (MDM) 23
20 Azure A staining 25
21 Azure A, MDM treatment 25
22 Azure A, hyaluronidase treated 25
23 Copper Phthalocyanin staining 26
24 to 25 Sudan black staining 28
26 Surface epithelium of the ovary 31
27 Surface epithelium, complete panorama 32
28 Apices of three surface epithelial cells 33
29 to 30 High magnification of several epithelial contact surfaces 34
<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Page</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>35</td>
<td>Surface vesicles of the epithelial cells</td>
</tr>
<tr>
<td>32</td>
<td>35</td>
<td>Lateral epithelial contact surfaces</td>
</tr>
<tr>
<td>33</td>
<td>38</td>
<td>Tunica albuginea</td>
</tr>
<tr>
<td>34</td>
<td>38</td>
<td>High magnification of an elastic fibre</td>
</tr>
<tr>
<td>35 to 36</td>
<td>40</td>
<td>Dark cells in the cortex</td>
</tr>
<tr>
<td>37</td>
<td>41</td>
<td>Intermediate dark cell</td>
</tr>
<tr>
<td>38</td>
<td>41</td>
<td>Light cell in the cortex</td>
</tr>
<tr>
<td>39 to 41</td>
<td>42</td>
<td>Light cells in the cortex</td>
</tr>
<tr>
<td>42 to 44</td>
<td>44</td>
<td>Lacunae border cells</td>
</tr>
<tr>
<td>45 to 47</td>
<td>47</td>
<td>Nucleated red-blood cells</td>
</tr>
<tr>
<td>48</td>
<td>49</td>
<td>Thecal cells, 0.3 mm oocyte</td>
</tr>
<tr>
<td>49</td>
<td>49</td>
<td>Theca interna cells, 2.0 mm oocyte</td>
</tr>
<tr>
<td>50 to 52</td>
<td>50</td>
<td>Theca interna cells, 1.0 mm oocyte</td>
</tr>
<tr>
<td>53 to 55</td>
<td>52</td>
<td>Theca externa cells, 1.0 mm oocyte</td>
</tr>
<tr>
<td>56 to 58</td>
<td>54</td>
<td>Various aspects of the follicle, 0.1 mm oocyte</td>
</tr>
<tr>
<td>59</td>
<td>55</td>
<td>Vitelline membrane, 0.2 mm oocyte</td>
</tr>
<tr>
<td>60</td>
<td>55</td>
<td>Follicle layers, 0.3 mm oocyte</td>
</tr>
<tr>
<td>61</td>
<td>56</td>
<td>Follicle cell transition zone, 0.3 mm oocyte</td>
</tr>
<tr>
<td>62 to 63</td>
<td>56</td>
<td>Zona radiata of a 0.3 mm oocyte</td>
</tr>
<tr>
<td>64 to 66</td>
<td>57</td>
<td>Basal FC cytoplasm and formation of the basal lamina, 0.4 mm oocyte</td>
</tr>
<tr>
<td>67 to 68</td>
<td>58</td>
<td>Follicle layers with early VM formation, 0.4 mm oocyte</td>
</tr>
<tr>
<td>69 to 71</td>
<td>59</td>
<td>Stages of VM formation in one oocyte, 0.4 mm</td>
</tr>
<tr>
<td>72 to 74</td>
<td>60</td>
<td>High magnification of VM formation, 0.4 mm oocyte</td>
</tr>
<tr>
<td>75</td>
<td>61</td>
<td>Pseudostratified follicle cells, 0.4 mm oocyte</td>
</tr>
<tr>
<td>76</td>
<td>61</td>
<td>Extensive VM formation, 0.4 mm oocyte</td>
</tr>
<tr>
<td>FIGURE</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>77 to 79</td>
<td>Various views of the FCs, 1.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>80 to 82</td>
<td>Vitelline membrane and zona radiata, 1.0 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>Base of the zona radiata</td>
<td></td>
</tr>
<tr>
<td>84 to 85</td>
<td>Variations in 1.1 mm oocyte follicle cells</td>
<td></td>
</tr>
<tr>
<td>86 to 87</td>
<td>Light micrograph of the follicle layers, 1 to 2 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>88 to 89</td>
<td>Basement lamina and lamella, 1.5 to 1.8 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>90 to 92</td>
<td>Basal to median levels of the pseudostratified follicle cells, 1.5 to 1.8 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>93 to 94</td>
<td>FC-VM interface with pore canals in VM, 1.5 to 1.8 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>95 to 96</td>
<td>Zona radiata and cortical layer, 1.5 to 1.8 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>98 to 100</td>
<td>Nuclear region and nearby protein yolk precursors (PYP), 0.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>Light micrograph, 0.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>Nucleus, 0.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>Nucleolar-like body in the ooplasm, 0.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>104</td>
<td>Nucleolus, 0.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>105 to 107</td>
<td>Mitochondrial mass and surrounding ooplasm, 0.1 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>Nuclear region, 0.2 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>Central ooplasm and organelles, 0.2 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>Nucleus, 0.3 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>111 to 112</td>
<td>Light micrograph of a 0.3 mm oocyte with lipid yolk</td>
<td></td>
</tr>
<tr>
<td>113 to 115</td>
<td>Ooplasm with various mitochondria and PYP, 0.3 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>116 to 117</td>
<td>Ooplasm with various PYP, 0.3 mm oocyte</td>
<td></td>
</tr>
<tr>
<td>FIGURE</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>118</td>
<td>Lipid yolk and forming PYP, 0.4 mm oocyte</td>
<td>94</td>
</tr>
<tr>
<td>119</td>
<td>Lipid yolk with a developing granular-vesicular body</td>
<td>94</td>
</tr>
<tr>
<td>120 to 122</td>
<td>Various lipid yolk particles and organelles, 0.4 mm oocyte</td>
<td>95</td>
</tr>
<tr>
<td>123</td>
<td>Lipid yolk with concentric rings of SER, 0.5 mm oocyte</td>
<td>96</td>
</tr>
<tr>
<td>124</td>
<td>Branching mitochondrion, 0.5 mm oocyte</td>
<td>96</td>
</tr>
<tr>
<td>125</td>
<td>Lipid yolk associated with SER, 0.5 mm oocyte</td>
<td>96</td>
</tr>
<tr>
<td>126</td>
<td>Various stages in PYP formation, 0.5 mm oocyte</td>
<td>97</td>
</tr>
<tr>
<td>127</td>
<td>Large lipid yolk bodies, 0.5 mm oocyte</td>
<td>97</td>
</tr>
<tr>
<td>128</td>
<td>Nuclear membrane, pores and SER in the ooplasm, 0.8 mm oocyte</td>
<td>98</td>
</tr>
<tr>
<td>129</td>
<td>Small nucleolus, 0.8 mm oocyte</td>
<td>98</td>
</tr>
<tr>
<td>130</td>
<td>Mitochondrial mass, 0.8 mm oocyte</td>
<td>98</td>
</tr>
<tr>
<td>131</td>
<td>Mitochondrial mass and SER, 0.8 mm oocyte</td>
<td>100</td>
</tr>
<tr>
<td>132</td>
<td>Polymorphic mitochondria, 0.8 mm oocyte</td>
<td>100</td>
</tr>
<tr>
<td>133</td>
<td>Ring-shaped mitochondrion and numerous loops of SER, 0.8 mm oocyte</td>
<td>100</td>
</tr>
<tr>
<td>134</td>
<td>Lipid yolk particles, 0.8 mm oocyte</td>
<td>100</td>
</tr>
<tr>
<td>135</td>
<td>Light micrograph, nucleus with lampbrush chromosomes and nucleoli, 1.0 mm oocyte</td>
<td>101</td>
</tr>
<tr>
<td>136</td>
<td>Annulate lamella, 1.0 mm oocyte</td>
<td>101</td>
</tr>
<tr>
<td>137</td>
<td>Mitochondrial mass, 1.0 mm oocyte</td>
<td>101</td>
</tr>
<tr>
<td>138</td>
<td>Mitochondrial mass, 1.0 mm oocyte</td>
<td>102</td>
</tr>
<tr>
<td>139</td>
<td>GVB with attached SER</td>
<td>102</td>
</tr>
<tr>
<td>140</td>
<td>Organelles and PYP, 1.0 mm oocyte</td>
<td>102</td>
</tr>
<tr>
<td>141</td>
<td>Golgi body and several SER configurations</td>
<td>104</td>
</tr>
<tr>
<td>142</td>
<td>A large mass of PYP in various stages of development</td>
<td>104</td>
</tr>
</tbody>
</table>
FIGURE

143  A large Golgi-like body, 1.0 mm oocyte 104
144  Two PYP systems, 1.0 mm oocyte 105
145  Extensive SER systems, 1.0 mm oocyte 105
146  Pinocytotic vesicles and lining bodies, 1.0 mm oocyte 105
147  Convoluted plasma membrane, 1.0 mm oocyte 105
148  Nuclear membrane, 1.1 mm oocyte 106
149  Nucleoli in a 1.1 mm oocyte 106
150  Lipid yolk and PYP systems, 1.1 mm oocyte 106
151 to 152  Large PYP, 1.1 mm oocyte 108
153  A developing GVB, 1.1 mm oocyte 108
154  A large dense PYP, 1.1 mm oocyte 109
155  Several Golgi and other organelles, 1.1 mm oocyte 109
156 to 157  Lining bodies and mitochondria with attached SER 109
158  Protein yolk platelets with a dense core, 2.0 mm oocyte 111
159  Protein yolk platelet with a crystallized core, 3.0 mm oocyte 111
160 to 161  Protein yolk platelets with crystallized cores, 4.0 mm oocytes 112
ACKNOWLEDGEMENTS

I wish to express my appreciation to my supervisor Dr. P. Ford for his support and his patience throughout the preparation of this work. I am also grateful to Dr. C.V. Finnegan for the use of his laboratory and to Dr. A.B. Acton and Dr. P. Larkin for their advice and support.

My special thanks is extended to Mrs. Maureen Douglas for her invaluable assistance and advice during the research, preparation and correction of this manuscript. To Mr. L.L. Veto I also owe a special debt of thanks for his assistance and encouragement.

My thanks also go to the captains and crews of the Investigator No. I and the A.P. Knight, the Fisheries Research vessels used to obtain my specimens.

Finally, I would like to dedicate this work to my wife Shelley, for her patience, prodding, encouragement and assistance.
INTRODUCTION

Yolk synthesis and deposition have been the subject of numerous investigations in the past two decades. Yolk, both protein and lipid, has been described as arising within various animal oocytes under the influence of the mitochondria, Golgi, endoplasmic reticulum, nuclear extrusions, or directly out of the cytoplasm and occasionally by a combination of two or more of these methods (Raven, 1961; Nørrevang, 1968; Beams & Kessel, 1973). In addition to these intraoocytic methods, yolk formation may occur extraoocytically, the precursor materials for yolk being synthesized elsewhere and entering the oocyte by micropinocytosis with subsequent incorporation into developing yolk bodies (Raven, 1961; Nørrevang, 1968; Bellairs, 1971; Kessel, 1971; Massover, 1971; Boyer, 1972). Thus the primary directive of this study was to examine the development of the oocytes of the dogfish *Squalus acanthias* ultrastructurally and determine the method of vitellogenesis.

A previous study on the morphology of the yolk of the dogfish *Mustelus laevis* Risso (Grodzinski, 1958) indicated the presence of an internal platelet in the protein yolk sphere with properties more reminiscent of an amphibian platelet than a teleost platelet. Thus the ultrastructure of the yolk platelet was also examined to determine if these properties were reflected in the structure of the various platelets.

While considering the possibility that yolk material was synthesized elsewhere and transported to the ovary for uptake into the oocyte it became necessary to examine the follicular envelope of the oocyte to establish its effectiveness as a barrier to the passage of yolk material, as well as any other possible functions. This follicular envelope or wall was examined in *Squalus acanthias* by Lance & Callard (1969).
who found it to consist of a zona radiata, a vitelline membrane, a single layer of columnar epithelium—the granulosa, the theca interna and the theca externa. TeWinkel (1972) examined the oocyte follicle (@ 4.0 mm) of the smooth dogfish *Mustelus canis* and described a zona radiata, a pseudostratified columnar granulosa, a basement membrane and a theca. Considering these discrepancies in two dogfish species, the first point was to establish the histochemical and ultrastructural composition of this follicular envelope.

The thecal layer has often been described as a connective tissue layer (Lance & Callard, 1969; Dumont, 1972; Peel & Bellairs, 1972; TeWinkel, 1972) in the lower vertebrates possibly with steroid activity in the theca interna (Lance & Callard, 1969; TeWinkel, 1972). With these seemingly contradictory facts in mind the theca was examined to determine its properties and function.

The basement membrane, consisting of the basal lamina and its underlying collagenous basal lamella (Nadol et al., 1969), poses an interesting problem. First of all, the literature disagrees on the presence of this complex beneath the follicular epithelium of two dogfish species, including the species under investigation where it was not observed (Lance & Callard, 1969). However, preliminary light microscopy for this study indicated the presence of a well developed basement lamina and lamella. Further, it was thought that only fibroblasts were capable of forming a basement membrane complex but recently, basal laminae have been found beneath epithelia lacking underlying fibroblasts (Hay & Revel, 1969; Bernfield & Wessels, 1970) while other evidence (Hay & Revel, 1969; Beisswenger & Spiro, 1973) has directly implicated epithelial cells in basal lamina formation. So, investigations were carried out on the
basal lamina-lamella complex to determine its chemical composition and to determine from which portion of the follicular envelope it was formed.

The granulosa or follicular sheath is a layer of epithelial cells found around animal oocytes that in *Squalus* has been implicated in steroid synthesis (Lance & Callard, 1969) while generally follicle cells have been shown to synthesize yolk materials (Raven, 1961; Davidson, 1968) for the oocyte and to transport extraoocytic yolk precursors into the oocyte (Raven, 1961; Davidson, 1968; Bellairs, 1971). The granulosa of *Squalus acanthias* was examined to clarify these possible functions.

The vitelline membrane was thought to be formed by the oocyte (Raven, 1961) but in many recent studies (Nørrevang, 1968; Bellairs, 1971; Cummings, 1972; Dumont, 1972; Sahota, 1973) the vitelline membrane was observed to be formed by the follicle cells. In teleosts, however, there is evidence for both hypotheses (Chaudhry, 1956; Yamamoto, 1963). The zona radiata of elasmobranchs were described by Balfour (1885) and Wallace (1903) who believed them to be separate membranes in the follicular envelope. Recent electron microscopical studies have demonstrated that the zona radiata is formed by microvilli arising from the egg surface (Schjeide, Wilkins et al, 1963; Yamamoto, 1963; Press, 1964; Bellairs, 1965, 1971). Thus, important areas of investigation are the examination of the vitelline membrane to determine its chemical composition, its relationship to the oocyte relative to the existence of a zona radiata, and its development.

Additional observations are also reported on the ovarian surface epithelium, the tunica albuginea, the ovarian cortex, the lacunae border cells, and the red blood cells as little if any previous work had been done on these aspects of the spiny dogfish ovary.
MATERIALS AND METHODS

EXPERIMENTAL ANIMALS


Ontogenetically, the ovaries arise in the dorsolateral lining of the peritoneum, one on each side of the dorsal mesentery in the genital ridges. The primordial germ cells, generally assumed to arise from yolk sac endoderm migrate into the peritoneum and the genital ridges then differentiate into ovarian tissue.

The paired ovaries of immature (93.5 cm ± 5.5 cm; Ketchen, 1972) *Squalus acanthias* are soft, oval bodies each measuring 4 to 6 cm in length, 3 to 4 cm in width and 1 to 1.5 cm in thickness. In the mature ovary the presence of 2 to 6 ripe eggs measuring 2.5 to 4 cm in diameter increases the overall dimensions of the ovary. Morphologically, it is a compact ovary similar to those found in many teleosts as well as in reptiles, birds and mammals.

PREPARATION FOR HISTOCHEMICAL ANALYSIS

The ovaries were removed from the living animal and fixed in 10% neutral buffered formalin (Lillie, 1954) at ambient temperature, dehydrated through a graded series of ethanols, cleared in benzene and embedded in Paraplast (Fisher Scientific Co.). Sections of 7 μm thickness were cut on an AO Spencer "820" Microtome and mounted on albuminized glass slides.

For the preservation of lipids, the material was embedded in Cryoform (International Equipment Co.) and frozen. Sections were cut at
16 μm on an IEC CTF Microtome-Cryostat, picked up on glass coverslips and air dried.

HISTOCHEMICAL PROCEDURES

Ten histochemical techniques were employed to demonstrate proteins, lipids, nucleic acids and carbohydrates.

A modified dinitrofluorobenzene (DNFB) technique was used to demonstrate proteins according to the method of Tranzer and Pearse (1964). DNFB is a general protein reaction that complexes with N-terminal α-amino groups, the phenolic hydroxyl group of tyrosine and the sulphydryl group of cysteine producing the coloured reaction product (Sanger, 1945; Pearse, 1968; Chayen et al, 1969).

Trichrome periodic acid-Schiff (PAS) staining was used to demonstrate mucoproteins, neutral mucopolysaccharides and acidophilic proteins (Hotchkiss, 1948). The reaction is based on the presence of a 1:2 glycol group or its equivalent amino or alkyl amino derivative which the periodic acid can oxidize into two aldehydes with subsequent attachment of the recoloured fuchsin of the Schiff's reagent.

Methyl green-pyronin was used to detect RNA (Jordan & Baker, 1955; Culling, 1963). This is a competitive staining reaction wherein a planar monovalent cationic dye such as pyronin or toluidine blue will react with nucleic acids having freely accessible purine and pyrimidine bases (single stranded RNA), whereas non-planar divalent cationic dyes such as methyl green will stain polymerized DNA selectively (Brachet, 1954; Scott, 1967). It should also be noted that pyroninophilia may also be due to other polymeric acid substances such as acidic mucopolysaccharides (Chayen et al, 1969).
Toluidine blue (1% aqueous) was used after RNase to detect RNA (Brachet, 1940a, 1942, 1944; Pearse, 1968) and as a control for hyaluronidase treatment.

Alcian blue (AB) was employed to demonstrate acid mucopolysaccharides (Steedman, 1950) as was the dialyzed iron technique (Hale, 1946). Alcian blue is a phthalocyanin derivative that stains the uronic groups of acidic and sulphated mucopolysaccharides at an acid pH. Alcian blue, a cationic dye, is thought to form reversible electrostatic bonds between the cationic dye molecules and the anionic sites on the glycosaminoglycans (mucopolysaccharides). Hale's also stains acidic mucopolysaccharides at a low pH, but this is due to the affinity of free acidic groups for colloidal Fe+++ at a pH of 1.8-2.0.

Gomori's aldehyde fuchsin as modified by Halmi and Davies was used to stain for sulphated mucopolysaccharides (Pearse, 1968).

The cationic thiazine dye Azure A (pH 1.5) (McConnachie & Ford, 1966; Pearse, 1968; Kvist & Finnegan, 1969) was also used to demonstrate sulphated mucopolysaccharides. Cationic dye binding is thought to be electrostatic in nature and it therefore depends upon the pH of the staining medium and the pK value of the anionic groups involved. Lowering the pH (below 2.0) decreases the dissociation of carboxyl groups and therefore at pH 1.5, only the sulphated groups are ionized and can bind the dye (Szirmai, 1963).

The copper phthalocyanin technique of Kluver & Barrera (1953) was used to stain phospholipids. The dye is an amine salt of a sulphonated CuPC that may have 1-4 substituted sulphonyl groups per molecule and one of a number of bases. The mechanism of staining is thought to be an ion association reaction that links the base, (CuPC)SO$_3$H, to the free oxygen
atom of the phospholipid phosphate (Pearse, 1968).

To demonstrate the presence of lipid in cryostat sections, Sudan black B was used (McManus, 1946). The stain is used in a 70% ethanolic solution and the specificity arises from the increased solubility of the colourant in the tissue lipids.

Several enzyme methods were employed to increase the specificity of some of the staining reactions. Malt diastase (Nutritional Biochemicals Corp.) was used to remove glycogen prior to staining with PAS. Ribo-nuclease (Sigma Chemical Corp.) was used before the methyl green-pyronin and toluidine blue procedures. Testicular hyaluronidase (Sigma Chemical Corp.) was used to remove hyaluronic acid, chondroitin sulphate A and chondroitin sulphate C; toluidine blue and Azure A staining followed.

Methylation and methylation-demethylation (saponification) blocking and unblocking reactions (Culling, 1963) were used in conjunction with AF and Azure A staining. Methylation blocks anionic groups by forming methyl esters on carboxyl groups and hydrolyzing sulphate esters. Demethylation unblocks carboxyl groups only by hydrolyzing the methyl esters (Culling, 1963).

The preceding techniques are outlined in Appendix A.

PREPARATION FOR ELECTRON MICROSCOPY

Ovaries of the dogfish Squalus acanthias were bathed in Karnovsky's (1965) glutaraldehyde-paraformaldehyde fixative, dissected out and placed in fresh fixative. Individual oocytes or clusters of small oocytes were selected and placed in fresh fixative for 1-2 hours at ambient temperature. The fixative was buffered to a pH of 7.2 with 0.2 M cacodylate buffer (pH 7.4). The samples were washed twice in buffer and stored. Post-
fixation in OsO₄ (1% in 0.2 M Cacodylate buffer, pH 7.2) varied from 1 to 2 hours depending on the thickness of the specimen. Subsequently, the oocytes were washed, dehydrated, embedded in Spurr's (1969) medium (Polysciences, Inc.) and sectioned at 600 Å (silver-grey) on a Porter-Blum MT-2 ultramicrotome. For light microscopy, thick (1 μm) sections were placed on glass slides and stained with 1% toluidine blue in 1% sodium borate while for electron microscopy, thin sections collected on carbon coated grids were stained for 1 hour in methanolic uranyl acetate and 1 hour in Reynolds lead citrate (1963). Thin sections were subsequently examined with a Hitachi HU-7S electron microscope.
RESULTS

THE OVARY

The ovary of Squalus acanthias can be divided into four main regions: the medulla, an inner stroma of connective tissue with numerous blood vessels; the cortex, a broad peripheral layer containing the ova; the tunica albuginea, a fibrous connective tissue layer surrounding the cortex; and the germinal epithelium, the outer columnar epithelium of the ovary (Figures 7, 8, 10, 16). An important feature to note, however, is that developing ova are found only on the dorsal surface of the ovary. This coincides with the location of the ostium tubae, the common opening to the oviducts which is found on the dorsal aspect of the fused oviducts.

The developing ova are encircled by four distinct layers. These layers are the non-cellular vitelline membrane (VM); the granulosa or follicular cell layer; the follicular basement membrane (FBM); and the theca. The FBM is subdivided into an outer (basal lamella) and an inner (basal lamina) component.

HISTOCHEMICAL ANALYSIS

PROTEIN

The DNFB reaction (Figures 1, 2) shows the ooplasm to be proteinaceous while the clear vesicles in the ooplasm represent lipid lost during preparation. The VM is proteinaceous while the follicle and thecal cells show only a small amount of protein. The follicle basement membrane (basal lamina) stains moderately. Within the cortex there would appear to be two types of cell, one containing dark red granules and staining more intensely than a second lighter cell type lacking granules. The
1. DNFB staining of a 0.4 mm oocyte. Large arrowheads indicate cytoplasmic bridges anchoring the oocyte (O) which is surrounded by numerous lacunae (L). Small arrowheads indicate the basement lamina while the arrow points to nucleoli in the oocyte nucleus (N). Other symbols: S = stroma. 100 μm bar.

2. DNFB staining of a 0.8 mm oocyte. Arrowheads correspond to the previous figure. Note the reaction of the vitelline membrane (VM) and the mitochondrial mass (M). Numerous lipid droplets (Li) are present as clear vesicles in the ooplasm. 100 μm bar.
cytoplasm of the RBC stains darkly red and contains a few clear vesicles possibly lipid in nature. The tunica albuginea also gives a strong positive reaction, here demonstrating collagen, as a result of the complete reduction provided by titanous chloride rather than that of sodium hydrosulphite or stannous chloride (Tranzer & Pearse, 1964).

MUCOPROTEINS & NEUTRAL MUCOPOLYSACCHARIDES

Figures 3, 4 and 5 are representative of the Trichrome-PAS technique, a method which will give blue-black nuclei, yellow RBC's and acidophilic proteins in addition to the deep red reaction of mucoproteins and neutral mucopolysaccharides. Once again the ooplasm displays its proteinaceous and lipid nature. The nucleus (Figure 5) contains several nucleoli. The vitelline membrane stains intensely positive demonstrating its mucoprotein or neutral mucopolysaccharide nature. The cytoplasm of the follicle cells varies in its response for acidophilic proteins from negative to positive while the cytoplasm of the thecal cells does not respond to the Trichrome-PAS at all. The inner component of the FBM (the basement lamina) is slightly pink, positive to PAS, a reaction that may indicate the presence of a sialic acid (Culling, 1963; White, Handler & Smith, 1968). The cortex again displays two types of cell. The first type has a deeply indented nucleus and stains very intensely for acidophilic proteins. The second type has a round nucleus and stains less intensely for the presence of acidophilic proteins. The full methol (Trichrome-PAS) is a stain that is also specific for RBC's and they stain intensely for acidophilic proteins. The tunica albuginea (Figure 7) gives a slightly positive acidophilic reaction. The tunica can also be seen to send projections into the cortex. Another interesting feature is the presence of PAS
3. PAS-trichrome staining of a 0.8 mm oocyte. Note the reaction of the VM and the basement lamina (small arrowheads) for neutral MPS. The mitochondrial mass (M) responds strongly for acidophilic proteins. Lipid (Li) droplets are still noted in the ooplasm (o). 100 μm bar.

4. PAS-trichrome staining of a 0.5 mm oocyte. The reaction of the basement lamina (BL) is clearly indicated as is that of the VM. Note light cells (large arrowheads) and dark cells (arrow) in the stroma. Other symbols: FC = follicle cells, T = theca. 100 μm bar.
5. PAS-trichrome staining of a 0.8 to 1.0 mm oocyte. This is a second technique used as a control for the dichrome PAS method. Note nucleoli (small arrowheads), nucleated red blood cells (arrow) and a cytoplasmic bridge (large arrowhead). 100 μm bar.

6. PAS-dichrome, diastase control. Not clearly indicated here is a slight positive PAS reaction in the ooplasm and in the cytoplasm of the stromal (S) and thecal (T) cells. The small arrowhead indicates the basement lamina while the arrow points to the nucleus of a lacuna border cell. 100 μm bar.

7. PAS-dichrome diastase. The ooplasmic and cytoplasmic PAS reactions of the control are removed with diastase treatment. The reactivity of the VM and the BL is undiminished. Note the surface epithelium (SE) of the ovary and its underlying tunica albuginea (TA). 100 μm bar.
positive material located intercellularly within the cortex. Frequently a "flocculent" material that is slightly PAS positive can be observed in the lacunae external to the theca.

The Dichrome technique, Figure 6, offers a few features obscured by the full Trichrome method. The ooplasm, the cytoplasm of the stromal and thecal cells of the control all show a slight positive PAS reaction. Figure 7 illustrates the effect of malt diastase on Dichrome PAS staining. Here, the ooplasm and the stromal cytoplasm do not show a positive reaction to PAS. The intensity of the staining of the VM and the FBM is only slightly reduced. This is an artifact and is not indicative of the presence of glycogen in these membranes.

**RIBONUCLEIC ACID**

The methyl green-pyronin technique, Figures 8 and 9, was used to demonstrate the presence of RNA. The ooplasm is pink (proteinaceous) and vacuolated indicating the lipid lost during preparation. The nucleoli stain intensely red. The VM and the outer component of the FBM (basal lamina) stains brightly orange. The nuclei of the follicle cells and all other cells stain with methyl green indicating the presence of DNA. The cytoplasm of the follicle cells exhibits a moderate degree of pyroninophilia. The cytoplasm of the stroma cells would seem to respond differentially with the dark cells having the greater degree of pyroninophilia. The cells of the theca are only slightly pyroninophilic. The tunica is either negative or only slightly pyroninophilic. The surface epithelium of the ovary is covered by a coating of material that is also pyroninophilic (Figure 8).

Pyroninophilia by itself is an indicator of the presence of RNA
8. Methyl Green-Pyronin staining of an oocyte at the surface epithelium (SE). There is a pyroninophilic response in the follicle cells, the dark cells of the stroma, and in and on top of the surface epithelial cells. There is a positive reaction in the basement lamina (large arrowheads) and a negative reaction in the vitelline membrane. The arrows indicate the intercellular canals of the surface epithelium. 100 μm bar.

9. Methyl Green-Pyronin staining of a 0.8 mm oocyte demonstrating the positive reaction of the nucleoli and a faint response in the chromosomes (small arrowheads). Other symbols: BL = basement lamina, PC = follicle cells. 100 μm bar.
but only material staining blue with toluidine blue or red with pyronin and removable by treatment with ribonuclease is RNA.

Figures 10 and 11 demonstrate the toluidine blue reaction for nucleic acids. The use of 1% aqueous toluidine blue after RNase is an alternative to Brachet's (1940a, 1942, 1944) technique with methyl green-pyronin. Toluidine blue will stain metachromatically when the acidic substances are present as long polymeric molecules with their acidic groups closely packed as in acid mucopolysaccharides (Sylven, 1954). In these figures, the ooplasm is slightly orthochromatic although in oocytes larger than 1.0 mm the reaction is negative. The oocyte nucleus shows only a faint trace of orthochromasia in keeping with its size and distribution of nuclear material. The nucleoli, however, are deeply orthochromatic. The VM is negative, exhibiting neither orthochromasia nor metachromasia. The basement lamina exhibits marked metachromasia while the basement lamella is negative. The follicle cell and thecal cell nuclei react orthochromatically as do the stromal cell nuclei and epithelial nuclei. However, the former with less condensed chromatin do not stain as darkly as the latter. The cytoplasm of the follicle cells reacts orthochromatically and has the appearance of a fine homogeneous network of fibrous material. The same can be said of the cytoplasm of the thecal cells. The cytoplasm of the stromal cells is also orthochromatic but the reaction is slightly darker and its texture coarser. The tunica albuginea (not visible) exhibits a very faint metachromasia. This faint metachromasia can also be seen at the border of the lacunae. The RBC nuclei are markedly orthochromatic while the cytoplasm of these cells is non-reactive to the stain.

Ribonuclease treatment followed by methyl green-pyronin almost
10. Toluidine blue for nucleic acids and acidic mucopolysaccharides. This section clearly indicates the orthochromatic (blue) reaction of the nucleic acids in the nuclei of all cells and in the cytoplasm of the follicle, stromal (S), and surface epithelial (SE) cells. The metachromatic response (purple) of the basement lamina (BL) indicates acid mucopolysaccharides. 100 μm bar.

11. Toluidine blue for nucleic acids and acidic mucopolysaccharides. This section more clearly illustrates the reaction of the follicle, thecal, and stromal cells for nucleic acids. 100 μm bar.

12. Toluidine blue–RNase. After RNase treatment, all cytoplasmic (non-nuclear) orthochromasia is removed. The basement lamina retains its metachromasia and therefore lacks RNA. 100 μm bar.
completely removes the pyroninophilia of the nucleoli. The ooplasm is negative while the staining of the follicle cells is reduced. The basement lamina has lost a considerable amount of pyroninophilia while the stromal cells are now negative. All other elements are unchanged.

Hot trichloroacetic acid extraction (Humanson, 1972) was also used. It produces essentially the same results (not shown) as RNase except that it is not as specific. TCA also reduces the methyl green staining of the nuclei.

In contrast to these results, Figure 12 shows an RNase treated section stained with toluidine blue. Here the nuclei and only the nuclei stand out against a blank background. All other cytoplasmic and ooplasmic staining has been removed. The follicular basement lamina retains its metachromasia undiminished. Hot TCA extraction (not shown) as before is less specific. Here, all the elements present exhibit a reduction in staining. Most noticeable, however, is the fine trace of cytoplasmic orthochromasia still remaining in most of the stromal cells. The nucleoli of the oocyte also show a reduction in stain intensity.

ACIDIC & SULPHATED MUCOPOLYSACCHARIDES

The alcian blue reaction can be seen in Figures 13a, b, c. The ooplasm is negative for the presence of acid mucopolysaccharides (AMPS) having only a faint bluish background color. The VM is also negative. The FBM is positive for AMPS and stains bright blue-green. Occasionally a positive reaction can also be seen between the cells of the theca. The tunica albuginea is the only other element to show a positive reaction to alcian blue and it is slightly less intense than the reaction of the FBM. The surface epithelium also appears to be covered in a substance
13. Alcian blue reactivity of several 0.6 to 0.8 mm oocytes. A metachromatic (blue-green) response demonstrates the presence of acidic and sulphated mucopolysaccharides in the basement lamina (arrows, Figures a and b), the tunica (TA, Figure c), and possibly in the theca (T, Figure a). Small arrowheads (Figure b) indicate lampbrush chromosomes lying between numerous nucleoli in the nucleus (N). 100 μm bars.
that demonstrates alcianophilia.

The Hale's colloidal iron reaction is indicated by Figure 14. Here, the ooplasm (except for the non-staining lipid vesicles), the follicle cells, the thecal cells and the stromal cells all react with this stain. This, however, is regarded as "background" staining. The most important features to note here are the negative VM and the FBM that gives a very intense reaction. Clearly the FBM has an inner and an outer component, the former being negative or only slightly positive while the latter is intensely positive.

Figures 15b and 16b indicate the effect of testicular hyaluronidase in saline on the staining reaction of toluidine blue. There is a slight reduction in the orthochromasia of the stroma cells that is unexpected but it is obviously some acidic molecule that is hyaluronidase labile. But, the most important aspect of this method involves the FBM. Hyaluronidase treatment almost completely removes the metachromasia of the basement lamina.

According to Spicer and Meyer (1960), aldehyde fuchsin, Figures 17 & 18, stains sulphated mucins preferentially. The staining reaction is a combination of the carboxyl groups with an intermediate metastable species of the active dye molecule pararosaniline. The major reaction product is found in the basal lamina. Secondary reactions are also indicated in the tunica and in the granules of the dark cells.

Following methylation, staining was totally blocked in all areas except for numerous short fibres in the tunica and the basement lamina. It must be noted that the staining reaction in the basement lamina was markedly reduced.

Saponification or demethylation, Figure 19, restored moderate
14. Hale's Colloidal Iron reaction in a 1.0 to 2.0 mm oocyte. The vitelline membrane (VM) and the basement lamina (large arrowheads) do not respond to this reaction while the basement lamella (small arrowheads) clearly does respond. 100 μm bar.
15a. Toluidine blue control for hyaluronidase. Water-mounted.
16a. Note the metachromatic (purple) response of the basement lamina (arrows). Other symbols: AC = abdominal cavity, TA = tunica albuginea. 100 µm bars.

15b. Toluidine blue-hyaluronidase. Water-mounted.
16b. Hyaluronidase treatment clearly removes the metachromatic response (arrows) of the basement lamina as well as diminishing the orthochromasia of all other components. Other symbols: L = lacuna, N = nucleus, O = oocyte, SE = surface epithelium. 100 µm bars.
17. Aldehyde fuchsin reactivity of a 1.0 to 2.0 mm oocyte. Strong response noted in the basement lamina (large arrowhead) and the granules of dark cells. Small arrowheads indicate nucleoli in the oocyte nucleus (N) while the arrow points to a lampbrush chromosome. 100 μm bar.

18. Aldehyde fuchsin response of the tunica albuginea. The elastic fibres (E) stain deeply with aldehyde fuchsin while the collagen (C) fibrils react less intensely. 100 μm bar.

19. Following methylation and demethylation (MDM), moderate aldehyde fuchsin staining is restored to the basement lamina (arrow) and to the dark cell granules (not clearly shown). The elastic fibres of the tunica (inset) also retain moderate staining. 100 μm bar.
staining to the basement lamina and the granules of the dark cells.

Azure A is a cationic thiazine dye similar to toluidine blue that is used to demonstrate sulphated MPS when used at the pH of 1.5 (Szirmai, 1963; McConnachie & Ford, 1966; Kvist & Finnegan, 1970). Dye binding is electrostatic and as the pH is lowered below pH 2.0 only the sulphated groups are ionized and can bind dye (Szirmai, 1963; Kvist, 1968). Azure A activity is indicated by Figure 20. It is evident that at this pH only the basement lamina is metachromatic. The metachromatic activity of this membrane also varies, being almost non-existent in oocytes under 1 mm and increasing in intensity as the oocyte enlarges.

Methylation-demethylation, which blocks both carboxyl and sulphate groups and subsequently unblocks only the COOH groups, affects Azure A staining as shown by Figure 21. It is obvious that both the orthochromasia and the metachromasia are only slightly reduced. Testicular hyaluronidase when applied to Azure A staining, Figure 22, has virtually no effect on the metachromasia of the basement lamina while the orthochromasia of all other elements is only slightly reduced.

PHOSPHOLIPIDS & LIPIDS

The copper phthalocyanin (CuPC) method of Kluver & Barrera (1953) was used, Figures 23a, b, to demonstrate the presence of phospholipids. The reaction of the ooplasm will vary from negative to only slightly positive depending upon the length of differentiation in 0.05% aqueous lithium carbonate. This indicates that the amount of phospholipid present at this stage is small. The mitochondrial cloud, however, gives a strong positive reaction (not shown). The nuclei of all cells stain with neutral red as do the nucleoli of the germinal vesicle.
20. Azure A reactivity of a 0.8 mm oocyte clearly indicates the presence of sulphated mucopolysaccharides in the basement lamina (FBM). Arrow indicates a mitotic figure while the small arrowheads point to a light cell (left) and a dark cell (right). 100 μm bar.

21. Azure A-MDM treatment indicates the presence of sulphated MPS in the basement lamina (arrow) by only faintly restoring the metachromasia. Several nucleoli can be seen in the nucleus (N). 100 μm bar.

22. Azure A-Hyaluronidase treatment has little effect on the metachromasia of the basement lamina (arrow). 100 μm bar.
Copper phthalocyanin treatment for phospholipids reveals strong positive reactions in the cytoplasm of the light (LC) and dark (D) cells with a moderate response in the thecal cells and the ooplasm. Other symbols: FC = follicle cells, L = lacuna, O = oocyte, T = theca, VM = vitelline membrane, arrow = basement lamina, large arrowhead = nucleated red blood cell. 100 μm bar.
The chromatin of the germinal vesicle is finely dispersed at this stage and thus does not stain with neutral red. The VM is negative to CuPC and only very slightly positive to neutral red. The basement lamina, on the other hand, gives a positive neutral red reaction while the basal lamella is negative to both CuPC and neutral red. The presence of phospholipid positive material in the granulosa cells is difficult to ascertain. There seems to be a slight reaction, but it is by no means as intense as the reaction of the stroma cells. Similarly, the reaction of the thecal cells is stronger than that of the follicle cells but not as strong as that of the stromal cells. In the stroma, the cytoplasm of the dark cells gives a strong positive reaction for phospholipid. The light cells, on the other hand, give a negative or only slightly positive reaction. The tunica albuginea also stains for phospholipid. The red blood cells are the most intensely reactive of all the elements found in these sections. The haemoglobin gives an intensely positive reaction for phospholipids while the nuclei stain brilliantly red.

Sudan black B, Figures 24, 25, has a high affinity for phospholipids (Baker, 1944, 1958; McManus, 1946; Pearse, 1968) and is used in a 70% ethanolic solution. The localization of the stain in cryostat sections is very interesting. The basement lamina is homogeneously grey while the VM is negative. The pertinent feature is the extreme localization at the apical portion of the follicle cells and the appearance of small lipid droplets traversing the vitelline membrane. There appears to be a localization of lipid along the oocyte-VM border. The central region of the oocyte shows the dense accumulation of lipid at this stage (approx. 3.0 mm in diameter).
24. Sudan Black B. Cryostat sections of a 3.0 to 4.0 mm oocyte. The vitelline membrane is a broad clear band dotted with small lipid droplets. The apical portion of the follicle cells (large arrowhead) is packed with lipid. The basement lamina is indicated by a blunt arrow. Other symbols: O = oocyte, S = stroma. 100 μm bar.

25. Sudan Black B. Cryostat sections of a 3.0 to 4.0 mm oocyte. The ooplasm indicates the presence of large quantities of lipid (black) droplets. 100 μm bar.
Table IA, IB, IC: A Table of the histochemical results obtained in this study.

Key to the Table:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>negative response</td>
</tr>
<tr>
<td>+/-</td>
<td>weak to negative response</td>
</tr>
<tr>
<td>+/-2</td>
<td>weak response</td>
</tr>
<tr>
<td>+</td>
<td>moderate response</td>
</tr>
<tr>
<td>++</td>
<td>good response</td>
</tr>
<tr>
<td>+++</td>
<td>strong response</td>
</tr>
<tr>
<td>++++</td>
<td>intense response</td>
</tr>
<tr>
<td>d</td>
<td>droplets in the VM</td>
</tr>
<tr>
<td>e</td>
<td>elastic fibres</td>
</tr>
<tr>
<td>m</td>
<td>metachromasia</td>
</tr>
<tr>
<td>o</td>
<td>orthochromasia</td>
</tr>
<tr>
<td>on</td>
<td>orthochromatic nucleus</td>
</tr>
<tr>
<td>onu</td>
<td>orthochromatic nucleolus</td>
</tr>
<tr>
<td>or</td>
<td>orange</td>
</tr>
<tr>
<td>r</td>
<td>red</td>
</tr>
<tr>
<td>y</td>
<td>yellow (protein)</td>
</tr>
<tr>
<td></td>
<td>DNPB</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
</tr>
<tr>
<td><strong>TUNICA</strong></td>
<td>+++</td>
</tr>
<tr>
<td><strong>STROMA</strong></td>
<td></td>
</tr>
<tr>
<td>LIGHT</td>
<td>+</td>
</tr>
<tr>
<td>DARK</td>
<td>+++</td>
</tr>
<tr>
<td><strong>THECA</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>FBM</strong></td>
<td></td>
</tr>
<tr>
<td>OUTER</td>
<td>-</td>
</tr>
<tr>
<td>INNER</td>
<td>++</td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>VM</strong></td>
<td>+++</td>
</tr>
<tr>
<td><strong>OOCYTE</strong></td>
<td>+++</td>
</tr>
<tr>
<td><strong>RBC</strong></td>
<td>++++</td>
</tr>
<tr>
<td><strong>NUCLEOLI</strong></td>
<td>++</td>
</tr>
<tr>
<td><strong>MITO. CLOUD</strong></td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>ALCIAN BLUE</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>TUNICA</strong></td>
<td>++</td>
</tr>
<tr>
<td><strong>STROMA</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>LIGHT</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>DARK</strong></td>
<td>++ o</td>
</tr>
<tr>
<td><strong>THECA</strong></td>
<td>+/-</td>
</tr>
<tr>
<td><strong>FBM OUTER</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>INNER</strong></td>
<td>+++</td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>VM</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>OOCYTE</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>RBC</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>NUCLEOLI</strong></td>
<td>...</td>
</tr>
<tr>
<td><strong>MITO. CLOUD</strong></td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>AZURE METH. A pH 1.5</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>TUNICA</td>
<td>-</td>
</tr>
<tr>
<td>STROMA LIGHT</td>
<td>-</td>
</tr>
<tr>
<td>DARK</td>
<td>+ oonu</td>
</tr>
<tr>
<td>THECA</td>
<td>+ o</td>
</tr>
<tr>
<td>FBM OUTER</td>
<td>-</td>
</tr>
<tr>
<td>INNER</td>
<td>+/2 ++ m</td>
</tr>
<tr>
<td>FC</td>
<td>+ o</td>
</tr>
<tr>
<td>VM</td>
<td>-</td>
</tr>
<tr>
<td>OOCYTE</td>
<td>-</td>
</tr>
<tr>
<td>RBC</td>
<td>...</td>
</tr>
<tr>
<td>NUCLEOLI</td>
<td>+ o</td>
</tr>
<tr>
<td>MITO CLOUD</td>
<td>-</td>
</tr>
</tbody>
</table>
ELECTRON MICROSCOPICAL OBSERVATIONS

THE OVARIAN EPITHELIUM

The surface of the ovary is pitted by shallow invaginations that lead to the larger oocytes (Figures 8, 16) as well as to the ovulated follicles and is covered by a "germinal epithelium". This term persists although the evidence favours the extragonadal origin of the primordial germ cells (Bloom & Fawcett, 1968; Hoar, 1970). This epithelium, possibly cuboidal in living tissue, is columnar in fixed material and is devoid of cilia contrary to the observations of TeWinkel (Mustelus canis, 1972) and Metten (Scylliorhynus canicula, 1941).

The plasma membrane is thrown into shallow folds along the surface of each cell while numerous clear and filled vesicles (180-250 μm X 360-400 μm) are observed just beneath the membrane (Figures 26, 27, 28, 29, 30, 31, 32). Frequently, both types of vesicle can be seen fused with the plasma membrane exposing their contents to the abdominal cavity. The material within the filled vesicles is filamentous. The cytoplasm of these cells is filled with numerous small vesicles (140 μm), microfilaments (36 to 45 Å in diameter), granular and agranular endoplasmic reticulum, often seen on the same cisternae, mitochondria and Golgi bodies. The most abundant organelle is the free ribosome (Figures 26 to 32). The nuclei are constricted into a varying number of lobes with only a narrow band of condensed chromatin present that is applied to the inner leaflet of the nuclear membrane. The nucleolus has a fibrillar core and an extensive granular cortex. The outer leaflet of the nuclear membrane, which is occasionally studded with ribosomes and blebs as if giving off vesicles, is separated from the inner membrane by a wide (100 to 150 Å) perinuclear cisterna. Nuclear pores are seen infrequently.
26. Surface epithelium of the dogfish ovary. The dominant features of this region are the large mucus filled channels (ICC) between the columnar cells and the large nuclei of the epithelial cells. The cells are filled with microfilaments (large arrowhead), Golgi bodies (G), mitochondria (M) and ribosomes. In this figure, several of the membrane to membrane relationships are indicated, particularly the convoluted plasma membrane (concentric membranes) in the upper right. Other symbols: NU = nucleolus, P = plug of detritus. 1 µm bar.
A panorama of the surface epithelium of the dogfish ovary from the collagen (C) of the tunica albuginea to the apical plasma membrane. The apices of four epithelial cells can be seen separated by three membrane contact surfaces each of which ends in a tight junction (TJ). and which may or may not have desmosomes along its length. At the very apex of each cell, numerous vesicles can be seen (V, arrows) that often open into the abdominal cavity. Also found in the apex of each cell are multitudes of ribosomes (R), E.R. and Golgi bodies (G). The nucleus is highly constricted and has a peripheral band of condensed chromatin. The basal aspects of these cells rest on a clearly defined basement membrane or lamina (BL) with numerous pinocytotic vesicles (PV) found in the cytoplasm and opening into the space between the cell and the B.L. The configuration of the intercellular channel (ICS) is shown as is the continuity of this channel with the clear zone between the B.L. and the cell. 1 μm bar.
28. High magnification of the apex of three surface epithelial cells and the intercellular channel (ICC). Two tight junctions (TJ) and several desmosomes (D) are indicated as are the numerous surface vesicles (V, arrows). Other symbols: AC = abdominal cavity, CPM = convoluted plasma membrane, G = Golgi, M = mitochondria, N = nucleus, P = pseudopodia, R = ribosomes. 1 μm bar.
29. Higher magnification of the apex of two surface epithelial cells. Of particular interest are the tight junctions (TJ) and desmosomes (D) found holding the two cells together. Also evident are the many surface vesicles (arrow) filled with a flocculent material, the microfilaments (MF), mitochondria (M) and small, smooth vesicles (SV). Other symbols: AC = abdominal cavity, N = nucleus. 1 μm bar.

30. Higher magnification of the apex of two surface epithelial cells. A well defined tight junction (TJ) can be seen as well as a region of convoluted plasma membrane (CPM) between the two cells. Numerous ribosomes (R) fill the cytoplasm. The arrow indicates one of many large surface vesicles while the arrowhead points to a fibrous material on the exposed surface of the cell. 1 μm bar.
31. An apical protuberance of a surface epithelial cell filled with surface vesicles some of which are opening (arrows) into the abdominal cavity (AC). Other symbols: large arrowhead = microfilaments, F = folds of the plasma membrane. 1 μm bar.

32. The apical region of at least three cells indicating the various relationships of their lateral cell membranes, CPM = convoluted plasma membrane, D = desmosomes. Other symbols: large arrowheads = microfilaments. 1 μm bar.
due to the angle of the section. In typical fashion, these epithelial cells rest on a basal lamina (basement membrane) a moderately dense, homogeneous band that varies in thickness, depending on the angle of the section, from 250 to 500 Å wide. The basal lamina follows the contour of the basal cell membrane but is separated from it by a light zone 500 Å wide. The bulk of the present literature indicates that this membrane is composed of tropocollagen embedded in an acid mucopolysaccharide matrix and that this membrane is a product of the epithelial cells and not a condensation of the connective tissue ground substance (Kurtz & Feldman, 1962; Pierce et al, 1964). Supporting this statement is the presence of numerous vesicles in the basal cytoplasm along with others undergoing "reverse pinocytosis" at the cell membrane (Figure 27). The junctional complexes of these epithelial cells are represented by zonula occludentes (tight junctions) and macula adherentes (desmosomes). The former (Figures 26 to 30) are found nearest the abdominal cavity and are characterized by the absence of an intercellular space (I.C.S.) in this region. Beneath the zonula occludens, the I.C.S. widens (360 Å) and then the membranes may exhibit one or more of three phenomena. First, the lateral cell membranes may run unimpeded to the intercellular channel (I.C.C.) (Figures 26, 27). Second, the membranes may be thrown into a series of concentric folds (Figures 26, 30, 32). Finally, there may be several desmosomes present (Figures 26, 27, 29, 32). In any event, the I.C.S. abruptly widens into an intercellular channel (Figures 26, 27, 28) that persists to within 100 nm of the basal lamina. Within this channel is a flocculent material that is probably protein or protein-polysaccharide in nature as well as a certain amount of membranous detritus. In Figure 26, a "plug" of mucoid debris can be seen trapped...
in a fold of the surface epithelium. At the basal aspects of these cells there are no junctional complexes evident. In one case, Figure 27, the channel is continuous with the light zone between the plasma membrane and the basal lamina.

THE TUNICA ALBUGINEA

The tunica albuginea which lies beneath the surface epithelium is most commonly described as a fibrous layer of connective tissue (Nelsen, 1953; Bloom & Fawcett, 1968; Mossman & Duke, 1973). TeWinkel (1972) describes this layer in Mustelus canis as being a collagenous connective tissue layer with interwoven smooth muscle. In Squalus, the tunica is composed primarily of "collagen fibres", elastic fibres and fibroblasts (Figures 18, 27, 33, 34). The "collagen fibres" which are 1 to 4 μm in diameter depending on their location are composed of unit fibrils of collagen 500 to 800 Å in diameter and of indeterminate length. The unit fibrils are cross-striated, with transverse bands repeating every 520 Å. The elastic fibres also vary in thickness from 0.5 to 2.0 μm and they are composed of fine filaments 50 to 60 Å in diameter. The fibroblasts in the tunica are typical of quiescent cells with elliptical nuclei, a few surface folds, small Golgi vesicles and a few granular E.R. cisternae. Also of interest are the vesicles that fuse with the plasma membrane surrounding a "collagen fibre". However, contrary to TeWinkel's findings, there is no evidence for the existence of smooth muscle in the tunica of Squalus acanthias.

THE CORTEX

The cortex of the ovary consists of a richly cellular stroma
33. The various elements of the tunica albuginea, collagen (C), elastic fibres (EF) and fibroblast-like cells. 1 μm bar.

34. High magnification of an elastic fibre (EF) demonstrating the 50 to 60 A filaments found within the fibre. The small arrowhead indicates fibres of similar diameter found freely in the tunica. 1 μm bar.
interspersed with blood vessels and large lacunae. Within the cortex there are three different types of cell: "dark" cells, "light" cells and lacunae border cells. Also within this region are numerous capillaries, some large blood vessels and a network of intercellular collagen.

The dark cells (Figures 35, 36, 37) have polymorphic nuclei with a band of chromatin apposed to the inner nuclear membrane and small patches dispersed centrally in the nucleoplasm. The outer nuclear membrane is free of ribosomes and is separated from the inner membrane by a space 550 to 650 Å wide. The cytoplasm of these cells is filled with numerous small empty vesicles (650 Å) and many large vesicles (approx. 800 nm). The large vesicles (dark bodies) can be either empty, partially filled with a fine granular material, completely filled, or any of these with a varying number of fibrous or crystalloid inclusions. These dark bodies resemble the granules of mammalian eosinophilic leucocytes complete with their crystalloid inclusions (Fawcett, 1966). There are also a few mitochondria and free ribosomes present but no E.R. or Golgi bodies. The cytoplasm is electron dense and is filled with a fine granular material. The plasma membranes of adjoining cells are separated by a space of 70 Å. Where three or more cells meet, the intercellular space can be filled with an electron dense substance. Figure 37 depicts what may be a dark cell except for the lack of a peripheral band of chromatin and the absence of dense crystalloids in the dark bodies. The presence of four Golgi bodies enclosing forming dense bodies is another feature that is not typical of a dark cell.

The second type of cell found in the cortex is the light cell (Figures 38, 39, 40, 41) which has a spherical or ovoid nucleus with a centrally located nucleolus and a chromatin pattern similar to the
35. A dark cell found in the cortex of the ovary. Note its characteristic lobed nucleus (N) and the dark bodies in the cytoplasm (FDB = forming dark body, LV = large vesicle with a small amount of dark body material). Also characteristic of a dark cell are the crystalloids (large arrowheads) found in the dark bodies. The cytoplasm of a dark cell is also filled with numerous small vesicles (V). 1 μm bar.

36. A dark cell with its characteristic contents and lobed nucleus. The large arrowhead indicates two nuclear pores. Another interesting feature to note is how loosely these cells seem to be anchored to one another. Other symbols: LC = light cell. 1 μm bar.
37. An intermediate dark cell with dark bodies (DB) but no crystalloids, a lobed nucleus (N) without a peripheral band of chromatin, and a cluster of Golgi bodies (G) an organelle not noted in a dark cell. Other symbols: M = mitochondria. 1 μm bar.

38. A light cell in the ovarian cortex. This cell type is characterized by an ovoid nucleus (N) with an outer nuclear membrane that is covered with ribosomes and is actively contributing (arrowheads) to the numerous large R.E.R. cisternae found in the cytoplasm. Other symbols: M = mitochondria, SV = large membrane bound smooth vesicles seemingly in transition to large filled vesicles (V). 1 μm bar.
39. Two light cells partially separated by a bundle of collagen fibres (C). The extent of the R.E.R. in the cytoplasm is evident as are several nuclear pores (arrow). Other symbols: small arrowheads = expanded outer nuclear membrane studded with ribosomes, M = mitochondria, N = nucleus. 1 μm bar.

40. A light cell with a nucleolus (NU) and a cluster of distorted smooth vesicles (SV) and outlying dense vesicles (DV), an arrangement similar to that of a Golgi body. 1 μm bar.

41. A cluster of several light cells separated by collagen fibres. Other symbols: RER = rough endoplasmic reticulum. 1 μm bar.
dark cell. The outer nuclear membrane is studded with ribosomes and is well removed from the inner membrane (360 Å to 4000 Å). The cytoplasm of these cells is filled with large vesicles of R.E.R. which encloses a light, flocculent material. Figures 38 & 39 suggest that these large vesicles of R.E.R. are formed by pinching off folds of the outer nuclear membrane, a theory consistent with the belief that the E.R. and the nuclear membrane are closely related if not continuous (Fawcett, 1966; Brown & Bertke, 1969). The cytoplasm contains a few mitochondria, polyribosomes, small vesicles and microtubules. Additionally, the cytoplasm of a light cell is less electron dense than that of a dark cell due to the absence of the fine granular matter found in the dark cells. Within the light cell, Golgi bodies are absent or highly distorted. However, a few medium-sized vesicles filled with a moderately electron dense granular substance can be seen lying close to several distorted lamellae (Figures 38, 40). Occasionally within a light cell one or more lipid drops can be found.

THE INTERCELLULAR SPACE

Frequently, the intercellular space will widen and the area will be occupied by a variable number of collagen fibres and an amorphous ground substance (Figures 39, 41) or large lipid droplets (Figures 42, 44).

THE LACUNAE BORDER CELLS

Within the cortex are numerous lacunae or sinusoids each lined by a special type of cell hereafter termed a lacunae border cell L.B.C. (Figures 42, 43, 44). Superficially, the L.B.C. resembles the simple squamous endothelial cell of a capillary wall, thickened in the region
42. Three lacunae border cells, a type found lining the numerous lacunae (L) of the cortex. These cells are filled with free ribosomes, microfilaments (F), mitochondria (M) and vesicles of various sizes. One type of vesicle (small arrowhead) can be found in the basal region where it is either continuous with the plasma membrane or free in the cytoplasm. These cells rest on a basal lamina (BL). Other symbols: D = desmosome. 1 \( \mu \text{m} \) bar.

43. Two lacunae border cells and their basal lamina (BL) lying "over" numerous collagen fibres (C) and two dark cells. The vesicular content and the basal pinocytosis (small arrowheads) of these cells is also evident. Rarely, vesicles can be seen opening into the lacuna (arrows). The surface fold (MF) is a configuration implicated in pinocytotic activity. 1 \( \mu \text{m} \) bar.

44. A lacuna border cell "overlying" two light cells (LC). Other symbols: C = collagen fibres with periodicity, F = filaments, L = lacuna, M = mitochondria. 1 \( \mu \text{m} \) bar.
of the nucleus and attenuated elsewhere. Unlike the capillary cell, the L.B.C. have few, if any, Golgi bodies or centrioles (Bloom & Fawcett, 1968), but the cytoplasm is packed with free ribosomes, microfilaments (30 to 50 Å in diameter), a few profiles of granular endoplasmic reticulum and a few mitochondria. These organelles are scattered throughout the cytoplasm rather than concentrated near the nucleus as in capillary cells. Also quite numerous in the cytoplasm of these cells are vesicles of medium (210 nm) to large (270 nm) size. The 210 nm vesicles are either clear or filled with a moderately electron dense finely granular material (Figures 42, 43) while the larger vesicles are free of any electron dense matter. One of the most conspicuous and characteristic features of the lacunae border cells is the presence of many small vesicles 600 to 700 Å in diameter (Figures 42, 43) which seem to arise from a large number of saccular invaginations of the plasma membrane that open onto the lacunar and basal surfaces of these endothelial cells. These vesicles are most numerous at the basal surface where they open into the "light zone" that separates the plasma membrane from the basal lamina. These vesicles indicate that a great deal of micropinocytotic activity is going on but none of these small vesicles have ever been observed fusing with any of the larger ones. The basal lamina (150 to 200 Å thick) is similar in all respects to that of other epithelia except that it is about one half the thickness of the basal lamina of the surface epithelial cells described earlier. As is the case with the latter, the lamina is probably composed of tropocollagen (Bloom & Fawcett, 1968). The lacunar plasma membrane rarely shows vesicles opening onto the surface (Figure 43) but several vesicles of this size range (600 to 800 Å) can be seen just beneath the plasma membrane (Figures 42, 43). Additionally, the
surface membrane possesses a few broad but short pseudopodia as well as the occasional narrow protuberance that resembles the surface fold generally implicated in pinocytotic activity (Figure 43). At the junctions of these cells the adjoining membranes come to within a few hundred angstroms of each other but for the most part remain distinctly separate. Typically, the apical region of the junction is characterized by the presence of a desmosome. The only other form of junctional complex between these cells is the occasional interdigitation of the plasma membranes (Figures 42, 43). Underlying the basal lamina is a basement lamella of an amorphous ground substance and an ordered array of collagen fibrils which seems to be typical of all epithelial cells (Bloom & Fawcett, 1968; Nadol et al, 1969).

**THE RED-BLOOD CELLS**

The nucleated red blood cells of *Squalus* can be seen in Figures 45, 46, & 47. These elliptical cells are about 24 μm long by 10 to 12 μm wide and 2.0 to 2.5 μm thick. The nucleoplasm is granular, homogeneous and electron dense. There is no obvious pattern of condensed chromatin. The nuclear membrane has a wide perinuclear cisterna with the occasional nuclear pore that bridges the cisterna. The haemoglobin is finely granular and homogeneous with the exception of a few inclusion vesicles. Unlike human erythrocytes (Tanaka & Goodman, 1972) with relatively few small clear vesicles, the blood cells of *Squalus* possess numerous large vesicular inclusions (280 to 550 μm) that typically contain whorls of membrane-like material (Figures 45, 46). This myelin-like configuration is brought about by the precipitation of phospholipids during fixation (Matthews & Martin, 1971). In one of the red blood cells (Figure 45) a
45. Two red blood cells and a nucleated capillary wall cell. There are myelin-like figures and "Golgi" bodies (G) present in these cells. 1 μm bar.

46. Three red blood cells, the lower with a portion of its nucleus showing. There is a marginal band of microtubules (arrow) present and several myelin-like vesicles (MV). 1 μm bar.

47. High magnification of the marginal band of Figure 46 here clearly indicating the microtubular (MT) nature of the band. 1 μm bar.
Golgi-like body can be seen. A marginal band of microtubules is present encircling the flattened elliptical cell at its equator, lying just beneath the cell membrane. This band is composed of 15 to 20 microtubules about 250 Å in diameter (Figures 46, 47). Figure 45 also shows a capillary wall cell. It is similar to a lacuna border cell except that it lacks large filled vesicles, small vesicles that fuse with the basal plasma membrane and its basal lamina that is thinner and less conspicuous than that of the lacuna border cell.

THE THECAL CELLS

Around a 0.3 mm oocyte, the thecal cells are elongate with flattened nuclei while the condensed chromatin is applied to the inner nuclear membrane (Figure 48). The nucleoplasm is either light or dense depending on the density of the cytoplasm with the density of the innermost cells obscuring any cytoplasmic detail while the more distal cells possess numerous Golgi bodies, vesicles of all size ranges, a few mitochondria, microtubules and microfilaments. There are only a few ribosomes present and no E.R. cisternae. The occasional lipid drop can also be found in the thecal cells at this stage. The thecal cells appear to be grouped into "islands" by the presence of an extremely dense, granular, intercellular matrix. Within that matrix are numerous profiles of collagen. A similar situation can be seen within the ovaries of the hamster and gerbil except the matrix is structureless (McReynolds et al, 1973). When the oocyte has attained a diameter of 1.0 mm the cells of the theca can be differentiated as either interna or externa.

The cells of the theca interna (Figures 50, 51, 52) have retained the flattened nuclei and chromatin pattern of the earlier stages. The
48. The thecal cells of a 0.3 mm oocyte undifferentiated as to interna or externa. The nuclei are flattened and the cytoplasm contains numerous Golgi (G) and clear vesicles. A granular matrix (GM) is evident separating the thecal cells from the follicle and the cortex. Other symbols: BL = basement lamina, C = collagen, LC = light cell. 1 μm bar.

49. The theca interna cells of a 2.0 mm oocyte with numerous E.R. cisternae and mitochondria (M). 1 μm bar.
50. Theca interna cells of a 1.0 mm oocyte with numerous free ribosomes, some on cisternae (arrows = RER) while other areas are clear (= SER). A well developed granular matrix (GM) is present. 1 μm bar.

51. Theca interna cells of a 1.0 mm oocyte with many free ribosomes but no R.E.R. Mitochondria (M) with granules and plate-like cristae are noted as are large vesicles containing granules (arrow) similar to those of the granular matrix (GM). 1 μm bar.

52. A theca interna cell of a 1.1 mm oocyte. Note the granular matrix (GM) and its collagen fibres external to the cell. Within the cell are numerous clear vesicles (LV, V) derived from either the Golgi (G) or the nuclear membrane (small arrowheads). A pair of centrioles (CE) is also evident in this cell. 1 μm bar.
most conspicuous feature of the cytoplasm of these cells is the presence of numerous large, clear vesicles (0.5 μm). Figure 52 suggests that these vesicles may have originated from the Golgi apparatus or from blebs of the nuclear membrane. Vesicles of other sizes can also be seen, the smallest of which (0.09 μm) are closely associated with the Golgi bodies. There is very little S.E.R. present and even less R.E.R. The numerous polysomes and free ribosomes often give the impression of R.E.R. when they lie close to some of the vesicles and the few S.E.R. tubules (Figures 50, 51). Mitochondria are occasionally seen and at this stage their cristae are plate-like. Microtubules of various lengths and the occasional droplet of lipid are also present in these cells. The dense granular matrix surrounding "islands" of thecal cells is still present and occasionally vesicles within the cells can be seen filled with a similar granular substance (Figure 51). The intimate association of this intercellular matrix and the collagen fibres can be seen in Figures 50, 51, and 52.

The theca externa cells of a 1.0 mm oocyte (Figures 53, 54, 55) have nuclei that are more spherical than those of the theca interna. The chromatin pattern is similar but there is a greater quantity of it in the core of the nucleus. These cells lack the large clear vesicles that are common to the cells of the theca interna. The vesicles that are present (0.1 to 0.3 μm) can be either clear, coated or filled with an electron dense material. In the latter case, this material resembles the granular matrix. The occasional vesicle can also be seen emptying into the intercellular space. Free ribosomes and polysomes are present as are frequent R.E.R. cisternae. Another interesting feature of these cells is the intricate relationships of their plasma membranes and the
53. A theca externa cell of a 1.0 mm oocyte. Note the absence of the large clear vesicles that characterize the theca interna. Several ribosomes and polysomes can be seen as well as some R.E.R cisternae. Other symbols: small arrowhead = cytoplasmic vesicle opening into the intercellular space, large arrowhead = SER continuous with the nuclear membrane. 1 \( \mu m \) bar.

54. Two theca externa cells of a 1.0 mm oocyte with their intricate membrane relationships clearly indicated and a portion of a theca interna cell in the lower left on the other side of the granular matrix (GM). Also evident in the cytoplasm are microtubules, small vesicles and mitochondria (M). 1 \( \mu m \) bar.

55. The cytoplasm of several theca externa cells of a 1.1 mm oocyte surrounded on the right by the granular matrix (GM) which separates the "thecal island" from the cells (LC) of the cortex. The interrelationship of the cells of the externa is a characteristic missing in the cells of the theca interna. Other symbols: R = ribosomes. 1 \( \mu m \) bar.
large intercellular spaces.

At the 2.0 mm (Figure 49) stage, the cells of the theca interna have acquired more cisternae of E.R. than at the 1.0 mm stage. In most cases, these cisternae are only partially studded with ribosomes. The number of mitochondria present has also increased slightly.

THE BASAL LAMINA-LAMELLA COMPLEX

The basement lamina-lamella complex is present around the smallest stage examined (0.1 mm). Thus any protein or lipid material en route to the oocyte must cross them. At this stage (Figures 56, 57, 58), the basement lamina is approximately 0.4 μm wide. It is a dense homogeneous band closely applied to the plasma membrane of the follicle cell. The basal lamella, however, is about 2.1 μm wide and it is filled with numerous collagen profiles. There is a loose organization of the collagen fibrils and scattered cross and longitudinal section can be seen. By 0.2 mm oocyte diameter, the lamina has attained an average width of 0.7 to 1.0 μm. Concomitant with this increase in width is the appearance of a linear fibrillar pattern within the membrane itself. This pattern is most prominent in the outermost 0.3 μm. The basal lamella has narrowed slightly (1.75 μm) and a granular component is now present. Along the plasma membrane of the follicle cell are small dense patches that appear to be localized thickenings of the membrane. At 0.3 mm diameter (Figures 60, 61) the average thickness of the lamina is approximately 1.0 μm. The fibrils (200 Å) are slightly more prominent while an intimate relationship between the lamina and the collagen fibres of the basal lamella seems to be developing. In the 0.4 to 0.5 mm oocytes observed, the basement lamina varied in thickness from 0.35 to 1.4 μm. The fibrillar
56. The follicle of a 0.1 mm oocyte composed of a homogeneous basement lamina or membrane (BM) and its outlying basement lamella (BL), a follicle cell layer (FC) and a vitelline membrane lying between the follicle cells and the oocyte (O). Note the ribosomes on the outer nuclear membrane of the F.C. nucleus, the projections of the ooplasm into the F.C. (large arrowheads) and the pinocytotic activity (arrow) at the base of the ooplasmic microvilli. 1 μm bar.

57. A demonstration of the variation in follicle thickness within a single oocyte (re: previous figure). Note the number of vesicles within the F.C., the V.M. and the oocyte. BM = 0.4 μm, VM = 0.5 μm. 1 μm bar.

58. A typical follicle cell of a 0.1 mm oocyte filled with numerous free ribosomes, rough endoplasmic reticulum (ER), mitochondria (M) and Golgi (G). The edge of a nucleus (N) with nuclear pores (arrow) is indicated as is the basement lamina and the basal lamella (BL). The large arrowheads indicate the projection of other follicle cells or the oocyte into this follicle cell. Note the microvillous nature of the oocyte's (O) plasma membrane. BM = 0.4 μm, VM = 0.5 μm. 1 μm bar.
59. The vitelline membrane (VM) of a 0.2 mm oocyte showing its fine fibrous nature. The apical aspect of the follicle cell (FC) is filled with ribosomes and mitochondria and often projections of the F.C. into the oocyte can be seen (arrow). Other symbols: large arrowheads = ooplasmic villi. 1 μm bar.

60. The follicle of a 0.3 mm oocyte. The basement lamina (BM) is quite thick and contains 200 Å fibrils (arrows). Follicular penetrations of the vitelline membrane (arrow) are noted and when observed in the oocyte (O) they usually terminate with a desmosome (D). BM = 1.05 μm, VM = 0.4 to 0.7 μm. 1 μm bar.
61. The follicle of a 0.3 mm oocyte with follicle cells that have numerous free ribosomes, mitochondria, and microtubules (arrow). This area of the follicle may be the region of transition from the animal to the vegetal pole. The occasional desmosome was noted along the follicular villi (large arrowhead). Other symbols: BM = basement lamina, O = oocyte. BM = 0.7 μm, VM = 0.4 to 0.8 μm. 1 μm bar.

62. The zona radiata (PZ) of a 0.3 mm oocyte, a band of ooplasmic vesicles or the tips of the ooplasmic microvilli found within the V.M. peripheral to the oocyte (O). The large arrowhead indicates a dense vesicle found within the oocyte. 1 μm bar.

63. The zona radiata (PZ) of the same oocyte 180° removed from the previous figure. 1 μm bar.
The basal lamina (BM), lamella (BL) and basal cytoplasm of the follicle cells (FC) of a 0.4 mm oocyte. The small arrowhead indicates the banding pattern of the collagen of the lamella. The outer membrane of the nucleus can be seen giving off ribosome studded vesicles (arrows) which form part of the R.E.R. component found in the basal cytoplasm. Also found in the cytoplasm are numerous vesicles filled with a component similar to the basement lamina (large arrowheads). BM = 1.06 μm. 1 μm bar.

The basal follicle cell cytoplasm filled with vesicles (large arrowheads) that are of similar density and texture to the basal lamina (BM). Note too, the rough E.R. and the mitochondria. BM = 0.88 μm. 1 μm bar.

The basal lamina around a 0.4 mm oocyte demonstrating its fibrillar pattern and interspersed granules. The basal lamella (BL) is filled with interwoven collagen fibres and here a foot-like process from a thecal (T) cell can be seen. BM = 1.4 μm. 1 μm bar.
67. The follicle of a 0.4 mm oocyte showing early stages in the formation of the vitelline membrane (arrows) with both ooplasmic and follicular projections or villi. The apical cytoplasm of the F.C. is filled with ribosomes, Golgi (G) and mitochondria. Other symbols: SER = smooth endoplasmic reticulum in the cortical ooplasm. EM = 0.6 to 0.7 µm, VM = 0.0 to 0.2 µm. 1 µm bar.

68. The follicle of a 0.4 mm oocyte in the early stages of vitelline membrane formation. Numerous ooplasmic and follicular villi are seen (arrows) with the latter often surrounded by a halo of microfilaments. The apical cytoplasm of the F.C. is filled with Golgi (G), smooth vesicles and ribosomes. In the basal cytoplasm, vesicles (large arrowhead) that contribute to the formation of the basement lamina can be seen. EM = 0.7 µm, VM = 0.0 to 0.2 µm. 1 µm bar.
69. The follicle of a 0.4 mm oocyte (O) with no vitelline membrane evident. The basement lamina (BM) is completely fibrillar. These follicle cells are filled with Golgi (G), small vesicles (arrowhead) and some R.E.R. Other symbols: T = theca. BM = 0.3 µm. 1 µm bar.

70. A different region of the same oocyte with a well developed vitelline membrane (VM) and the developing villar nature of the oocyte. Other symbols: arrowhead = stream of material penetrating to the S.E.R from the base of the villi, M = mitochondria, N = nucleus, R = ribosomes. BM = 0.35 µm, VM = 0.28 µm. 1 µm bar.

71. Another region of the same 0.4 mm oocyte follicle with extensive R.E.R. in the F.C. cytoplasm and several cellular or oocytic projections (arrowheads). Note the well developed S.E.R. band in the oocyte and the lipid (L) yolk particle. BM = 0.37 µm, VM = 0.5 µm. 1 µm bar.
72. Development of the vitelline membrane in a 0.4 mm oocyte. In areas of follicle cell-oocyte (0) contact, desmosomes can be seen (large arrowheads). The cytoplasm of the F.C. is filled with ribosomes, vesicles, Golgi (G) and mitochondria (M) and some microfilaments (arrow). VM = 0.0 to 0.15 μm. 1 μm bar.

73. Finely fibrous vitelline membrane forming in patches between oocyte (0) and follicle cell villi (large arrowhead). 0.4 mm oocyte. Note also the microfilaments (arrows) associated with the villi. VM = 0.0 to 0.3 μm. 1 μm bar.

74. Note the patchy formation of the vitelline membrane and its fibrous nature (large arrowhead) in a 0.4 mm oocyte. The dominant feature of these three figures is the abundance of organelles in the F.C. near the V.M. and the lack of them in the oocyte (0). VM = 0.0 to 0.2 μm. 1 μm bar.
75. A panorama of several pseudo-stratified follicle cells (FC) from the basement lamina (BM) to the oocyte (O). 0.4 mm oocyte. The vitelline membrane is almost non-existent and desmosomes (D) can be seen in areas of membrane to membrane contact. Similar structures are noted between follicle cells. The cytoplasm of the F.C.s is filled with ribosomes (R), polyribosomes, several vesicles, mitochondria and E.R. Oocytic villi (arrow) can be seen and they are surrounded by microfilaments. BM = 1.2 μm. 1 μm bar.

76. The thickest vitelline membrane (VM) observed around a 0.4 mm oocyte. The V.M. is finely fibrous and is pierced by pore canals (arrow) or villi from either the oocyte or the follicle cell. Other symbols: G = Golgi, N = nucleus, VI = ooplasmic villi. VM = 2.5 to 4.5 μm. 1 μm bar.
pattern of the lamina (B.M.) now involves the entire membrane and at times this pattern is augmented by the appearance of granules between the layers (Figures 64, 65, 66, 68, 69). The basal lamella (B.L.) is about 0.6 to 0.8 μm wide, its granular content has increased as has the complexity of the relationship of the collagen fibres both among themselves and with the basement lamina (B.M.). At 0.6 mm oocyte diameter, the basement lamina has an average thickness of 0.8 to 1.0 μm, there has been little change. By 0.8 mm diameter, the lamina has an average thickness of 1.1 μm and the fibrillar pattern is becoming less evident. At a diameter of 1.5 to 1.8 mm (Figures 88, 89, 90), the B.M. varies in thickness from 4.7 to 5.5 μm. The fibrillar pattern of the earlier stages is absent and the membrane is homogeneously granular. The B.L. is about 6.0 μm wide and is loosely filled with an interwoven array of collagen fibres and numerous clusters of 0.025 μm granules. There is also a finer component that seems to be derived from the B.M. itself. At the basement lamina-lamella interface, the collagen fibres are so arranged that some of them have their origin in the substance of the basement lamina. The former orderly arrangement of these fibres is no longer evident, a "herringbone" pattern having taken its place.

THE FOLLICLE CELLS

The follicle or granulosa cells of the 0.1 mm oocyte (Figures 56, 57, 58) are low cuboidal and one layer thick. This corresponds to the findings of Lance & Callard (1969) who noted that all elasmobranchs with a single cell-type granulosa belong to the order Selachii while those with a two cell-type granulosa belong to the order Batoidei. Typical of epithelia cells, they rest on a basement membrane and face a lumen (the
oocyte). The nuclei are oval with a finely dispersed chromatin pattern and slight condensations bordering the nuclear membrane. The cytoplasm contains numerous mitochondria with plate-like cristae, free ribosomes and scattered vesicles (300 to 800 Å). Rough E.R. is present though not extensive and is concentrated in the mid to basal regions of the cell. A few S.E.R. tubules are also evident. In the basal region, several of the vesicles (800 Å) are filled with material having the same electron density and texture as the basement lamina. But, none of these vesicles can be observed fusing with the plasma membrane. Another interesting feature of these cells is the presence of several dense double membrane bound vesicles in the apical region of the cell (Figures 56, 58). These "vesicles", however, are projections of the ooplasmic membrane into the follicle cells. Similar phenomena have been reported by Yamamoto in Oryzias (1963). In a 0.4 mm oocyte these ooplasmic projections are quite evident as are projections of the follicle cells into the oocyte (Figures 60, 67, 68, 73). Often associated with the ooplasmic projections is a "halo" of microfilaments (Figures 68, 72, 73, 74, 75). The Golgi bodies, vesicles and S.E.R. are still prominent in the apical regions of these cells (Figures 67, 68, 69, 72). Rough E.R. is scattered throughout the cytoplasm but more highly concentrated in the basal portion of the cell (Figures 64, 65, 71, 74). In the basal region next to the lamina, electron dense vesicles (500 to 1600 Å) are still present (Figures 64, 65, 66, 68). Free ribosomes and polysomes are also evident as are small desmosomes (Figures 68, 72, 73, 75) which are located between adjacent cells and sometimes in areas where the membranes of the oocyte and the follicle cell meet. Another interesting feature of this stage is the varying thickness of the B.M. (lamina) (0.75 to 1.5 μm) from one
side of the oocyte to the other. This corresponds respectively to the vegetal-animal axis of the oocyte and to a decrease in the density of the cytoplasmic and nuclear material of the follicle cells of the animal hemisphere. The animal pole is turned inward relative to the surface of the ovary (Wallace, 1904; Raven, 1961). In a 1.1 mm oocyte, the cytoplasmic and ooplasmic projections are less evident. The basal portions of the follicle cells still contain numerous dense vesicles (120 to 360 nm), mitochondria and R.E.R. cisternae (Figures 77, 78, 84). None of these vesicles, however, are seen fusing with the plasma membrane. In the apical region (Figures 77, 79, 85), Golgi bodies are prominent as are occasional R.E.R. cisternae. Also present in the apical region at this stage are lipid droplets (Figures 77, 79, 84, 85) 0.6 to 0.7 μm in diameter. By 1.5 to 1.8 mm, the follicle cell layer has changed considerably. The cells are becoming columnar (see also Yamamoto, 1963; Bellairs, 1965; Cummings et al, 1971) or pseudostratified columnar, with the nuclei displaced towards the basement lamina (Figures 90, 91, 92). Tangential sections produce a multilayered follicular epithelium. These cells are identical to the follicle cells of smaller oocytes in all other aspects. The cells closest to the B.M. (Figure 90) are filled with numerous small vesicles of varying electron densities. The most dense vesicles are closest to the basement lamina. Also within these cells are several mitochondria, free ribosomes and vesicular R.E.R. Microtubules (Figures 90, 92) are plentiful, forming a latticework throughout the cell. The intercellular membranes are quite porous and even show the occasional intercellular bridge (Figures 90, 91, 92). At or above the level of the nuclei the follicle cell cytoplasm now contains Golgi bodies and R.E.R. cisternae in greater quantities than are found in the basal regions.
77. The follicle cells of a 1.1 mm oocyte. The basal aspect (upper right) is filled with mitochondria (M), dense vesicles and R.E.R. The apical region is filled with numerous Golgi (G), clear vesicles and some R.E.R. Other symbols: L = lipid, N = nucleus, VM = vitelline membrane. BM = 3.0 to 3.5 μm, VM = 10 to 15 μm. 1 μm bar.

78. The basal aspect of a follicle cell (FC) of a 1.1 mm oocyte. The layered nature of the basement lamina (BM) is evident as are the dense vesicles (large arrowheads) in the F.C. BM = 2.5 μm. 1 μm bar.

79. The apical aspect of a follicle cell of a 1.1 mm oocyte. Unlike the previous figure, the cytoplasm is filled with mitochondria (M) and R.E.R. The V.M. is to the lower left. 1 μm bar.
80. The vitelline membrane of a 1.1 mm oocyte (0) pierced by pore canals (large arrowhead) for the passage of villi. The zona radiata is beginning to take shape at the oocyte's surface. 1 μm bar.

81. The zona radiata of a 1.0 mm oocyte (0). At the base of the zone are broad villi (VI) with some pinocytotic activity evident. The large arrowhead indicates a pore canal. 1 μm bar.

82. An extensive zona radiata in a 1.0 mm oocyte probably produced by the angle of sectioning. The broad villi bases (OB) terminate in smaller vesicle-like tips (OV) buried in the substance of the V.M. 1 μm bar.
83. The broad villi (OB) of the zona radiata (PZ) of a 1.0 mm oocyte also depicting the pinocytotic activity at the oocyte's surface (arrows). 1 μm bar.

84. Three follicle cells of a 1.1 mm oocyte each differing in background densities. The large arrowhead indicates a dense vesicle of similar composition to the lamina (BM). Other symbols: arrows = pore canals, L = lipid, VM = vitelline membrane. EM = 3.0 to 3.5 μm, VM = 10 to 15 μm. 1 μm bar.

85. A follicle cell of a 1.1 mm oocyte containing several dense lipid bodies, mitochondria (M), ribosomes (R), rough endoplasmic reticula (RER) and numerous small vesicles (large arrowheads). 1 μm bar.
86. A low magnification, thick section of the follicle of a 1 to 2 mm oocyte (O). The angle of the section broadens the vitelline membrane (VM) and gives a pseudostratified appearance to the tall, columnar follicle cells (FC) with their basal nuclei. The basement lamina (B) and the lamella lie between the F.C.s and the "islands" of theca cells. 370X.

87. Higher magnification of the follicle cells, vitelline membrane (VM), the oocyte (O) and the intervening zona radiata (PZ). 600 X.
88. The basement membrane or lamina (BM) of a 1.5 to 1.8 mm oocyte. The texture of the membrane is granular and in the lower left several F.C. vesicles of similar composition can be seen. At the lamina-lamella interface it is obvious that the collagen fibres of the B.L. have their origin in the basement lamina. BM = 4.7 μm. 1 μm bar.

89. The basement lamella (BL) of a 1.5 to 1.8 mm oocyte. The collagenous nature of these fibres is clearly evident (arrow) and intermingled within the herringbone pattern are clusters of granules (G) of unknown origin. The fibres of the B.L. are also closely associated with the theca interna cells (T). 1 μm bar.
90. The basement membrane (BM) and several follicle cells of a 1.5 to 1.8 mm oocyte. The numerous dense vesicles (arrow) of the F.C. are closely associated with the basement lamina (BM) interface. Of particular interest is the large cytoplasmic continuity between two follicle cells (large arrowhead). 1 μm bar.

91. Numerous pseudostratified follicle cells (FC) at the level of their basal nuclei (N). The basement lamina of this 1.5 to 1.8 mm oocyte is to the upper left. 1 μm bar.

92. Two follicle cells above the level (closer to the oocyte) of the nuclei with several Golgi (G), short E.R. cisternae and numerous microtubules (arrows). The small arrowheads indicate breaks in the lateral cell membranes. The V.M. is to the lower right. 1 μm bar.
(Figure 92). As the oocytes increase in size (greater than 2.0 mm), this follicular cell arrangement persists. In the apical regions there is an increasing amount of lipid present and subsequent to this is the separation of the follicle cells and the vitelline membrane. There are, however, cytoplasmic bridges that traverse this gap and connect some follicle cells with the vitelline membrane.

**THE VITELLINE MEMBRANE**

The relationship between the follicle cells, the cell membrane of the oocyte, the zona radiata and the V.M. is intimate and complex (Figures 86, 87). Any consideration of the formation of the V.M. must include each of the aforementioned elements.

In the smallest oocytes examined (0.1 mm) the vitelline membrane (V.M.) is well developed. It is a homogeneously dense structure of 0.5 to 0.7 µm thickness (Figures 56, 57, 58). The oocyte plasma membrane is highly folded and it is evident that some of these projections or villi traverse the substance of the V.M. (Raven, 1961; Bellairs, 1965; Mørreyang, 1968), approach and even penetrate the neighbouring follicle cells. At the base of these projections, several small clear vesicles (Figures 56, 57) can be seen passing into the ooplasm. The material of the V.M. occupies fully the space between the F.C. and the oocyte. This is quite unlike chorion formation in the teleost *Oryzias* (Yamamoto, 1963) where the forming membrane is deposited in a wide intercellular space.

By 0.2 mm, the V.M. has taken on a fibrous appearance (Figure 59). The extent of its penetration of both the oocyte and the follicle cells is evident, as is the close relationship of the follicle cells and the oocyte. The close proximity of the oocyte villi to the follicle cells
and the presence of follicle cell cytoplasm beneath the V.M. attests to this. The vitelline membrane varies in thickness from non-existent to 0.5 μm.

At 0.3 mm the V.M. was observed at thicknesses ranging from 1.0 to 3.0 μm (Figures 60, 61, 62, 63). The structure of the membrane is unaltered and numerous follicular penetrations are still evident. At the base and occasionally along the sides of these projections desmosomes or desmosome-like figures can be seen (Figures 60, 61). Similar observations have been made in a variety of higher vertebrates (Mørrevang, 1968) and in birds particularly (Bellairs, 1965). The zona radiata is quite obviously that area created by the macro- and microvilli of the oocyte plasma membrane. The extent of this zone varies within a single oocyte and from oocyte to oocyte (Figures 60, 62, 63). The penetration of clear and dense vesicles into the cortical ooplasm also varies with the development of the V.M. and the Z.R. (Figures 60, 61, 62, 63). Also present in the cortical ooplasm are a few smooth E.R. vesicles and cisternae.

In 0.4 mm oocytes, there are conflicting data as to the thickness and therefore the development of the vitelline membrane. In one specimen the V.M. varies in thickness from non-existent to 3.0 to 4.0 μm (including the zona radiata). The specimen with the thickest V.M. (3.0 to 4.0 μm) is also fibrous and displays numerous pore canals (Figure 76). Many macrovilli are present on the ooplasmic border and interspersed among these villi are vesicles of ooplasm that spread out into the vitelline membrane. The spaces between the macrovilli are devoid of fibrous V.M. material but are partially filled with a flocculent substance.

In a second specimen, the V.M. varies from non-existent to 0.5 μm
thick (Figures 69, 70, 71). Where the V.M. is absent, the oocyte and the follicle cells are separated by a space of approximately 70 Å. Local thickenings or electron dense patches are also evident along this interface. The boundary of this interface is not smooth, as the two plasma membranes interdigitate extensively. Also of interest are the gaps in the plasma membranes of neighbouring follicle cells (Figures 69, 70). Presumably, as observations are made nearer to the opposite pole the thickness of the V.M. increases. The interface of the V.M. and the follicle cells is remarkably smooth while the ooplasmic surface displays its characteristic villi. Again, not all the spaces between the villi are filled with the material of the V.M. Characteristic projections of follicular material into the ooplasm are still present (Figures 69, 71). Approximately 2 µm beneath the ooplasmic membrane is the beginning of a band of S.E.R. vesicles. This band also contains a few mitochondria and Golgi bodies. Between this band and the base of the ooplasmic villi are "paths" of electron dense material (Figures 70, 71).

The most interesting specimens of this stage are only beginning to form the vitelline membrane (Figures 67, 68, 72, 73, 74, 75). There are frequent desmosomes present along the apposed F.C. and oocyte plasma membranes. The fine fibrous nature of the V.M. is noticeable but often it is difficult to discern. The membrane does not form evenly around the oocyte but forms in clumps between the ooplasmic projections (Figures 73, 74). Often in areas where these patches are located the F.C. plasma membrane can not be seen. Interestingly, the F.C. cytoplasm adjacent to the projections and V.M. patches contains many fine microfilaments (Figures 67, 68, 73) 25 Å in diameter. Above all, the most striking feature of this and the other 0.4 mm specimens is the abundance of cellular
organelles within the follicle cells close to the forming V.M. and the lack of them near the V.M. in the oocyte.

When the oocyte has reached a diameter of 1.0 to 1.1 mm the vitelline membrane has changed. The V.M. is now about 10 to 15 µm thick and it occasionally appears to be granular (Figures 80, 81, 82). Pore canals are still present, but there are also holes or clear vesicles in the membrane that are the same size as the pore canals (Figures 80, 81, 84). The interface of the follicle cells and the V.M. is relatively even and only infrequently interrupted by interdigitations. In the specimens observed, there are no desmosomes present along this interface. At the ooplasmic border the relationship between the V.M. and the oocyte is more complex than previously noted. Although some specimens still exhibit simple macrovilli, villi and vesicles, others display an intricate array of ooplasmic "buds", intermingled smaller ooplasmic vesicles and a band of ooplasmic vesicles (zona radiata) penetrating the V.M. (Figures 80, 81, 82, 83). The spaces between the larger "buds" and the smaller vesicles is only partially filled with a material similar to that of the vitelline membrane. The larger "buds" contain vesicles of moderately electron dense material derived by micropinocytotic activity (Figure 83).

At a diameter of 1.5 to 1.8 mm the V.M. is about 50 to 70 µm thick. The entire membrane is fibrous and is extensively pierced by pore canals which in turn are filled with a fibrous material (Figures 93, 94, 95). At the F.C. interface, the fibres of the V.M. are closely associated with the F.C. plasma membrane and in some instances may continue 500 to 900 Å past the plasma membrane (Figures 93, 94). The microfilaments within the pore canals extend into the follicle cells a distance of approximately 1.0 µm. In the follicle cells and closely associated with the areas
93. The vitelline membrane-follicle cell (VM-FC) interface of a 1.5 to 1.8 mm oocyte. The V.M. is fibrous and often fibres can be seen extending into the follicle cell. Note the pore canals (PC) which here are F.C. villi. 1 μm bar.

94. The fibrous nature of the V.M. is evident with similar fibres found within the pore canals (PC) and F.C. cytoplasm (arrows). Vesicles (V) are also noted in the follicle cell closely associated with the pore canal and the membrane interface. 1 μm bar.

95. The vitelline membrane (VM) penetrated by pore canals (PC) of various orientations (arrows). 1 μm bar.
96. The zona radiata (PZ) of a 1.5 to 1.8 mm oocyte. It is composed of vesicles and lies within the vitelline membrane (VM) external to the oocyte and the broad oocyte villi (OB). 1 μm bar.

97. The cortical region of a 1.5 to 1.8 mm oocyte (O) showing pinocytotic vesicles (arrow) and vesicles inside of and outside of (double arrows) a lining body (LB). Note the "paths" (S) of electron dense material that penetrate into the oocyte to a depth of 7 μm. Lining bodies are also illustrated in Figures 146, 147, 156, 157. 1 μm bar.
where microfilaments penetrate the follicle cells are numerous vesicles 500 to 700 Å in diameter (Figure 94). The pore canals (Figure 95) that penetrate the V.M. do not have any particular orientation, crossing each other at various angles. As the pore canals approach the oocyte (Figure 96), they are no longer discernable from the elements of the vesicular band surrounding the oocyte. Depending on the angle of the section, this band is from 2.0 to 5.0 μm wide. The ooplasmic membrane is still highly convoluted and the spaces between the macrovilli only partially filled with V.M. material. Within the cortical region of the oocyte the "paths" of electron dense material first reported in a 0.4 mm oocyte are quite extensive (Figure 97). These "paths" anastomose freely and penetrate to a depth of 6 to 7 μm beneath the oocyte membrane. Also seen throughout this cortical region and even deeper into the oocyte are numerous double membrane bound structures. These structures are filled with a fibrous material reminiscent of that within the pore canals. Some of the larger structures also exhibit vesicles 500 Å in diameter (Figure 97) similar to those found near the F.C.-V.M. border.

When the oocytes have reached a diameter of 2.5 mm the vitelline membrane has an average thickness of 60 to 90 μm. The membrane has a fine granular appearance and it no longer has gaps between the oocyte villi. The villi are on the whole tall (2.2 μm) and regular with the appearance of a striated band. The vesicular band (Z.R.) is no longer evident although small (440 Å) canal-like structures are still visible.

THE OOCYTE NUCLEUS

The nucleus of a 0.1 mm oocyte (Figure 101) has numerous nucleoli and lampbrush chromosomes and is therefore a primary oocyte in mid-
98. Nuclear region of a 0.1 mm oocyte. Large arrowheads indicate ribosomal particles in the nucleoplasm and ooplasm. Arrows indicate E.R. connections with a G.V.B. Note the pores (P) and blisters (B) of the nuclear membrane. 1 µm bar.

99. Nuclear region of a 0.1 mm oocyte with numerous yolk precursor bodies nearby. Notice also the proximity of the S.E.R. vesicles to the nuclear membrane. 1 µm bar.

100. Nuclear region of a 0.1 mm oocyte demonstrating the production of smooth membrane bound vesicles (B) from the nuclear membrane. 1 µm bar.
diplotene (Bloom & Fawcett, 1968; Davidson, 1968; Bellairs, 1971). The nuclear membrane or envelope of the dogfish oocyte is typical, in that it is composed of two membranes 50 to 90 Å thick separated by a perinuclear cisterna 350 to 700 Å wide (Figures 98, 99, 100) (Yamamoto, 1964; Fawcett, 1966). The ooplasmic side of the membrane is devoid of ribosome-like particles thus resembling the smooth surfaced vesicles found in the neighbouring ooplasm. The outer nuclear membrane is generally not parallel to the inner membrane but displays numerous "blisters" or "blebs" at irregular intervals along its length, the blebs being extremely close to or nearly continuous with the smooth surfaced vesicular E.R. in the ooplasm. The inner nuclear membrane follows a less tortuous path and in contrast is twice as thick as the outer nuclear membrane. At this stage, the nuclear envelope is covered with nuclear pores 500 to 600 Å in diameter, irregularly distributed being clustered (16/μm²) in certain areas of the nuclear membrane and sparse elsewhere (4/μm²).

The nucleoli of a 0.1 mm oocyte are granular in nature and consist of 150 to 200 Å particles. The structure of the largest (20 μm diameter) observed nucleolus (Figures 102, 104) is reticular with the outer 4 μm being less densely packed, thus giving the impression of active disintegration. In the nucleoplasm between the nucleoli and the nuclear membrane there are numerous electron dense particles 150 to 200 Å in diameter. Particles of the same size range can also be observed in the ooplasm opposite the nuclear pores. Another interesting phenomenon observed (Figure 103) is a large (800 nm) nucleolar-like body in the ooplasm.

The nuclear morphology of a 0.2 mm oocyte is similar (Figure 108) with the exception of a slight increase in the number of smooth vesicles
101. Cross-section of a 0.1 mm oocyte demonstrating its relationship to the other elements of the ovary, note particularly the surface epithelium (SE), the tunica (TA) and a nucleolus (NU) within the nucleus of the oocyte. 450 X, 10 μm bar.

102. Nucleus of a 0.1 mm oocyte (Figure 101) showing the granular nature of the nucleolus (NU), the ribosomal particles (R) and the pores (P) of the nuclear membrane. 1 μm bar.

103. Ooplasm of a 0.1 mm oocyte with a nucleolar-like body (NU-B) present amidst several G.V.B.s. 1 μm bar.
Nucleolus (NU) of a 0.1 mm oocyte indicating its granular and reticular nature, its fragmentation and dispersal of $150 \text{ Å}$ particles as well as its extreme size. 1 \( \mu \text{m} \) bar.
next to the outer nuclear membrane.

The smooth, even nuclear membrane of 0.3 mm oocytes (Figure 110) reflects an increase in nuclear volume. Nuclear pores are abundant (25 to 30 / \( \mu m^2 \)) and the nucleoplasm still contains 150 to 200 Å particles.

By 0.8 mm diameter (Figures 128, 129) the most notable change in the nuclear envelope is the frequency (50 to 60/ \( \mu m^2 \)) of the nuclear pores. They are approximately 600 to 700 Å in diameter and have a centre-to-centre spacing of 120 to 180 nm. In the nucleoplasm, there are dense patches of a finely granular material adjacent to the pores but clear areas between the pores. As in the earlier stages the nucleoplasm contains abundant electron dense particles (150 to 200 Å) which occur singly and in clusters. Clusters of similar particles are also found in the ooplasm. At this stage there is a perinuclear band devoid of smooth E.R. vesicles and cisternae. The granular nucleoli have increased in number, and range in size from 2.6 to 25 μm.

At 1.0 mm, light microscopy (Figure 135) reveals the smooth outline of the nucleus and its large size (350 to 400 μm). Within the nucleoplasm, several lampbrush chromosomes can be seen, as well as 12 to 13 (per section) nucleoli 6 to 30 μm in diameter. Clustered around the nucleus are numerous mitochondria and lipid vesicles and a solitary stack of annulate lamellae (Figure 136).

In a 1.1 mm oocyte the ultrastructure of the nucleus is virtually the same as it was in a 0.1 mm oocyte, the only differences being the overall increase in nuclear diameter, the number of nucleoli and the irregularity of the nuclear membrane (Figure 148). The frequency of nuclear pores is at least equivalent to that of an 0.8 mm oocyte with
105. Central ooplasm of a 0.1 mm oocyte with the outer fringe of a 9 to 10 μm mitochondrial cloud (MM) shown. Note also the abundance of smooth membrane bound vesicles within the cloud and in the ooplasm. The large G.V.B. also shows some dissolution of its contents. 1 μm bar.

106. A portion of the mitochondrial mass (MM) of a 0.1 mm oocyte demonstrating the intimate association of the smooth endoplasmic reticulum of the cloud with the mitochondria as well as the outlying granular and granular-vesicular bodies (GVB). 1 μm bar.

107. Central ooplasm of a 0.1 mm oocyte with extensive S.E.R. near the mitochondrial mass. Small arrowhead indicates a myelin-like figure within a G.V.B.; note the expanded Golgi (G) lamellae. 1 μm bar.
108. Nuclear region of a 0.2 mm oocyte with several pores in the membrane and numerous vesicles (V) at the ooplasmic side of the membrane. Numerous ribosomes (R) and S.E.R. are also present in the perinuclear ooplasm. The mitochondria (M) display stretched (dumbbell) or constricted configurations. 1 μm bar.

109. Central ooplasm of a 0.2 mm oocyte with extensive S.E.R. in vesicular and tubular configurations, Golgi bodies (G) with expanded lamellae and mitochondria (M). The parallel alignment of cristae and the slightly biconcave center may indicate the mitochondrion is beginning to divide. 1 μm bar.
almost every fragment of available space occupied. At high magnification (Figure 1H9) the nucleoli of this stage appear to be bipartite, having intermingled granular and amorphous regions.

THE MITOCHONDRIAL MASS

In a 0.1 mm oocyte the distribution of mitochondria through the ooplasm is sparse with the exception of a densely packed mass of mitochondria (Figures 105, 106) located midway between the central nucleus and the vitelline membrane. This "cloud" of mitochondria is 9 to 10 μm in diameter and consists of both mitochondria and vesicular smooth E.R. The mitochondria range from 0.3 to 0.8 μm in diameter and have plate-like or tubular cristae. In some instances, the cristae align themselves in closely apposed rows and frequently appear to fuse together.

This "cloud" persists in all sizes examined (pre-vitellogenic) with increasing dispersion within the ooplasm. Variations in mitochondrial morphology are also noted. In a 0.2 mm oocyte, the incidence of mitochondria within the ooplasm has risen. In addition to the "normal" mitochondria, several unusual configurations are seen. One form of mitochondrion displays its cristae aligned in long parallel rows (Figure 109). The second (Figure 108) displays several forms of constriction. In one case the mitochondrion is stretched extremely thin in the centre and presents a "dumb-bell" shape. Another type of constriction is reminiscent of binary fission of unicellular organisms. In a 0.3 mm oocyte the mitochondria of the ooplasm display few unusual features, with the exception of an occasional ring-shaped mitochondrion (Figures 113, 114). In a 0.5 mm oocyte the numbers of ooplasmic mitochondria have increased considerably over the 0.1 mm oocyte (Figures 123, 126)
and branched mitochondria occur (Figure 124). By 0.8 mm the ooplasmic mitochondria are quite numerous but their distribution is not uniform and does not follow any particular pattern. The mitochondrial mass of a 0.8 mm oocyte is quite large, at least 15 to 20 μm in diameter (Figure 130), and consists of polymorphic mitochondria and extensive smooth E.R. systems both vesicular and cisternal (Figures 131, 132, 133). There are, however, three basic types of mitochondria within the "cloud": ring, dumb-bell and normal. In a 1.0 mm oocyte the number of mitochondria in the ooplasm is similar to that of 0.5 to 0.8 mm oocytes, however, the mitochondrial mass (Figures 137, 138) is not as densely packed and the amount of S.E.R. has decreased. Within the mitochondria, membranous whorls can now be seen in the matrix and they may or may not be continuous with the cristae. Similar whorls are also found in the ooplasmic mitochondria (Figure 141).

No further ultrastructural observations were made on the mitochondrial mass but in light microscopic observations this structure was still present in oocytes up to 4.0 mm in diameter. At this stage the mass had axes of 0.5 mm and 0.2 mm.

LIPID YOLK

In the earliest oocytes examined there is little lipid yolk present, however, in 0.3 to 0.4 mm oocytes numerous lipid droplets can be found (Figures 111, 112, 118, 120, 121, 122). These droplets are densely osmiophilic, ovoid and lack a limiting membrane. Their average size is 2 μm x 3 μm although numerous smaller droplets are found, the latter either reflecting the angle of sectioning through a larger droplet or demonstrating the formation of a lipid droplet. These droplets are
110. Nucleus of a 0.3 mm oocyte with a smooth even membrane and abundant nuclear pores (P). Ribosome-like particles are found on either side of the nuclear membrane. 1 µm bar.

111. Cross-section of a 0.3 mm oocyte indicating the relationship of the oocyte (O) with its band of lipid yolk (LY) to the vitelline membrane (VM, small arrowhead), the basement lamina (large arrowhead) and the interposed follicle cell layer. Other symbols: Li = lipid found intercellularly in the stroma, LC = light cells, DC = dark cells. 380 X, 10 µm bar.

112. Cross-section of a 0.3 mm oocyte. High magnification. The interdigitation of the V.M. and the oocyte is seen as is a thecal layer (T) and the basal lamina (large arrowhead). 608 X, 10 µm bar.
distributed in the ooplasm (Figures 111, 112) midway between the nucleus and the vitelline membrane. Frequently, only the vesicles of the S.E.R. are observed in close association with the lipid droplets, but occasionally, mitochondria and/or Golgi are also found nearby.

In 0.5 mm oocytes (Figures 125, 127) the association of the lipid yolk droplets with vesicles and cisternae of S.E.R. is more pronounced than that of 0.4 mm oocytes. Infrequently, a Golgi body can be found near a droplet and in one instance (Figure 123) a droplet was found surrounded by concentric rings of S.E.R. cisternae and numerous mitochondria.

In 0.8 mm oocytes with the increased development of the mitochondrial mass (Balbiani body), the lipid drops closest to this body (Figures 132, 133) become more intimately associated with the mitochondria, however, the chains of S.E.R. vesicles are still the organelles most often associated with the lipid yolk droplets (Figures 133, 134). In 1.0 and 1.1 mm oocytes, this association of the lipid droplets with ooplasmic organelles is markedly reduced. However, where smaller droplets are observed, the smooth E.R. vesicles can still be found close at hand (Figures 139, 150).

Further ultrastructural observations were hampered by inadequate fixation, however, normal lipid yolk drops were observed in 3.0 mm oocytes. These droplets were quite large, 3 μm x 4 μm to 5 μm x 7 μm, and appeared to be bipartite having a less dense core.

**PROTEIN YOLK**

Unlike lipid yolk, the precursors of protein yolk can be found in 0.1 mm oocytes as multigranular (M.G.B.), multivesicular (M.V.B.) and granular-vesicular bodies (G.V.B.).
The multigranular bodies are the least numerous and are the smallest of the three types. These membrane-bound bodies have a clear background, vary in size from 120 nm to 250 nm and may contain any number of electron dense granules (yolk granules) 100 to 500 Å in diameter (Figures 99, 105, 106). Similar bodies (intracisternal granules) have also been found in crustacean (Kessel, 1968) and trout oocytes (Beams & Kessel, 1973).

Multivesicular bodies are also found in 0.1 mm oocytes (Figure 99). They are structures of moderate size (0.75 to 1.0 μm in diameter) bound by a membrane that may or may not completely encircle the body. Within this body are numerous smaller (50 to 150 nm) membrane-bound vesicles and a finely granular product.

The third and most numerous form of protein yolk precursor found in 0.1 mm oocytes is the granular-vesicular body (G.V.B.) (Figures 99, 105, 106). These are the largest of the three types (1 to 2 μm in diameter) and contain the same structures found in the M.V.B.s and M.G.B.s and in addition, fractions of broken or dissolving vesicular membranes that resemble myelin figures (Figures 98, 99, 107). Myelin-like figures have also been noted in the protein yolk precursors of Rana esculenta & Rana temporaria (Kress & Spornitz, 1972), Ciona intestinalis (Kessel, 1966) and Triturus (Spornitz & Kress, 1973). The G.V.B.s are more electron dense than either the M.G.B.s or the M.V.B.s due to the dissolution of the vesicles and granules within the body and their subsequent recondensation as a homogeneously granular matrix (Figures 98, 99, 105, 106). Multivesicular bodies which correspond to the M.V.B.s and G.V.B.s described herein are also reported in many other oocytes (Rana pipiens & Xenopus, Spornitz & Kress, 1971; Oryzias, Yamamoto, 1964; Ciona, Kessel, 1966; Norrevang, 1968; Prostheceraeus floridanus, Boyer, 1972; Triturus,
The oocytes of this size (0.1 mm) possess extensive cisternae of vesicular smooth E.R., many of the vesicles being larger (250 nm) than the ones found within the G.V.B.s (100 nm). Also, there are many smaller S.E.R. vesicles associated with the various yolk precursor bodies (Figures 105, 106) and others that contain yolk granules. Thus, one source of material for the G.V.B.s is from the S.E.R. Further, there are other large S.E.R.-like bodies (300 to 600 nm) that are formed by repeated fusion with the smaller S.E.R.-M.G.B.s and therefore represent early stages in the formation of a protein yolk precursor body (Figures 99, 106).

There are several other sources of small vesicles for the yolk precursor bodies, firstly from the nuclear membrane as previously described (pp. 79 and 82 & Figures 98, 100) and secondly from the Golgi apparatus. The small Golgi vesicles (500 to 700 Å) are similar in size to the vesicles found within the M.V.B.s and the G.V.B.s (Figures 106, 107) and are frequently seen in intimate association with a developing protein yolk precursor (Figure 106). Occasionally, one or more of the central lamellae of a dictyosome are expanded (Figure 107) which may indicate a tangential sectioning plane or the formation of a yolk precursor vesicle. Finally, vesicles may be derived from pinocytosis which is difficult to substantiate as pinocytotic vesicles are identical in appearance to vesicles from other sources.

In a 0.2 mm oocyte small vesicles associated with the nuclear membrane are plentiful (Figure 108). As in the 0.1 mm oocytes, ribosomes are only thinly dispersed in the ooplasm. Smooth E.R. is abundant and the Golgi bodies are still producing large and small vesicles (Figure 109).

At 0.3 mm most of the granular-vesicular bodies have become more
electron dense and possess finely granular or dense cores (Figures 113, 114, 115). Other G.V.B.s can be seen intimately associated with vesicles and small granular bodies and often their limiting membranes are broken or highly irregular (Figures 114, 115, 116). The S.E.R. is still abundant but occasionally long chain-like forms are found (Figures 113, 116). The mitochondria from the mitochondrial cloud are beginning to spread throughout the ooplasm and Golgi bodies, when seen, are associated with mitochondria, a G.V.B. and the long tubules of S.E.R. (Figure 116). This is a pattern that is still evident in larger oocytes.

In addition to the well developed G.V.B.s of the 0.3 mm dogfish oocyte there are also numerous new M.V.B.s and G.V.B.s forming (Figure 117). In some instances these developing bodies consist primarily of myelin figures while others are typical M.G.B.s, M.V.B.s and G.V.B.s.

By 0.4 mm, lipid yolk bodies are well developed. Their association with the S.E.R., mitochondria and Golgi has been noted previously. Also, G.V.B.s in various stages of development are often found in close association with the lipid yolk (Figures 118, 119, 121). Occasionally, a forming G.V.B. can be detected without a limiting membrane or with a partial one but in such cases there is usually a cluster of S.E.R. vesicles within 700 nm distance (Figures 119, 121). The growth of the G.V.B. by fusion of smaller vesicles with the limiting membrane (Figure 118) and the incorporation of granular and membranous elements through G.V.B. membrane discontinuities (Figure 121) can still be demonstrated. In 0.4 mm oocytes, new configurations of S.E.R. (Figures 118, 121) are seen: the S.E.R. appears as an elongated cisterna with expanded ends which are normally devoid of any contents and as such are reminiscent of certain of the large vesicular elements that contain the granules.
113. Ooplasm of a 0.3 mm oocyte with a well developed granular-vesicular body (GVB), several mitochondria, one ring-shaped mitochondrion (M) and several chain-like strands of S.E.R. 1 μm bar.

114. Ooplasm of a 0.3 mm oocyte demonstrating initial coalescence of the G.V.B.s and the irregular or broken nature of the limiting membrane (large arrowheads). A ring-shaped mitochondrion (M) is also shown. .1 μm bar.

115. Ooplasm of a 0.3 mm oocyte demonstrating various stages in the formation of a G.V.B. Large arrowheads indicate the partial absence of limiting membranes. 1 μm bar.
116. Ooplasm of a 0.3 mm oocyte revealing developing G.V.B.s, mitochondria (M), a Golgi body (G) and S.E.R. tubules. Arrow indicates a break in a G.V.B. membrane for the uptake of more material. Small arrowheads indicate the presence of material within the S.E.R. of similar size to that found in the G.V.B.s 1 μm bar.

117. Ooplasm of a 0.3 mm oocyte in a region of forming multivesicular and granular-vesicular bodies. Note particularly the frequency of myelin-like figures within the bodies. 1 μm bar.
118. Ooplasm of a 0.4 mm oocyte with numerous lipid yolk (LY) droplets and closely associated S.E.R., note the expanded cisternae of S.E.R.. Also evident are numerous protein yolk precursor bodies often with incomplete limiting membranes and fusing vesicles (large arrowhead). The arrow indicates the granular and vesicular nature of a G.V.B. 1 μm bar.

119. Ooplasm of a 0.4 mm oocyte clearly demonstrating the lack of a limiting membrane about the lipid yolk bodies. Also featured is a developing G.V.B. with its vesicular and granular (large arrowheads) composition. 1 μm bar.
120. Ooplasm of a 0.4 mm oocyte with a small lipid droplet surrounded by vesicles of S.E.R. Also present are a few mitochondria and a G.V.B. 1 μm bar.

121. Ooplasm of a 0.4 mm oocyte showing a large lipid droplet with S.E.R. and a G.V.B. nearby. Note especially the incomplete membranes around the developing G.V.B.s (large arrowheads) and the expanded ends of the S.E.R. cisternae. 1 μm bar.

122. Ooplasm of a 0.4 mm oocyte in a region of numerous lipid yolk droplets that surround a complex Golgi body, a few mitochondria and some S.E.R. vesicles. 1 μm bar.
123. A lipid yolk droplet in a 0.5 mm oocyte that is surrounded by concentric rings of S.E.R. and numerous mitochondria. 1 μm bar.

124. A branching mitochondrion found in a 0.5 mm oocyte, a configuration taken to indicate multiplication of mitochondrial numbers. 1 μm bar.

125. Lipid yolk particles in a 0.5 mm oocyte demonstrating their association with S.E.R. vesicles. Note also the developing G.V.B.s and their incomplete membranes. 1 μm bar.
126. Two large G.V.B.s in a 0.5 mm oocyte clearly indicating the membranous and granular elements of which they are composed. Also of interest are the vesicles apposed to the G.V.B. that contain single granules. The smaller G.V.B.s are more densely packed and one of them has nearly completed the dissolution and recondensation of its contents (large arrowhead). The arrows indicate chains of S.E.R. vesicles. 1 μm bar.

127. Several large lipid yolk droplets in a 0.5 mm oocyte clearly demonstrating the lack of a limiting membrane as well as the intimate relationship with the S.E.R. 1 μm bar.
128. The nuclear membrane of a 0.8 mm oocyte indicating the increased frequency of nuclear pores as well as the presence of 150 Å particles on both sides of the membrane. In the ooplasm is a large S.E.R. vesicle at the hub of several S.E.R. chains. Other symbols: N = nucleus, P = nuclear pores, R = 150 Å ribosomal particles. 1 μm bar.

129. A small nucleolus in the nucleus of a 0.8 mm oocyte. Note that the electron dense particles in the nucleoplasm are the same size as those found within the nucleolus. 1 μm bar.

130. A segment of the mitochondrial mass of a 0.8 mm oocyte with S.E.R. cisternae intermingled with the polymorphic mitochondria. 1 μm bar.
of a M.G.B. or a G.V.B. (Figure 118).

In a 0.5 mm oocyte the protein yolk precursors are much the same as in all previous stages (Figures 125, 126). There are, however, very dense precursor bodies that have nearly completed dissolution and recondensation of their granular and membranous components as well as many large (3.2 μm) G.V.B.s.

In a 0.8 mm oocyte small M.G.B.s (200 to 450 nm) are found scattered throughout the S.E.R. of the mitochondrial cloud (Figure 131). Developing G.V.B.s are found in the immediate vicinity of most lipid droplets (Figures 133, 134) with mitochondria nearby. The chains of S.E.R. vesicles that are associated with the lipid droplets are also associated with the developing G.V.B.s (Figure 133). Near the nucleus (Figure 128) are several chains of S.E.R. with expanded vesicles similar to those described in 0.4 mm oocytes.

In 1.0 mm oocytes the formation or development of protein yolk precursors (G.V.B.s) follows several pathways. Firstly, the pattern of mitochondria, Golgi, S.E.R. chains and G.V.B.s persists (Figures 140, 141) with the Golgi providing vesicular elements and the S.E.R. providing vesicular and granular elements (Figures 139, 141). Secondly, there often exists large masses of vesicular S.E.R., M.G.B.s, M.V.B.s and G.V.B.s (Figure 142). Within these masses can be seen the addition of free vesicles, granules and small M.G.B.s to the G.V.B.s via discontinuities in the limiting membrane and the fusion of moderately sized (500 to 600 nm) M.G.B.s to yield a larger body and the incorporation of small vesicles (50 to 100 nm) into a large (1.4 to 1.7 μm) G.V.B. by membrane to membrane contact. A third possible source of large vesicles (500 to 900 nm) for protein yolk precursors may be from a Golgi-like
131. A segment of the mitochondrial mass of a 0.8 mm oocyte with large quantities of S.E.R. present as well as numerous small multigranular bodies (large arrowheads). 1 μm bar.

132. Polymorphic mitochondria in the ooplasm of a 0.8 mm oocyte with numerous S.E.R. cisternae and a lipid yolk particle. Other symbols: L = lipid, D = dumbbell-shaped mitochondrion, R = ring-shaped mitochondrion. 1 μm bar.

133. A ring-shaped mitochondrion in the ooplasm of a 0.8 mm oocyte with numerous tubules of S.E.R. coursing about and closely associated with both the lipid and the developing G.V.B.s (large arrowheads). 1 μm bar.

134. Numerous lipid yolk particles in the ooplasm of a 0.8 mm oocyte with S.E.R. cisternae around the particles. Note also the highly granular G.V.B. 1 μm bar.
135. A 1 µm thick cross-section of a 1.0 mm oocyte demonstrating the lampbrush chromosomes and numerous (NU) nucleoli within the nucleus (N). 100 µm bar.

136. A solitary stack of annulate lamellae found within a 1.0 mm oocyte near the nucleus. 1 µm bar.

137. A portion of the mitochondrial mass of a 1.0 mm oocyte. 1 µm bar.
138. A segment of the mitochondrial mass of a 1.0 mm oocyte that now appears less tightly packed, but now has numerous myelin-like figures within the mitochondria. 1 μm bar.

139. A granular-vesicular body in the ooplasm of a 1.0 mm oocyte showing the accumulation of a fluffy component and the attachment of an E.R. cisterna containing several granules. S.E.R. is still found associated with lipid yolk. 1 μm bar.

140. The ooplasm of a 1.0 mm oocyte showing the frequently observed relationship of mitochondria, Golgi, G.V.B.s and S.E.R. (small arrowheads). 1 μm bar.
body (Figure 143). Similar clear vesicles with yolk granules have been found elsewhere in smaller oocytes e.g. Figures 141, 144. A final possible source of the limiting membrane for a protein yolk precursor body are the chains of S.E.R. vesicles. These chains or tubules with occasional expansions along their length course throughout the oocyte (Figure 145) often looping back on themselves. When this occurs (Figure 144), the membranes delineate an area less electron dense than the surrounding ooplasm but of similar density to membrane bound bodies lying nearby. The presence of yolk granules within these bodies and within the vesicles of the S.E.R. indicate that these vesicles may be one of several sources of yolk body precursors.

Another source of vesicles and the material they are transporting to the yolk precursor bodies is from the oocyte membrane via pinocytosis (Figures 146, 147). But these vesicles show no distinguishing features and thus cannot be differentiated from vesicles arising from the Golgi, the nuclear membrane or the S.E.R. In this region near the oocyte-V.M. border are numerous lining body complexes and streams of electron dense matter that has diffused through the oocyte membrane.

In 1.0 mm oocytes, ribosomes are not numerous but are found more frequently than in earlier oocytes and normally occur as polyribosomes (Figures 142, 144, 145).

In oocytes 1.1 mm in diameter two methods of protein yolk precursor formation are indicated. First, there are numerous clear vesicles possibly derived from expanded S.E.R. cisternae (0.4 mm oocytes) or from a Golgi-like body (1.0 mm oocytes) that possess either granules (M.G.B.s) or granules and membranous elements (G.V.B.s) (Figures 151, 151 i, 152, 153). Secondly, there is further evidence that the S.E.R.
141. An unusual section of a Golgi body (G) in a 1.0 mm oocyte, with a myelin-like figure in a dumbbell mitochondrion. Also present is a multigranular body (GB) and several chains of S.E.R. tubules that are beginning to loop and also contain granules. 1 μm bar.

142. A large mass of protein yolk precursor bodies demonstrating the addition of free vesicles, granules and small M.G.B.s to the G.V.B.s via membrane (arrowheads) discontinuities. The large central G.V.B.s also undergoing dissolution and recondensation of its contents. 1 μm bar.

143. One of many Golgi-like bodies found in 1.0 mm oocytes, note the expanded lamellae. 1 μm bar.
144. Two different protein yolk precursor systems in a 1.0 mm oocyte: in one, multigranular bodies (GB) are formed within a membrane bound vesicle that is either translucent or opaque; in the second, chains or tubules of S.E.R. loop on themselves delineating an area of similar density to that of the opaque G.B.. Note also the occurrence of granules within the S.E.R. cisternae and a few polyribosomes. 1 μm bar.

145. Extensive S.E.R. membrane systems beneath the V.M. of a 1.0 mm oocyte that eminate or terminate in expanded cisternae (EC) similar to the vesicles of certain protein yolk precursor bodies. Other symbols: M = mitochondria, R = ribosomes. 1 μm bar.

146. Numerous pinocytotic vesicles (PV) beneath the plasma membrane of a 1.0 mm oocyte. Note also the paths or streams of electron dense material amidst the P.V. and the lining bodies (LB). 1 μm bar.

147. The highly convoluted plasma membrane of a 1.0 mm oocyte demonstrating the formation of pinocytotic vesicles (PV and small arrowheads). Other symbols: LB = lining body. 1 μm bar.
148. The nuclear membrane of a 1.1 mm oocyte studded with numerous pores (P). The nucleus (N) is filled with many polyribosome-like figures. Other symbols: O = oocyte. 1 μm bar.

149. Two nucleoli (NU) within the nucleus of a 1.1 mm oocyte that are bipartite with amorphous (A) and granular (G) regions. The arrow indicates the fragmentation of the nucleolus producing polyribosomal figures (R). 1 μm bar.

150. Two lipid yolk (LY) particles in a 1.1 mm oocyte intimately associated with the S.E.R. Note the large vesicle (upper arrowhead) with a few granules in it and the circle of S.E.R. forming a vesicle (lower arrowhead). 1 μm bar.
chains encircle an area of ooplasm which then may become less electron
dense and evolve into a G.V.B. (Figures 150, 152, 153). The S.E.R.
vesicles that are to form the limiting membrane may or may not contain
granules to be added to the G.V.B. The first step in the transformation
is the dissolution of the ends of the vesicles followed by the end to
end fusion of adjoining membranes which is presumed due to the breaks
in the limiting membrane of other early G.V.B.s (Figures 152, 153, 154).
This transformation, however, requires the loss of one half of the
vesicular membrane either by incorporation into the limiting membrane,
addition to the G.V.B. as one of the many membranous fragments, or
complete dissolution. Following the formation of the membrane, the body
then begins to acquire the yolk granules and membranous elements characteristic
of a G.V.B. with a finely granular matrix (Figures 153, 154).

Polyribosomes are still only sparsely distributed (Figures 150, 152,
155, 156) and when observed often resemble free yolk granules.

Another interesting feature of oocytes in this size range is the
relationship of the S.E.R. chains with the mitochondria, Golgi and the
lining bodies. The general proximity of the S.E.R. and the mitochondria
notwithstanding, membrane contact between the two organelles is often
observed (Figures 156, 157). Further, the S.E.R. tubules have also been
observed in direct continuity with the vesicles of a Golgi body (Figure
155) and with a granular product in the cisternae similar to that found
in many of the smaller (500 to 700 Å) Golgi vesicles. Membrane to
membrane contact between the S.E.R. tubules and the lining bodies has
also been observed (Figures 156, 157). The lining bodies as previously
described are follicle cell processes and one can readily surmise the
transfer of some follicle cell product to the S.E.R. of the oocyte via
151. Several large, translucent vesicles (GB, GVB) in a 1.1 mm oocyte that contain granular and vesicular elements. 1 μm bar.

152. A large G.V.B. in a 1.1 mm oocyte lying beside a forming vesicle of similar size and density. The latter appears to be forming from a small granular body (GB) and a loop of S.E.R. that contains granules (large arrowhead). 1 μm bar.

153. A forming granular-vesicular body (GVB) in a 1.1 mm oocyte. Alongside is a poorly developed S.E.R. loop (arrowhead) that encircles several granules (GR) and an area of similar density to that of the G.V.B. 1 μm bar.
154. A large protein yolk precursor (GVB) in a 1.1 mm oocyte. Its outer limiting membrane is incomplete allowing incorporation of granular and vesicular elements. S.E.R. vesicles are seen in contact with the limiting membrane. 1 μm bar.

155. Two of several Golgi (G) bodies found in a 1.1 mm oocyte with small dense vesicles (large arrowheads) approaching the forming face of the body. Of particular interest is the relationship of a strand of S.E.R. with the forming face of the body. Other symbols: M = ring-shaped mitochondrion, R = polyribosomes. 1 μm bar.

156. In the region of the oocyte plasma membrane of a 1.1 mm oocyte showing the fibrous vitelline membrane (VM), several pinocytotic vesicles (PV) and a few lining bodies (LB). Also evident is the attachment of the S.E.R vesicles to a mitochondrion (large arrowhead) and to a lining body. 1 μm bar.

157. The cortical region of a 1.1 mm oocyte with numerous lining bodies (LB), pinocytotic vesicles (PV), smooth E.R. tubules and mitochondria. The relationship of the S.E.R. with the mitochondria and lining bodies is again evident (large arrowheads). Also found between the oocyte plasma membrane and follicle cell plasma membrane (the lining body complex) are numerous 400 to 500 Å granules. 1 μm bar.
the lining body. In Figure 157, note the numerous vesicles clustered about the base of the lining body and the 400 to 500 Å granules in the intercellular space of the lining body.

Figures 156 & 157 are of the cortical region of a 1.1 mm oocyte and in them one can observe numerous pinocytotic vesicles and the occasional coated vesicle. The latter resemble the coated vesicles of cockroach (Anderson, 1964) and mosquito (Roth & Porter, 1964) oocytes as they take up yolk via pinocytosis.

By 2.0 mm diameter, some of the primary yolk precursors (G.V.B.s) have formed dense cores and resemble typical protein yolk platelets (Figure 158). That is, they have an outer limiting membrane, a light matrix often with vesicles or granules, and a dense core (Kessel, 1968; Nørrevang, 1968; Massover, 1971; Spornitz & Kress, 1971, 1973; Dumont, 1972; Kress & Spornitz, 1972). A crystalline lattice pattern was not observed in yolk platelets of 2.0 mm oocytes.

In 3.0 and 4.0 mm oocytes yolk platelets with a crystalline lattice pattern can be found (Figures 159, 160, 161). Thus the tripartite platelet described for 2.0 mm oocytes has changed only in that the dense core now shows a crystalline pattern with a periodicity of 90 to 100 Å. It is the presence of this crystalline pattern that defines these organelles as yolk platelets (Massover, 1971). Further, the complete crystallization of the core, in amphibian oocytes, is interpreted as indicating a mature yolk platelet (Karasaki, 1963; Spornitz & Kress, 1971, 1973; King et al, 1972). The centre to centre periodicity of dogfish yolk platelets is similar to that of vesicular yolk platelets of Rana catesbiana (85 Å; Massover, 1971), Rana esculenta and R. temporaria (95 Å; Kress & Spornitz, 1972) and Triturus vulgaris (110 Å; Spornitz & Kress, 1973). At no time
158. Protein yolk platelet (PYP) formation in a 2.0 mm oocyte. By this stage, the contents of a G.V.B. have dissolved and reformed as a dense core (CO) bound by their outer limiting membrane (LM). Between the L.M. and the core is a light matrix in which granules and vesicles are still evident. 1 \( \mu m \) bar.

159. A protein yolk platelet in a 3.0 mm oocyte with its outer limiting membrane and a 90 to 100 \( \AA \) crystalline lattice pattern in its core (arrows). 1 \( \mu m \) bar.
160. Two protein yolk platelets found in a 4.0 mm oocyte. The outer limiting membrane (LM) of the platelet and its crystalline core (arrow) are quite evident. 1 μm bar.
were intramitochondrial yolk platelets similar to those of certain amphibia observed (Massover, 1971).
DISCUSSION

HISTOCHEMISTRY

OOCYTE

Fixation and embedding difficulties restricted this study to oocytes less than 5 mm in diameter thus restricting any discussion of yolk formation in *Squalus acanthias* to the earliest stages (Raven, 1961). Cytologically (lampbrush chromosomes, numerous nucleoli) these oocytes were found to be in mid- to late diplotene of the first meiotic division. The presence of nucleoli in the germinal vesicle indicates rRNA synthesis (Davidson et al, 1964; Brown, 1966; Van Gansen & Schram, 1972) which was substantiated by RNase treatment. The reaction of the nucleoli with aldehyde fuchsin indicating sulphated mucopolysaccharides is unexpected, but Stich (1951) found polysaccharides in the oocyte nucleoli of *Ascaris*, *Cyclops* and *Diaptomus* while Yamamoto (1956b) found them in the early oocytes of *Liopsetta*. The cytoplasm of the oocyte presents no unexpected results staining for protein, RNA and phospholipids. The vacuoles present in paraffin sections are lipid in nature as demonstrated by the intense Sudan black reaction. *Mustelus canis* (TeWinkel, 1972) oocytes, 0.25 to 3.0 mm, also give a positive reaction for lipids with Oil red O. The ooplasm of early *Xenopus* oocytes (Dumont, 1972) also stains for protein, RNA and lipid. A polysaccharide reaction that does not occur in *Squalus* was noted in *Xenopus* oocytes. The mitochondrial mass (yolk nucleus) which has been observed in young oocytes of arachnids (van Bambake, 1898) through to teleosts (Livni, 1971) is rich in protein, RNA, and phospholipids. This corresponds to the findings of both Raven (1961) and Dumont (1972) as well as Guraya (1968) who reviewed the
hypochemistry of yolk nuclei. Electron micrographs (Figures 106, 130, 137) reveal that this structure is composed of densely packed mitochondria and in keeping with the terminology of Ward (1962), Balinsky & Devis (1963) and Dumont (1972) it is referred to as a mitochondrial mass or cloud.

**VITELLINE MEMBRANE**

The vitelline membrane occupies an area between the ooplasm and the follicle cells and it is generally conceded that it is formed from the oocyte (Raven, 1961). This point, however, cannot be resolved with light optics and will be dealt with later. Histochemically, the V.M. reacts only for the presence of protein and polysaccharides or neutral mucopolysaccharides. The aldehyde fuchsin reaction can be regarded as non-specific. The presence of protein and polysaccharide either together or by themselves has been demonstrated by Yamamoto (1956b) in Liopsetta; Arndt (1960b) in cyprinoids; Tandler (1957), Franchi (1960) and Wartenberg & Stegner (1960) in the zona radiata of human eggs and Dumont (1972) in Xenopus. Dumont notes that the reaction is first most intense for polysaccharides, later giving way to protein with a slight polysaccharide component. Bellairs et al (1963) studied the composition of the avian vitelline membrane and found it to be a glycoprotein containing hexoses, hexosamines and sialic acid. The presence of sialic acid in large quantities in the V.M. of Squalus is doubtful since it did not give a positive reaction with alcian blue, Hale's or toluidine blue.

**FOLLICLE CELLS**

The transfer of a lipoprotein precursor molecule from the blood to the follicle cell to the oocyte has been well documented in the domestic
hen (Flickinger & Rounds, 1956; McIndoe, 1959; Schjeide et al, 1963 &
1970; Husbands, 1970; Christie & Moore, 1972; Gormal & Kuksis, 1973), in
Xenopus (Wallace, Jared & Nelson, 1970) and in an insect (Telfer & Melius,
1963). In view of this, the lipid droplets seen traversing the V.M. in
Squalus may be considered a physical manifestation of a similar phenomenon.
Supporting this previous statement is the presence of dense lipid droplets
in the apical region of the follicle cells, an area that had previously
remained unstained, and free lipid droplets located in and external to the
theca. Bonhag (1955a, b, 1956, 1958) also found that in insects lipid is
transferred to the oocyte from follicle cells and trophocytes. The presence
of phospholipid in the follicle cells is indicated as is the presence of
some protein and RNA. However, there is no morphologically apparent transfer
of this RNA to the oocyte as in the housefly and Drosophila (Bier, 1963).
Follicle cells are generally rich in RNA (Raven, 1961) - a condition that
would point to a synthetic activity. The demonstration of RNA in the
follicle cells of Squalus definitely indicates some form of proteinaceous
synthetic activity. The presence of sulphated mucopolysaccharides
(Chondroitin sulphate A or C) in the follicle cells may be explained as
the reaction of precursor molecules for the synthesis of the follicular
basement membrane. One would then expect a metachromatic response from
Azure A (pH 1.5) but this does not occur. Instead there is a slight
orthochromatic response that is only slightly labile to methylation-
saponification and hyaluronidase indicating that there are precursor
molecules for the basement lamina present but the acidic groups of the
polymeric molecules are not closely packed. This would account for the
BASEMENT LAMINA-LAMELLA COMPLEX

The follicular basement membrane (F.B.M.) is a unique structure. TeWinkel (1972) studying Mustelus canis mentions the basement membrane, while Lance and Callard (1969) studying Squalus acanthias omit mentioning it completely. It is present in Squalus and one can observe two components to the membrane. Such a membrane is associated with the basal surface of epithelia and it should be expected here as the follicle cells can be considered specialized epithelial cells (Bloom & Fawcett, 1968). The inner component (closest to the oocyte) is termed the basement lamina while the outer component is referred to as an amorphous ground substance (Bloom & Fawcett, 1968) or as the basement lamella (Nadol et al, 1969).

The basement lamella reacts negatively to all but Hale's and aldehyde fuchsin. The reaction with Hale's is difficult to explain because it should produce the same results as alcian blue. Additionally, the aldehyde fuchsin reaction indicating sulphated mucopolysaccharides should be supported by Azure A (pH 1.5) metachromasia and it is not. In contrast to these results, the ground substance of epithelia is described as consisting of unit fibrils of collagen embedded in an amorphous protein-polysaccharide matrix (Bloom & Fawcett, 1968). One would then expect the ground substance to stain with DNFB and PAS. The basement lamella is often identified as a product of the connective tissue fibroblasts (Bloom & Fawcett, 1968), here found in the theca, and should then correspond to the staining reactions of the theca. Confirmation of this can be found in the tables.

The basal lamina is considerably more interesting. Its minor components are protein, lipid, neutral mucopolysaccharides, mucoproteins and/or sialic acid. An epithelial basement lamina is also PAS positive (Bloom & Fawcett, 1968). Its major components are acid and sulphated
mucopolysaccharides (anionic and sulphated anionic glycosaminoglycans). The glycosaminoglycans that are present are hyaluronic acid, chondroitin sulphate A and chondroitin sulphate C, as indicated by the hyaluronidase labile metachromasia of toluidine blue. Hyaluronic acid is a highly hydrated molecule and such a composition would make it very viscous (Schubert & Hamerman, 1956). As such, it could endow a structural function to the anionic glycosaminoglycans by providing rigidity to the lamina as well as the physiological function of controlling molecular diffusion (Rogers, 1961). The persistence of a slight amount of metachromasia following hyaluronidase treatment may indicate the presence of a sialic acid fraction (Culling, 1963; Kvist & Finnegan, 1970).

Aldehyde fuchsin has a very high affinity for the strongly acidic sulphonate groups of anionic glycosaminoglycans (Spicer & Meyer, 1960). However, it cannot be specific for sulphonate groups, as witnessed by the persistence of staining following methylation and a slight increase following demethylation. This staining of carboxyl groups following demethylation is contrary to the findings of many other investigators (Scott & Clayton, 1953; Spicer & Meyer, 1960; Spicer, 1962) but finds support in the work of Ortman et al (1966) wherein aldehyde fuchsin solutions were found to be unstable and would give rise to intermediate compounds that react with carboxyl groups (Kvist & Finnegan, 1970). The persistence of staining after methylation is in disagreement with all of the previously mentioned investigators and indicates that here the aldehyde fuchsin solution may also be staining a sialic acid fraction or there may not be complete blockage of the sulphate or carboxyl groups.

The incomplete blocking of Azure A staining after the methylation-demethylation sequence indicated that, at pH 1.5, not all the metachromasia
and orthochromasia was due to the presence of sulphated mucopolysaccharides. Although it had been reported that most non-sulphated glycosaminoglycans are orthochromatic when stained with cationic thiazine dyes (Sylven & Malmgren, 1952; Sylven, 1954; Walton & Ricketts, 1954), highly polymerized or concentrated hyaluronic acid can give a metachromatic response (Meyer, 1955, 1956; Schubert & Hamerman, 1956). The reduction in Azure A metachromasia following hyaluronidase indicates that at least some of the metachromasia is due to chondroitin sulphate A and/or chondroitin sulphate C and supports the results following methylation-demethylation. It has also been recorded that faint azurophilia persisting after hyaluronidase digestion indicates the presence of chondroitin sulphate B (McConnachie & Ford, 1966). Thus, the basal lamina is composed mainly of chondroitin sulphate A and/or chondroitin sulphate C with a moderate amount of hyaluronic acid and possibly some sialic acid with some chondroitin sulphate B.

**THECA**

The theca interna, not distinguishable from the externa in these early stages, has been reported to consist mainly of connective tissue cells (Lance & Callard, 1969) in *Squalus acanthias* or of a narrow layer of cells and blood capillaries adjacent to the granulosa and the surrounding band of collagenous material in *Mustelus canis* (TeWinkel, 1972). Functionally, the theca has been implicated along with the granulosa in steroid biosynthesis (Lance & Callard, 1969; Livni, 1971). The histochemical data presented indicate the presence of small quantities of glycogen, protein, RNA, phospholipid and possibly some acidic and sulphated mucopolysaccharides. It would appear to have little biosynthetic activity (other than steroid, Lance & Callard, 1969) although it may contribute some acidic and
sulphated mucopolysaccharides to the outer component of the basement membrane, the basal lamella.

**STROMA**

The distinction between light and dark cells in the stroma is at best a tenuous one, light optics and section thickness combine to make distinguishing these two cell types difficult. Nevertheless, cells with ovoid nuclei have been designated as light cells, while dark cells are those with pleomorphic nuclei. Generally, both types present the same picture as the thecal cells, except that the dark cells stain more intensely than either the light cells or the thecal cells. The stroma of *Mustelus* is described as consisting of an exceptionally loose network of connective tissue membranes carrying blood vessels and presumably, lymphatic channels. (TeWinkel, 1972). Thus, results similar to those of the theca, which is also a connective tissue layer, would be expected. The response of a small percentage (approximately 2 to 4 %) of the dark cells with aldehyde fuchsin is extremely interesting. The observed reaction indicates the presence of both sulphated mucopolysaccharides and non-sulphated mucopolysaccharides (hyaluronic or sialic acid) in the dark cells. Although such a response may indicate that there are two types of dark cell, the possible metabolic or functional significance of such a reaction is obscure. These cells are randomly scattered in the stroma, thus direct contribution to the formation of either the follicular membranes (lamina and lamella) or the elastic fibres in the tunica can be ruled out. Additionally, with such a strong aldehyde fuchsin reaction, one would then expect a supporting alcianophilic and/or a metachromatic response (toluidine blue, Azure A pH 1.5). Such is not the case. Cook (1972) describes aldehyde fuchsin as a "capricious"
stain requiring the use of a freshly opened bottle of paraldehyde (Moment, 1969) to achieve the desired results. The high level of background stain present has been attributed to an old and deteriorated staining solution (Cook, 1972) but as it was freshly prepared, the only remaining variable is the paraldehyde which was not freshly opened.

**TUNICA**

The tunica albuginea, a connective tissue layer beneath the surface epithelium and surrounding the cortex (Nelsen, 1953) is ignored by Lance and Callard (1969) in their report on *Squalus acanthias*, but is described by TeWinkel (1972) in *Mustelus canis* as a dense cortical collagenous, connective tissue layer with interwoven smooth muscle. The strong DNFB reaction indicates the presence of abundant protein but equating this protein to smooth muscle will have to await the demonstration of the latter with the electron microscope. The use of titanous chloride as a reducing agent in DNFB also provides for improved staining of such tissue components as collagen and elastic fibres (Pearse, 1968). The other carbohydrates present include sulphated and acidic mucopolysaccharides, results consistent with a connective tissue nature. Again the results of aldehyde fuchsin (AF) and Azure A staining are contradictory. The complete blocking of AF staining following methylation and demethylation is indicative of a very high affinity for the strongly acidic sulphonate groups of the sulphated mucopolysaccharides (Conklin, 1963; Spicer & Meyer, 1960; Spicer, 1962; Kvist & Finnegan, 1970). Upon this premise, an intense Azure A metachromatic response is expected. The lack of such a response would indicate that there were no chondroitin sulphates present. The cytological appearance of the tunica, Verhoeff's reaction (unreported data) for collagen as well as
TeWinkel's (1972) observation of collagen in *Mustelus*, all indicate that collagen is present in the tunica. The association of the chondroitin sulphates with the collagen of cartilage (Swanson, 1965), a good indication that it should be present here, argues that the Azure A reaction is erroneous. The only possible explanation for this phenomenon is that the molecules present are bound or masked, or the groups are not present in sufficient quantity to produce metachromasia (Sylven, 1954; Chayen et al, 1969). The presence of short elastic fibres in the tunica emphasizes that in all probability this layer is viscous and flexible. These fibres interwoven with the collagen of the tunica lend support to the components of the ovary as well as serving to bind the ovary together and thus preventing the numerous (4 to 8) large oocytes present prior to and at ovulation from fragmenting the ovary. The elastic fibres have the capacity to stretch and then snap back into their original state. Presumably, this property would be most important after ovulation has occurred to restore the ovary to its original shape and to close the gaps created by the ovulated eggs.
ULTRASTRUCTURE

OVARIAN EPITHELIUM

The term "germinal epithelium" persists even though the extragonadal origin of the primordial germ cells is well substantiated (Bloom & Fawcett, 1968; Hoar, 1970; Bellairs, 1971). Throughout my observations of the developing oocytes, there was never any evidence to suggest that this epithelium gave rise to the developing oocytes. Considering this and the basic similarities of the early dogfish and avian embryos, I feel that the primordial germ cells of Squalus can be found in the yolk sac region. The term "germinal epithelium" is inappropriate and will not be used in this study.

The absence of cilia on the epithelium is of interest considering its ciliation in at least two other dogfish species. Mustelus canis (TeWinkel, 1972) has a ciliated ovarian epithelium while Scylliorhynus canicula (Metten, 1941) has a ciliated abdominal cavity. In all probability this ciliation is responsible for the directed movement of an egg towards the ostia. Squalus eggs, however, are said to move passively toward the anterior end of the oviduct and become engulfed by the ostium tubae (Weichert, 1961). In accounting for this, one must consider one critical factor, Squalus acanthias has functional right and left ovaries and oviducts whereas the other species mentioned have but a single functional ovary and oviduct. From this, one can conclude that ciliation is a reflection of the atrophy of one ovary and oviduct and therefore it increases the probability that any ovulated egg will reach the ostium.

The cells of the ovarian epithelium are similar to most other typical epithelial cells (Bloom & Fawcett, 1968; Matthews & Martin, 1971). They are columnar with a basal, lobed nucleus having a prominent nucleolus and
apically located organelles. The numerous mitochondria, Golgi bodies and E.R. indicate active synthesis and this is manifest by the preponderance of microfilaments within the cells and the mucin-like vesicles, derived from the Golgi, at the surface of the cell. In all probability, the flocculent component of these vesicles functions as a protective or mucoid coating for the ovary similar to that found coating the intestinal epithelia of many mammals (Fawcett, 1966) and corresponds to the pyroninophilia (RNA) and alcianophilia (acidic and sulphated mucopolysaccharides) demonstrated histochemically. The microfilaments, which are far more numerous than in other epithelial cells (Bloom & Fawcett, 1968; Matthews & Martin, 1971) are easily accounted for when one recalls the size and number of ripe eggs found in a mature female. The microfilaments, in all likelihood, serve to maintain the integrity of the cells under the disruptive influence of the development and ovulation of several eggs 3 to 4 cm in diameter.

Quite unlike the surface epithelium of the domestic fowl (Dahl, 1972), there are few, if any, narrow surface crypts but there are extensive junctional complexes. The absence of definite crypts or epithelial ingrowths indicates that there is no contribution of epithelial elements to the cortical region either for use as follicle cells or as steroid producing thecal gland cells (Harrison, 1961; Narbaitz & Adler, 1966; Dahl, 1972).

The variety of junctional complexes found beneath the zonula occludentes presents an interesting problem—What purpose do they serve? Obviously they are intercellular attachment devices that contribute to the maintenance of cell shape and thus to the integrity of the surface epithelium as well (Fawcett, 1966; Matthews & Martin, 1971). The numerous regions of concentric folds are by no means unique to Squalus, but they
are considerably more complex than others observed between certain mammalian epithelial cells (Matthews & Martin, 1971). It is quite probable that they too serve as intercellular attachment devices but it is also probable that the extra surface area they provide may be utilized if the cells were stretched by a developing 2 to 4 cm oocyte.

The extensive intercellular channels found between individual cells is another feature unique to the dogfish ovary. As far as I have been able to discern, no such channels have been reported in any other vertebrate ovary. Their purpose is obscure but several possible explanations are offered. First, they may serve as a reserve mucoid source for coating the epithelial surface. Second, they may act as an area to receive membranous detritus which is then concentrated and eliminated into the abdominal cavity. Both explanations have faults however. In the first instance, the zonula occludentes would act as a seal to the passage of materials and fluids in either direction (Fawcett, 1966). In the second instance, although there is some evidence for membranous material in the canal and possibly an eliminated "plug" of matter at the surface, the elimination of waste in this manner is inefficient. The only possible channel out of the body is via the oviducts, a path that is functionally inappropriate.

The well developed basement lamina (membrane) that separates the epithelium from the tunica in Squalus was not observed beneath the epithelium of the smooth dogfish Mustelus canis (TeWinkel, 1972).

**TUNICA ALBUGINEA**

The nature of the tunica in Squalus is similar to that of Mustelus (TeWinkel, 1972) differing only in the absence of smooth muscle. The greater thickness of this layer relative to that of most adult mammals
(Mossman & Duke, 1973) reflects the need to maintain the integrity of the organ again under the disruptive influence of the large eggs found in adult ovaries. The collagen present offers resistance to a pulling force whereas the elastic fibres will return the tunica to its original shape.

Although the periodicity of the collagen fibres is somewhat less (520 Å vs 640 Å) than that of "native" collagen it is felt that this is not a significant difference. Elastic fibres have been described as "lacking visible fibrillar subunits" (Bloom & Fawcett, 1968) or consisting of an "amorphous electron-lucent substance with some longitudinal filaments buried within the amorphous elastin" (Matthews & Martin, 1971). To the contrary, the elastic fibres of the tunica show distinct 50 to 60 Å filaments oriented parallel to the long axis of the fibre. In view of this it would seem safe to conclude that these subunits are the contractile elements of the fibres.

**Cortex**

Typically, the ovarian cortex or stroma of non-mammalian vertebrates is described as consisting of fibroblast-like cells, connective tissue fibres and smooth muscle (hen, Peel & Bellairs, 1972), an exceptionally loose net of connective tissue membranes (*Mustelus canis*, TeWinkel, 1972), a connective tissue stroma (*Squalus acanthias*, Lance & Callard, 1969). However, the observations within this study are not consistent with such a viewpoint. What is indicated is a richly cellular cortex that is interspersed with blood vessels and large lacunae. This seems to parallel the mammalian cortex which is described as being "embryonic" in that it is compact, cellular and lacking in fibres (Mossman & Duke, 1973). Although this description matches our observations, one must hesitate before drawing
any conclusions as the descriptions are made from light micrographs and little if any electron microscopy has been done on the cells of the cortex. Although there is a distinct morphological division of the cells of the cortex into at least two types, the functional significance of the cells remains obscure. Histochemically, they resemble the thecal cells, a response not unexpected considering the thecal cells are probably modified cortical cells (Mossman & Duke, 1973). The bodies of the dark cells may, in fact, be lysosomes (Fawcett, 1966) but precisely why there are so many cells with such large numbers of lysosomes is unknown. The possibility that these bodies are not lysosomes also exists and without performing enzymatic histochemical tests we cannot say that these bodies are or are not lysosomes. The light cells are characterized by the extensive development of distended cisternae of granular E.R.: a phenomenon that is typical of glandular cells elaborating a protein rich secretory product (Fawcett, 1966). However, if these cells are producing a proteinaceous substance for export, there is no indication as to how it is transported from the cell or as to where it is transported. An obvious supposition would be that these cells are secreting a product that is finding its way into the developing oocytes as a constituent of the proteid yolk.

THE INTERCELLULAR SPACE

The collagen fibres that are found woven throughout the ovary may be a product of either or both of the light and dark cells. Their presence lends credence to the aforementioned discussion of the connective tissue nature of the cortex (Lance & Callard, 1969; Peel & Bellairs, 1972; TeWinkel, 1972).
LACUNAE BORDER CELLS

Undoubtedly, the lacunae border cells are a form of endothelium which more than superficially resembles that of a capillary wall. More appropriately, the lacunae border cells resemble the "continuous sinusoids" found in the liver of "certain" vertebrate species (Bloom & Fawcett, 1968). As such, these cells provide a protective epithelium for the cells of the cortex. Additionally, the numerous 600 to 700 Å vesicles may function in the micropinocytotic transfer of small quantities of fluid and solutes across the cell discharging it into the clear zone. The use of electron opaque tracers and enzymes like peroxidase have shown that such transfer does occur in capillary walls. However, the labelled vesicles are relatively few and many are in doubt as to whether this mechanism could account for either the selectivity of the process or the volume of material transported per unit time (Fawcett, 1966; Bloom & Fawcett, 1968). Whether or not one must worry about the volume transferred or the selectivity in this instance has not been determined.

The enormous surface area involved leads one to the conclusion that there is transfer of some substance or substances across this barrier and if any selectivity is involved it would be imposed by the basement lamina. If some substance is transferred across this cellular barrier, it is not intended for either the light or the dark cells specifically as both can be found underlying the lacunae border cells. The lacunae show no evidence of electron opaque materials and one therefore concludes that in vivo they are fluid filled channels.
RED BLOOD CELLS

The red blood cells of *Squalus* are typical nucleated red blood cells. The most unusual features noted are the presence of myelin-like bodies and a Golgi-like body. The myelin-like figures are likely to be precipitated phospholipids (Matthews & Martin, 1971) while the Golgi-like body is either an artifact or a degenerating Golgi apparatus. The microtubules of the marginal band function as stiffening, cytoskeletal elements that help to maintain the flattened form characteristic of nucleated red blood cells.

THECAL CELLS

The thecal layer is often described as a connective tissue layer (Dumont, 1972; Peel & Bellairs, 1972; TeWinkel, 1972) in the lower vertebrates. However, in mammals and many of the lower vertebrates, steroid activity has been ascribed to the theca interna (as well as the granulosa) (Lance & Callard, 1969; TeWinkel, 1972). The theca interna of the hen, on the other hand, is a connective tissue layer interspersed with luteal clusters that constitute the thecal gland, a steroid secreting structure (Peel & Bellairs, 1972).

The theca interna of *Squalus acanthias* has been described as consisting mainly of connective tissue cells (Lance & Callard, 1969) while that of *Mustelus canis* as consisting of a narrow layer of cells and blood capillaries adjacent to the granulosa and the surrounding band of collagenous tissue (TeWinkel, 1972). The theca externa of *Squalus* has not been described previously, whereas, in *Mustelus* it is composed of loose membranes and blood vessels peripheral to a network of tubules (TeWinkel, 1972).

What I have observed in *Squalus* ovaries differs markedly from this. Initially the thecal cells are fibroblast-like, undifferentiated as to
internae or externa. There is, however, an abundance of Golgi bodies and smooth vesicles that foreshadows the condition found in slightly larger oocytes when the theca can be divided into interna and externa. The presence of smooth membrane bound vesicles, Golgi bodies and flattened nuclei define the theca interna cells and lend credence to the possibility that these cells may in time secrete steroids. This is not unequivocal proof that these cells do or will secrete steroids. However, the lack of such smooth membrane-bound organelles would preclude the possibility of these cells ever producing steroids (Fawcett, 1966; Mossman & Duke, 1973).

The appearance of the cells of the theca externa indicates that they too are involved in the synthesis of a product. Based on the morphology of certain of the vesicles one may conclude that these cells are responsible, at least in part, for the formation of the dense granular matrix and possibly the collagen fibres found therein. There is also evidence to indicate that the cells of the cells of the theca interna contribute to this matrix.

In all, the thecal cells appear to be modified connective tissue cells with cytological characteristics similar to those of cells involved in protein and steroid synthesis. This dense matrix may act as a basement membrane separating the thecal cells from their associated follicle and the surrounding cortical cells. In this manner, we may have the formation of a thecal glend analogous to that found in the hen (Peel & Bellairs, 1972) and numerous mammals (Mossman & Duke, 1973).

**BASEMENT LAMINA-LAMELLA COMPLEX**

The basement membrane or basal lamina present around developing dogfish oocytes is uncommonly thick, having a counterpart only in Descemet's
membrane of the cornea (Fawcett, 1966) and the diabetic glomerular basement membrane (Siperstein et al, 1964). The average basement lamina is a finely filamentous sheath less than 100 nm thick (Fawcett, 1966; Hay & Revel, 1969). I have already noted such typical laminae associated with the surface epithelial cells and the lacunae border cells.

It is obvious from the data presented that the basal lamina (F.B.M.) is a product of the follicular epithelioid cells. Precisely, it is formed through the combined efforts of the granular E.R. and the Golgi vesicles located in the basal regions of the epithelial cells involved. Similar conclusions involving epithelial cells forming basal lamina as well as an extracellular collagenous matrix have been advanced concerning avian corneal epithelium (Hay & Revel, 1969), human glomerulus (Beisswenger & Spiro, 1973), the cuticle of the roundworms Lumbricus and Ascaris as well as the byssus threads of Mytilus edulis (Gross, 1963).

Basal laminae have been shown to exist beneath epithelia regardless of the absence of fibroblasts (Hay & Revel, 1969; Bernfield & Wessells, 1970), once the only cell type thought capable of secreting collagen or such a connective tissue matrix (Gross, 1963; Bloom & Fawcett, 1968). This gains importance when one considers that basal laminae (glomerular) are similar in composition to collagen (Vernier, 1964; Dische et al, 1965; Hay & Revel, 1969; Beisswenger & Spiro, 1973) but with 1½ times more hydroxyproline and 6 times more hydroxylysine than native (glomerular) collagen (Hay & Revel, 1969). Thus one would expect the basement lamina to exhibit staining properties similar to those of collagen and elastic tissue. Such a relationship has already been demonstrated histochemically (See Tables IA to IC & pp 29). Additionally, two carbohydrate components have been identified within glomerular basal laminae. One
component is a hydroxylysine-linked (collagen-linked) glucosylgalactose unit (Beisswenger & Spiro, 1973) while the other is a free sialofucohexosaminoglycan (Dische et al., 1965; Hay & Revel, 1969; Nadol et al., 1969). The histochemical data presented earlier record the occurrence of acid mucopolysaccharides within the basal lamina and possibly within the follicle cells. In the latter instance, however, the techniques employed were not sufficiently sensitive to unequivocally demonstrate the presence of acid mucopolysaccharides. Using the silver methenamine and ferritin-labelled antibody techniques, Vernier (1964) demonstrated that the epithelial cells of human glomeruli contained material similar to a component of the basement lamina and concluded that these cells were at least partially responsible for the synthesis and storage of basement lamina material. Farquhar (1964) observed E.R. cisternae of these same cells with a material that was ultrastructurally similar to the lamina. Based on these observations and conclusions, it is obvious that parallel observations made regarding basement membrane formation in *Squalus acanthias* should lead to parallel conclusions. The following conclusions by Dische (Hay & Revel, 1969) sum up the observations recorded herein and mirror the current dogma:

"Basement membrane is equivalent to a collagenous protein not organized above the level of tropocollagen but possibly possessing certain degrees of orientation with an amorphous substance between the collagenous protein molecules... The collagen molecules themselves are apparently linked to one or more (hexosamine-free) glycans... whereas in the amorphous matrix would be located (an acid mucopolysaccharide and a sialofucohexosaminoglycan)...the lowest level of organization (of collagen) is represented by basement membrane, (here) collagen does not show its characteristic striation due to the basic organization of tropocollagen into fibrils therefore the membranes do not show a fibrillary structure even in electron micrographs."
The basement lamella is the more distal portion of the basement membrane complex that typically contains a highly ordered array of collagen fibres crossing approximately at right angles (Nadol et al, 1969). In their studies of the skin of the teleost Fundulus, Nadol et al (1969) report observations of the basal lamella similar to those reported herein for the basal lamella of *Squalus*. To wit, the collagen fibres of the lamella are oriented in alternating longitudinal and circular layers with the layers crossing at 105 to 110°, producing a "herringbone pattern", the same pattern observed in 1.5 to 1.8 mm dogfish oocytes. Their most significant conclusion is that the collagen fibres of the lamella have their origin in the substance of the basement lamina where there are designated areas that direct the deposition of collagen in but one orientation, doing so continuously in direction if not in time.

The implications of this are two-fold. First, as the oocyte and basement lamina grow, so will the lamella, because the number of initiation sites on the lamina will increase. Secondly, the earlier conclusions concerning the collagenous nature of the lamina are reinforced. Also, one may consider the linear pattern observed within the basement lamina to be the ordering of the tropocollagen molecules into non-striated fibres. Whether this reflects the *in vivo* situation or is an artifact of preparation cannot be ascertained at this time. However, a 0.51 M NaCl solution has been shown to produce reconstituted collagen fibres that lack periodicity (Gross, 1963).

The intimate relationship between the collagen fibres of the basement labella and the theca must also be considered. The basic similarity of this arrangement and that of the collagen and matrix of the "thecal islands" leads one to believe that the thecal cells may be contributing to
the growth of the basal lamella. Significantly, Grobstein's labelling studies on the interface materials of epitheliomesenchymal interactions (1968) have shown that separately both epithelial and mesenchymal tissues can synthesize collagen. Epithelium, however, produces only small amounts of hydroxylated protein (Grobstein, 1968). But when an epitheliomesenchymal interface is treated (with label), it is noted that collagen production has increased. He concludes hydroxylated material is transferred from the mesenchyme to the epithelium or that in the presence of mesenchyme the epithelium may be stimulated to hydroxylation. In either case, one component of the macromolecular material accumulating at the epitheliomesenchymal interface probably comes from the mesenchyme. Although convincing morphological evidence for this is lacking, the circumstantial evidence is substantial. For instance, there are extensive collagenous deposits at the sites of epitheliomesenchymal interfaces i.e.: beneath the surface epithelium (the tunica albuginea) and beneath the follicle cells (the basal lamina-lamella complex). The origin of the other cells in the ovary is mesodermal (Balinsky, 1966) and one would expect collagen to be deposited here but in lesser amounts than where there is an epitheliomesenchymal interface. Therefore, although the majority of the evidence presented favours the production of the basement lamina-lamella complex from the epithelial cells one cannot completely eliminate the underlying tissues (mesodermal) for being responsible for at least part of the membranous complex.

**FOLLICLE CELLS AND THE VITELLINE MEMBRANE**

The activity of the follicle cells in basement lamina formation has been established but the ultrastructural evidence also points to several
other possible formative functions and their related phenomena. Generally, the follicular sheath resembles that of all vertebrates (Raven, 1961 and Nørrevang, 1968 for reviews; Yamamoto, 1963; Jollie & Jollie, 1964; Bellairs, 1965; Mossman & Duke, 1972; Dumont, 1972) and many invertebrates (Raven, 1961; Nørrevang, 1968; Anderson & Spielman, 1971; Cummings, 1972). The smallest oocytes examined (100 μm) showed a well developed low cuboidal granulosa layer while Peel and Bellairs (1972) noted a cuboidal epithelium around 0.5 to 1.0 μm hen oocytes. A similar situation may also be found around dogfish oocytes of that size.

The ultrastructural evidence for steroid activity in these early oocytes is circumstantial. However, histochemical demonstration of 3βHSD in the granulosa of Squalus acanthias oocytes was made by Lance and Callard (1969): the presence of a steroid dehydrogenase generally being accepted as evidence that the tissue is capable of steroid synthesis (Lance & Callard, 1969). The evidence of potential steroidogenic activity lies with the amount of smooth membrane present in the cell (Nicholls & Maple, 1972; Peel & Bellairs, 1972) and certainly the follicle cells of early dogfish oocytes do possess considerable amounts of smooth membrane vesicles and smooth endoplasmic reticulum. While the presence of such features alone does not constitute conclusive proof of steroid secretion their absence provides strong argument against such activity (Nicholls & Maple, 1972). On the other hand, any attempt to relate these ultrastructural features to endocrine activity are confused by the well established function of this layer in the transfer of materials to the oocyte and in the formation of the basement lamina and the vitelline membrane. From these data, it is impossible to state conclusively that the follicle cells of these stages are or are not secreting steroids.
The formation of the vitelline membrane (zona pellucida) by the follicle cells has been established in a variety of mammals (Nørrevang, 1968; Mossman & Duke, 1973), birds (Nørrevang, 1968; Bellairs, 1971), insects (Aedes, Anderson & Spielman, 1971; Ephestia, Cummings, 1972; Douglas Fir beetle, Sahota, 1973), and amphibia (Xenopus, Dumont, 1972). The situation in the teleosts is not as well defined with the zona pellucida being formed by the follicle cells in Trichiurus and Tracanthus (Chaudhry, 1956) and from the oocyte in Oryzias latipes (Yamamoto, 1963). Vitelline membrane formation in the elasmobranch Squalus acanthias resembles most of the higher vertebrates in that it is a product of the follicle cells. In those follicle cells just initiating V.M. formation there are extensive stacks of R.E.R., numerous mitochondria, free ribosomes and Golgi bodies. A similar situation has been noted in Ephestia (Cummings, 1972) where the follicle cells have extensive cisternae of R.E.R. and numerous Golgi at the beginning of V.M. formation. The only difference is that in Ephestia the V.M. is formed from secretion bodies assembled in the Golgi from material produced in the R.E.R. cisternae. Squalus, however, has microfibrils that closely resemble those of the V.M. within the peripheral regions of the follicle cells and these present all appearances of being intimately related to or added to the substance of the vitelline membrane. Lacking direct evidence of secretion bodies fusing with the V.M. (Cummings, 1972) it seems logical to assume that some unpackaged, monomeric secretion product of the follicle cells is being polymerized in the region of the developing vitelline membrane (Chiquoine, 1960) and is transformed into the protein-polysaccharide fibres of the vitelline membrane. The mucopolysaccharide nature of the vitelline membrane has also been reported by Harrison (1962) who speculated that the membrane probably functioned
as a transport highway for the appropriately selected molecules and as a barrier to others. A mucopolysaccharide coat over the oolemma of the cockroach and cricket (Anderson & Spielman, 1971) is also implicated in chemical selectivity. Thus, it is likely that the dual chemical composition (protein and mucopolysaccharide) of the vitelline membrane reflects two major functions—support and chemical selectivity. Adding further support to the theory that the follicle cells, not the oocyte, form the V.M. is the lack of sufficient ribosomes, R.E.R. and Golgi bodies in the cortical ooplasm to form a membrane of such magnitude (Cummings, 1972) and the absence of V.M. material in the deep crypts formed by the ooplasmic macrovilli.

The zona radiata is a portion of the follicular envelope first noted in elasmobranchs by Balfour (1885) and Wallace (1903). To them, the zona radiata or zonoid layer was composed of "radial fibres supported above and below by thin surfaces" a concept that does not hold true under electron microscopical examination. In this study and several others (Schjeide, Wilkins et al, 1963; Yamamoto, 1963; Press, 1964; Bellairs, 1965 & 1971) it is clearly evident that the zona radiata is a microvillar specialization of the oocyte's plasma membrane. The typical morphology of the zona radiata is that of finger-like microvilli (See Raven, 1961 and Nørrevang, 1968 for reviews) similar to that found around the smallest oocytes (100 µm) examined in this report. The unusual feature of this "layer" in larger Squalus oocytes is that it is composed of a band of membrane bound vesicles derived from the oocyte, the surface of which is thrown into numerous large "buds" and macrovilli. Structurally, the raising of the oocyte membrane into microvilli produces an increase in the surface area of the oocyte (Bellairs, 1971). Kemp (1956) estimated that the zona radiata of the frog
produced a 35% increase in surface area (Raven, 1961). Considering the extent of the modifications in *Squalus*, an increase in surface area of similar magnitude would seem in order. Functionally, this increase in surface area is taken to reflect the enhanced passage of materials through the oocyte membrane (Bellairs, 1971).

The raising of the oocyte and occasionally the follicle cell border into microvilli is a feature common to all classes of vertebrates (Raven, 1961; Yamamoto, 1963; Jollie & Jollie, 1964; Bellairs, 1965 & 1971; Williams, 1965; Davidson, 1968; Nørrevang, 1968; Dumont, 1972) and some invertebrates (Kessel, 1968, Cummings, 1972). Generally, these microvilli only penetrate the vitelline membrane (zona pellucida) stopping at or before the opposing plasma membrane. However, there are reports of follicle cell processes (microvilli) penetrating the cortical layer of an oocyte (Bellairs, 1965; Nørrevang, 1968) and one report of "prolongations of the follicle cell through the egg membrane forming direct protoplasmic connections with the ooplasm" in the Selachii (Wallace, 1904). For the most part, these processes are confined to large yolked eggs (birds and reptiles, Bellairs, 1965) but they have also been noted in the smaller amphibian eggs (Nørrevang, 1968). Therefore the findings of this report are consistent with previous work on the Selachii and with the other vertebrate classes having large yolked eggs.

These follicle cell processes have been examined the most carefully in birds (Press, 1964; Bellairs, 1965) and the terminology most frequently applied to these "contact areas" are "transosomes" and "lining bodies" respectively. Ultrastructurally, these processes are thought to have arisen from desmosomes with one membrane being peculiarly specialized (Bellairs, 1965; Nørrevang, 1968). But throughout the literature there is no general
agreement on their morphology or function. Bellairs (1965) notes the lining body resembles a desmosome but its membranes are not arranged in mirror-image pairs and that it is also larger than a desmosome. In *Squalus* oocytes, it is highly likely that the follicle cell processes of the small (0.1 mm) oocytes and the double membrane bound structures of the larger oocytes (1.5 mm) are manifestations of a lining body-like phenomenon. The relationship here of the developing processes with desmosome-like structures adds further credence to the theory that these "lining bodies" developed from regular desmosomes.

The functions of this phenomenon have been speculated upon and the two most logical are that they serve either for securing the oocyte in place or providing a means of transferring nourishment across the vitelline membrane (Press, 1964; Bellairs, 1965; Nørrevang, 1968). In the latter instance, several of the earlier studies hinted at an ooplasmic-follicular continuity via the follicle cell processes (Kemp, 1958) but later electron microscopic studies (Raven, 1961; Nørrevang, 1968) and this report show no such continuity. Thus, it must be noted that Wallace's (1904) conclusions were in error, there being no direct connection between the follicle cell and the oocyte. Other authors (Chiquoine, 1960; Adams & Hertig, 1964) contended that these processes may still be related to nutritional transport of materials unable to diffuse through the vitelline membrane with the material entering the oocyte via micropinocytosis. Nørrevang (1968), however, holds the opposite view that the presence of desmosomes in an area of contact would not permit the transport of materials into the oocyte and that it was more likely that the processes served as anchoring structures for the oocyte. A third possibility exists and that is that these "lining bodies" serve both functions. This is the view held in this report with regard to
the processes found in *Squalus* oocytes. The desmosome-like structures and
the variety of angles that the processes penetrate the oocyte and the depths
at which they are found support the "anchoring" statement. On the other
hand, the presence of small vesicles at the follicular origin of the
processes and within and without the "lining bodies" of the oocyte as well
as the paths or streams found associated with the lining bodies in the
cortical ooplasm indicate the transfer of material via the processes. The
streams of material probably represent some form of yolk precursor matter
that has been passed through the vitelline membrane via the pore canals or
lining bodies. Slauterback (1963) suggested that microtubules found in
secreting cells may act as a transport system and if this assumption is
extended to closely packed microfilaments one can readily envision some
material being channeled into the follicular opening of the pore canals,
transferred through the vitelline membrane and diffusing through the twin
plasma membranes of the ooplasmic lining bodies. In opposition to
Nørrevang (1968) it is obvious that the lack of desmosomes around the
entirety of the lining body is going to allow material to diffuse across
the membranes and into the oocyte.

In addition to the follicle cell processes found in *Squalus* oocytes
there are also ooplasmic microvilli that penetrate the follicle cell cyto-
plasm. This is a phenomenon infrequently recorded, being noted in but two
teleost species, *Lebistes reticulatus* (Jollie & Jollie, 1964) and *Oryzias
latipes* (Yamamoto, 1963). In the former instance, the "microprojections
of the ooplasmic processes pass through the pore canals and into the recesses
of the inner follicle plasma membrane". Once again there is oocyte and
follicle cell contiguity but never cytoplasmic continuity and thus only
indirect pathways for the passage of nutrients into the egg (Jollie &
In the latter instance, oocyte microvilli are said to pass through perforations in the zona pellucida and extend into the follicle cells forming complicated anastomoses. It is difficult, however, to tell from Yamamoto's micrographs that the microvilli actually penetrate the substance of the follicle cells and do not merely occupy an extracellular space. The existence of these ooplasmic microvilli in dogfish oocytes is transient, with none being observed in oocytes larger than 1.0 mm in diameter. Speculating on their function, one may assume that they do allow passage of some materials, possibly in a reverse flow, or they serve as additional anchors for a young oocyte until superceded by the more highly developed follicle cell processes or lining bodies.

One feature that is unique about these penetrating microvilli in Squalus oocytes is that there is no record of any other vertebrate or invertebrate oocyte having both ooplasmic and follicular cell processes that penetrate the substance of each other.

**OOCYTE NUCLEUS**

In all stages examined (0.1 to 4.0 mm) the oocyte nuclei were at diplotene of the first meiotic division. Even in the most immature females examined (60 to 90 cm) such was the case. This lead to the conclusion that the earliest phases of oogenesis are to be found in late embryos (20 months) or in the newly born young.

**A. NUCLEOLI**

The granular nature of Squalus oocyte nucleoli is a not uncommon feature. Raven (1961) notes that electron microscopy has often revealed a purely granular composition of oocyte nucleoli. The granules of the nucleoli are similar to those randomly distributed in the nucleoplasm.
averaging 150 Å in diameter and when the periphery of the nucleolus is examined it can be seen that the nucleolus is extruding these granules. Further, the 150 Å granules found in the ooplasm opposite the nuclear pores are, in all likelihood, these same granules that have passed through the nuclear membrane via the nuclear pores. It has been established that the hundreds of nucleoli present during the prolonged diplotene stage represent an amplification of the genome associated with the synthesis of large quantities of rRNA (Brown, 1966; Davidson, 1968; Bellairs, 1971; Kessel & Decker, 1971; Dumont; 1972). Thus these 150 Å particles in the nucleolus, nucleoplasm and in the ooplasm must be ribonucleoprotein (ribosomes) or the 18S and 28S ribosomal RNA molecules that are contained in inactive single ribosomes (Scheer, 1973). Lampbrush chromosomes, as found in the diplotene stages of this study have been implicated in rRNA synthesis as well as in DNA-like RNA synthesis (Rana, Kessel & Decker, 1971). The passage of nucleolar material into the ooplasm has been described in so many cases (sponges, hydroids, platyhelminthes, annelids, turbellarians, molluscs, arthropods and vertebrates) that it can hardly be doubted that it is of general occurrence (Raven, 1961). There are numerous theories on how this nucleolar material crosses the nuclear membrane (See Raven, 1961 for a review) but the most commonly accepted theory is that these 150 Å rRNA particles pass through the nuclear pores (Rhodonis, Anderson & Beams, 1956; Kemp, 1956b; Kessel & Beams, 1963b; Yamamoto, 1964; Williams, 1965; Priapulus, Nørrevang, 1968; Bellairs, 1971; Prostheceraeus, Boyer, 1972; Xenopus, Scheer, 1973; trout, Beams & Kessel, 1973).

It is also of interest to note that there is "abundant cytological evidence" (Raven, 1961) and electron microscopical reports that whole nucleoli have been extruded into the ooplasm (rat and some reptiles,
Bellairs, 1971; Thyone, Nørrevang, 1965). Thus, the 800 nm body observed in the ooplasm of a 0.1 mm oocyte is an extruded nucleolar body. A body this size would not pass through a nuclear pore and therefore the nuclear membrane would either have to break and reform or pinch off with the nucleolus contained inside. The latter possibility is less likely due to the lack of an encircling double membrane or the remnants of one.

B. NUCLEAR PORES

The complete or partial perforation of the nuclear envelope by pores is discussed in all papers focussed on the structure of the nuclear envelope of oocytes (Nørrevang, 1965). Regardless of species, both membranes of the nuclear envelope fuse in an area approximately 500 Å in diameter and may or may not have a constricting diaphragm (Nørrevang, 1968). In this regard, the nuclear pores of dogfish oocytes are quite typical. The presence or absence of an annular diaphragm was not established.

"In older oocytes—and always at the onset of vitellogenesis—nuclear pores cover the entire nuclear envelope..." (Nørrevang, 1968). Thus the increase in nuclear pore frequency reflects not only the approach of vitellogenesis but also the increase in the amount of rRNA synthesized in the nucleoli and transported to the ooplasm (Scheer, 1973).

C. NUCLEAR MEMBRANE

Throughout the stages examined the nuclear membrane shows only two major changes in morphology: the increase in the number of nuclear pores and the "blebbing" of the outer nuclear membrane. The latter is not an uncommon phenomenon, being reported in Priapulus (Nørrevang, 1965), trout (Beams & Kessel, 1973), Xenopus (Dumont, 1972), Triturus vulgaris (Spornitz & Kress, 1973), and numerous other species (Nørrevang, 1968). These vesicles or blebs from the nuclear membrane have been demonstrated to be
part of the endoplasmic reticulum (Nørrevang, 1965 & 1968; Beams & Kessel, 1973; Spornitz & Kress, 1973), either granular or agranular depending on the species. Further, the postmitotic nuclear membrane has been shown to reform by the coalescence of flat saccular elements of the endoplasmic reticulum (Bloom & Fawcett, 1968) and thus is considered to be an integral part of the endoplasmic reticulum. Although no extensive connections between the nuclear membrane and the vesicular smooth E.R. of the ooplasm were found in this study, the morphology of the membrane is indicative of at least a temporary connection. Given a rapidly developing oocyte and the abundance of smooth E.R. adjacent to the nucleus one can readily surmise that this is an active process not easily demonstrated.

A second phenomenon associated with the blebbing of the oocyte nuclear membrane is the formation of annulate lamellae (Nørrevang, 1968; Spornitz & Kress, 1973). The structure found in a 1.0 mm oocyte matches the classical description, wherein it consists "of parallel flattened vesicles, pierced by pores, very similar to those seen in the nuclear envelope" (Nørrevang, 1968). Such structures usually appear at the onset of vitellogenesis and are thought to play a role in protein yolk synthesis, a function that was neither confirmed nor denied in this report.

It is also of interest to note that the increasing irregularity of the nuclear membrane parallels the increase in nucleoli number and that this phenomenon has been recorded previously (Nørrevang, 1968; Dumont, 1972) and related to the further differentiation of the oocyte.

MITOCHONDRIAL MASS

Also known as a Balbiani body or a yolk nucleus, the mitochondrial mass has been described in a variety of oocytes and was thought to be
involved in the production of yolk. But electron microscopy has revealed that these bodies show a variety of structural units, being composed of either a single element or several elements in a complex structure (Raven, 1961; Yamamoto, 1964; Williams, 1965; Nørrevang, 1968). In trout (Beams & Kessel, 1973) the body is a prominent, granular mass, in Spisula and sea urchins (Nørrevang, 1965), endoplasmic reticulum is most prominent, in mammals, the Balbiani body consists mainly of Golgi vesicles, in birds, (Bellairs, 1971) the body, which disappears prior to yolk deposition, consists of smooth E.R., mitochondria and Golgi vesicles, in Xenopus, Triturus and Rana esculenta (Nørrevang, 1968; Dumont, 1972) and Pentastomida (Nørrevang, 1972) the Balbiani body is an enormous juxtanuclear mass of mitochondria intermingled with a few smooth membrane vesicles and granules. Spornitz and Kress (1973), however, maintain that Triturus vulgaris lacks a Balbiani body.

The juxtanuclear structure found in Squalus oocytes most closely resembles that found in many amphibians and will be referred to as a mitochondrial mass, as in Ward (1962), Balinsky & Devis (1963), Anderson & Beams (1964), and Dumont (1972).

In this study as well as many others (Yamamoto, 1964; Nørrevang, 1965 & 1968; Dumont, 1972) it has been shown that yolk formation is morphologically independent of the Balbiani body and it was urged that this name be dropped. Further, as these structures are not morphologically homologous it has also been considered unwise to use a general name for them. They do, however, exhibit certain common features; they appear in the immediate vicinity of the nucleus, mitochondria are often closely associated with them as are E.R. components, they contain protein and are often rich in RNA and they usually disappear prior to vitellogenesis (Raven, 1961:
Yamamoto, 1964; Bellairs, 1971; Dumont, 1972). Therefore, I feel that a
common term such as Balbiani body devoid of its implication in yolk
formation is more readily acceptable than a variety of descriptive terms,
one of which I have used extensively.

The increase in the number of mitochondria noted in early Squalus
oocytes is a normal feature of oogenesis in all vertebrates and invertebrates.
In Pentastomida (Nørrevang, 1972) the multiplication of mitochondria and
the concomitant increase in ergastoplasm are taken as the first signs
that the oocyte has entered its growth phase. In Priapulus the number of
mitochondria rises during oogenesis from 5 to 8 in the oogonium to about
40,000 in the mature oocyte with similar increases noted in other oocytes
(Nørrevang, 1968). The obvious question of the mechanism of mitochondrial
multiplication now arises. Based on the variety of mitochondrial config­
urations found in dogfish oocytes it is logical to presume that the mito­
chondria proliferate either by budding or by elongation and division of
pre-existing mitochondria. This is a conclusion previously reached by
many authors. The dumb-bell and branched mitochondria described in this
report have also been noted in Priapulus, Drosophila, Lacerta, the rabbit,
and the rhesus monkey (Nørrevang, 1968). These variations are generally
believed to be signs of mitochondrial multiplication with the former case
reflecting multiple divisions and the latter a budding process. The
parallel arrays of cristae are either a further indication of an imminent
division or an indication of reduced metabolic activity (Fawcett, 1966).
Given that this configuration occurs in a metabolically active oocyte, the
latter supposition can be ruled out. The ring-shaped mitochondria are
unusual but they may be produced as a result of the sectioning plane
passing through the crook of a "question-mark" or dumb-bell shaped
mitochondrion.

Having already noted that the Balbiani body is not involved in yolk formation, many authors have speculated on the function of this structure. Livni (1971) found G-6-P dehydrogenase (a cytoplasmic enzyme), α-glycerophosphate dehydrogenase and succinate dehydrogenase (mitochondrial enzymes) in the mitochondrial Balbiani body of three teleosts. Many other authors (Yamamoto, 1964; Guraya, 1965; Abraham et al, 1966; Ulrich, 1969; Beams & Kessel, 1973) intimate that it forms or constitutes an essential precursor substance (usually RNA) which is necessary for oocyte growth and vitellogenesis. Other authors refuse to speculate on its function (Bellairs, 1971; Dumont, 1972) but allow that it is near enough to the nucleus to intercept essential materials moving into the ooplasm from the nucleus, thus hinting that the Balbiani body may indirectly be involved in vitellogenesis. Regardless of the confusion this body of organelles creates, its function in *Squalus acanthias* and various amphibian oocytes is that of a centre for the multiplication of mitochondria. These mitochondria then disperse and indeed the whole structure disperses prior to vitellogenesis allowing the mitochondria to associate with the numerous vesicles and cisternae of S.E.R. for the formation of proteid yolk. At no time were mitochondria observed with integral yolk platelets as in many amphibian oocytes (Ward, 1962; Massover, 1971; Kress & Spornitz, 1972). Thus, the mitochondrial mass or Balbiani body is involved in yolk formation, but only indirectly, in that it contributes essential prerequisites, either enzymes or ribonucleic acid.

**LIPID YOLK**

Of the three types of yolk found in most oocytes (Raven, 1961)
Squalus acanthias oocytes lack only carbohydrate (glycogen) yolk. The pertinent histochemical data are inconclusive but ultrastructurally no glycogen rosettes were observed in any of the stages examined.

Lipid or fatty yolk is formed before protein yolk (Raven, 1961) and in amphibians it is formed at the end of the "pre-vitellogenic" period (Kemp, 1956b). Lipid yolk is usually represented as a dense, smooth or irregular body without a limiting membrane (Raven, 1961; Yamamoto, 1964; Nørrevang, 1968; Bondi & Facchini, 1972). These characteristics describe the large dense bodies found in early dogfish oocytes.

The methods of formation of lipid yolk are as varied as the species that produce it. Numerous species (Lumbricus, Limnaea, Asterias, Rana, Raven, 1961; mollusc, crayfish, ascidians, Priapulus, Nørrevang, 1968; Oryzias, Yamamoto, 1964; Branchiobdella, Bondi & Facchini, 1972) report the formation of lipid yolk de novo (i.e. not-associated with any organelles). Other species (earthworms, birds, man, Raven, 1961; Arabacia, sea urchin, rabbit, Nørrevang, 1968) show its formation occurring in association with mitochondria or a juxtanuclear accumulation of mitochondria (Raven, 1961). Only two reports of direct transformation of mitochondria into lipid are known (Drosophila & dog, Raven, 1961). Formation of lipid yolk by or in association with Golgi is also reported frequently (Raven, 1961; Nørrevang, 1968).

From the available morphological data, it is difficult to ascribe the formation of lipid yolk in dogfish oocytes to any particular organelle. The relationship of the formed lipid elements with the vesicles of the smooth E.R. hints at some metabolic association. However, there is no direct evidence to support such a claim. Thus, it is probable that as in so many other species (Raven, 1961; Yamamoto, 1964; Nørrevang, 1968;
Bondi & Facchini, 1972) the lipid yolk in dogfish oocytes can be said to arise de novo in the ooplasm. This in turn can be further qualified if one considers Raven's (1961) conclusion that the time and place at which lipid yolk becomes visible does not necessarily correspond with the time and place of fat synthesis but probably represents a process of condensation and precipitation of soluble lipids which reach their saturation point and precipitate out possibly using various cell components as "condensation nuclei". In this instance, the lipid yolk may be using the S.E.R. as its condensation nuclei with further growth of the lipid droplet occurring independently of the smooth endoplasmic reticulum. This may explain the proximity of the S.E.R. to the lipid yolk and also why no direct contribution to the developing droplet has been seen.

Again, the morphological data is insufficient to conclusively explain the origin of this "dissolved lipid". It can come from but one of two sources, the first being from within the oocyte itself. However, this study has been unable to provide any evidence to substantiate this theory. Secondly, the lipid may be extra-oocytic in origin, travelling to the ovary from its point of origin via the blood (Raven, 1961; Nørrevang, 1968; Bellairs, 1971) ultimately penetrating the oocyte either by diffusion or by pinocytotic processes which have already been discussed in terms of the dogfish oocyte vitelline membrane. This is substantiated by the free lipid drops found in the cortex, theca and follicle cell layer. Additionally, the location of the lipid yolk in the mid to outer regions of the oocyte and the pronounced formation of lipid concomitant with development of ooplasmic microvilli are factors that also prompted Bondi & Facchini (1972) to conclude that the materials for lipid yolk penetrated the oocyte by diffusion.
The relationship of the mitochondria and the lipid droplets has previously been considered to be consistent with the interpretation that the mitochondria play an important role in lipid metabolism as the mitochondria have been shown to contain the enzymes involved in triglyceride metabolism (Fawcett, 1966; White, Handler & Smith, 1968). This relationship then provides a store of energy for immediate or later use and a potential source of short carbon chains for the synthesis of structural components which contain lipids, such as membranes. The latter is of importance during protein yolk formation for the outer limiting membrane of the yolk platelet and for possible contribution to the numerous membrane bound vesicles that constitute part of the primary yolk platelets.

PROTEIN YOLK

Protein yolk is the second type of yolk found in dogfish oocytes and although its precursor bodies are formed concurrently with lipid yolk, the platelets themselves do not become visible until well after lipid yolk has been formed.

The development of G.V.B.s and M.V.B.s as precursors to protein yolk platelets is a well established pathway observed in tunicates (Ciona, Kessel, 1966), anurans (Kress & Spornitz, 1972) and urodeles (Spornitz & Kress, 1973). In Ciona the abundant Golgi supply the large numbers of vesicles required while in Rana esculenta and R. temporaria the vesicles are derived from micropinocytosis, the S.E.R. and the Golgi and in Triturus vulgaris the vesicles of primary yolk are formed from the nuclear membrane or de novo in the ooplasm. Secondary yolk in T. vulgaris is formed from pinocytotically derived vesicles. In each instance the ooplasm has abundant free ribosomes.
In *Squalus acanthias* oocytes, vesicular yolk is formed by vesicles from each of these sources, a phenomenon unreported in any other yolk producing organism. A possible explanation for this phenomenon lies in the fact that only the S.E.R. is moderately well developed and therefore membranous contributions from all sources are required to produce the massive amounts of protein yolk found in dogfish oocytes.

Another factor that must be considered in dogfish vitellogenesis is the source of the yolk proteins incorporated into the platelets. It is generally accepted that in vertebrates the raw materials of yolk are formed in the liver and pass from there in the blood plasma to the ovary where they enter the oocyte by pinocytosis (Rudack & Wallace, 1968; Wallace & Dumont, 1968; Bellairs, 1971; Kress & Spornitz, 1972). This is supported by several lines of evidence. Firstly, the rate of deposition in bird's eggs is too great for the yolk to have been manufactured in the oocyte or in the follicle cells. In a hen, this would require each follicle cell to secrete a quantity of yolk equal to its own volume every half hour during the last phase of deposition. Secondly, the blood serum and liver of laying hens has been shown to contain large amounts of lipids and proteins with properties closely resembling those of similar material found in yolk, while these substances cannot be detected in male birds and non-laying hens (Heald & McLachlan, 1965; Husbands, 1970; Bellairs, 1971; Beuving & Gruber, 1971; Christie & Moore, 1972). Thirdly, there is no biochemical data to support the conception of an intraoocytic synthesis of yolk proteins particularly in the ranidae (Kress & Spornitz, 1972) and in urodeles (Spornitz & Kress, 1973). But there is considerable morphological and autoradiographic data to indicate that yolk is in part synthesized intra-oocytically (*Lebistes reticulatus*, Droller & Roth, 1966; *Brachydanio*
Thus this question arises, does *Squalus acanthias* produce its yolk autosynthetically (within the oocyte) or heterosynthetically (outside the oocyte)? Like many problems the answer is not clear cut. In all likelihood *Squalus* follows the vertebrate plan (Bellairs, 1971) and produces its yolk proteins in the liver and transports them to the oocyte. In support of this hypothesis one must consider the massive size of the dogfish liver, the size and numbers of the mature oocytes (similar to the hen's) and the pinocytotic vesicles found at the oocyte's plasma membrane. But in the smallest oocytes, pinocytosis is not occurring at a rate sufficient to account for the yolk precursor bodies (G.V.B.s) that are present. Thus, it is clear that the membranous elements which constitute the framework of the yolk platelets are produced autosynthetically. However, the contents of these membranous elements may be formed under the influence of the free ribosomes as is the case in the tunicate *Ciona* (Kessel, 1966), the crayfish (Beams & Kessel, 1963) and the lobster *Homarus* (Kessel, 1968), a proposal that is compatible with the role of the ribosome in protein synthesis. Alternatively, the small molecular precursors of protein yolk may pass by diffusion across the oocyte membrane to appear in the E.R., Golgi and possibly the mitochondria (Ward, 1962; Beams & Kessel, 1963; Kessel, 1968a, b,c; Anderson, 1969; Kessel, 1971; Massover, 1971; Boyer, 1972) ultimately finding their way to the granular-vesicular bodies. Indicative of this in dogfish oocytes are the intimate associations of the S.E.R. with the lining bodies, Golgi and mitochondria. In any event, these intraoocytic membranous elements serve a packaging role for the accumulation of yolk material over a period of time.
The formation of the limiting membrane of yolk platelet precursors from distended Golgi or S.E.R. cisternae has a counterpart in vitellogenesis in crayfish (Beams & Kessel, 1962, 1963a, b) and in Lebistes (Droller & Roth, 1966). In crayfish, large (300 to 800 Å) yolk granules are formed within the cisternae of R.E.R. later to pass to agranular parts of the E.R. where they aggregate into larger masses ultimately changing into finely granular yolk bodies. Further, in trout oocytes a system of precursor formation identical to dogfish oocytes has been noted. Here S.E.R. cisternae with intracisternal granules anastomose, fuse and swell to permit the accumulation of ICG or precursor yolk (Beams & Kessel, 1973).

The looping of S.E.R. tubules to form a limiting membrane has a similar counterpart in Priapulus oocytes. There the R.E.R. vesicles surround a small, irregular condensation of dense material, with the incomplete mantle they form creating a gradient, drawing in additional material. The limiting membrane is late to form but its relationship to the R.E.R. is taken as an indication that its formation is induced by the ergastoplasmic membrane (Nørrevang, 1968).

As previously mentioned, granular-vesicular bodies display figures indicative of the dissolution and reassociation of their contents as a granular matrix. Similar phenomena have been reported in all oocytes that have a vesicular form of yolk formation (Beams & Kessel, 1962, 1963a, b; Yamamoto, 1964; Kessel, 1966, 1968; Boyer, 1972; Dumont, 1972; Kress & Spornitz, 1972; Beams & Kessel, 1973; Spornitz & Kress, 1973). However, little notice has been given to this transformation of proteinaceous and lipid (membrane) precursors into a homogeneous mass. The fact that this transformation is possibly enzyme mediated has gone unmentioned and may explain the proximity of the mitochondria to the G.V.B.s.
One feature of vitellogenesis in dogfish oocytes that poses another question is when does the crystallization of the platelet occur? The fact that it is not directly related to the development of the G.V.B. is demonstrated by the presence of well developed electron dense G.V.B.s in 0.1 mm oocytes and a total absence of yolk platelets. However, the crystallization of the platelet at 3.0 to 4.0 mm may reflect the threshold level for a pinocytotically derived precursor required for crystallization. A similar phenomenon has been recorded in *Triturus vulgaris* (Spornitz & Kress, 1973) wherein crystallization of primary and secondary yolk platelets does not occur until micropinocytosis has begun.

The crystalline pattern in amphibian yolk platelets has been attributed to the arrangement of lipoprotein subunits. These units are phosvitin (MW 40,000) and lipovitellin (MW 210,000) (Wallace, 1963; Rudack & Wallace, 1968; Massover, 1971; Leonard et al, 1972) classically present in the ratio of two phosvitin to one lipovitellin hexagonally arranged to form the pattern observed. Recently, however, a freeze-etching study has suggested that the crystalline pattern is formed by lipovitellin dimers with one phosvitin molecule associated with each monomer of the lipovitellin dimer (Leonard et al, 1972). Analysis of the dogfish yolk platelet and comparison of it with the amphibian system was beyond the scope of this study. But considering the similarities of amphibian and elasmobranch yolk platelets, expecting such a molecular ratio (2:1 or 1:1) in elasmobranch yolk would not seem out of order. For a discussion of the properties of various vertebrate phosvitins see Clark (1972).

The formation and crystallization of phosvitin and lipovitellin in *Rana pipiens* was thought to involve a terminal phosphorylation mediated by an oocytic protein kinase (Wallace, 1963). But in a recent chromatographic
study (Wallace et al, 1972), the lack of any specific association of $^{32}$P-labelled material with either the lipovitellin or, particularly, the phosvitin after incubation in Na$_2$HPO$_4$-$^{32}$P was taken as precluding the previous suggestion. Further, incubation of vitellogenin, the phosvitin-lipovitellin precursor substance, with ATP ($^{32}$P) and purified oocyte protein kinase did not dissociate the phosphorylated product into lipovitellin and phosvitin. Thus, the processing mechanism for the conversion of vitellogenin into yolk proteins remains unknown (Wallace et al, 1972). Considering the similarity of amphibian and dogfish yolk (see below) these conclusions may hold true for Squalus as well.

The presence of a crystalline zone is regarded as being typical for yolk platelets (Massover, 1971; Spornitz & Kress, 1973) and even as defining the organelle as a yolk platelet (Massover, 1971). However, Spornitz & Kress (1973) also consider that the presence of a crystalline or paracrystalline arrangement in structures found in oocytes cannot alone be regarded as sufficient evidence that such structures are identical to yolk. This statement refers only to intramitochondrial crystalline inclusion bodies in the ranidae which have different center to center spacing of the crystal cylinders relative to the pattern of the common amphibian yolk (vesicular yolk) platelet (Spornitz, 1972). Thus it is safe to conclude that the crystalline structures found in dogfish oocytes are yolk platelets and based on the center to center spacing of the pattern that these platelets are identical to the vesicular yolk platelets of the amphibia. The term "identical" is used loosely and in all likelihood the similar crystalline lattice patterns of amphibian and elasmobranch yolk platelets reflects similar ratios of phosvitin to lipovitellin (Spornitz, 1972).

It is also of interest to note that yolk platelets with a crystalline
core (amphibian type) belong to the phylogenetically older vertebrate groups. These groups include the amphibia, the holosteans and now the elasmobranchs. The yolk of the phylogenetically newer groups, the teleosts, reptiles, birds and mammals lack a crystalline core and are referred to as the bird type (Kilarski & Grodzinski, 1969).

Based upon the data presented the following pathway for protein yolk formation in dogfish oocytes has been postulated. In the smallest oocytes (< 0.4 mm), small M.G.B.s and M.V.B.s are formed by and from Golgi vesicles, S.E.R. vesicles, nuclear membrane and micropinocytotic vesicles. These in turn fuse with one another forming granular-vesicular bodies. In larger oocytes (0.4 mm +) the outer limiting membrane of a yolk platelet precursor is formed either from distended Golgi or S.E.R. vesicles or from loops of small S.E.R. vesicles. In the former case these two vesicular bodies are empty and appear first as large M.G.B.s. Once these precursor membranes form or begin to form they take up M.G.B.s, M.V.B.s and small G.V.B.s. By either method granular-vesicular bodies are formed and these in turn display the breakdown and dissolution of their contents (i.e. myelin-like figures and homogeneous granular matrix). The subsequent recondensation of this material produces an electron dense granular matrix or core within the G.V.B.. The final phase in protein yolk platelet formation is the crystallization of this core.
SUMMARY

TUNICA ALBUGINEA

The protein reaction suspected to indicate the presence of smooth muscle as in the tunica of the smooth dogfish Mustelus canis (TeWinkel, 1972) was not corroborated by the electron micrographs. The histochemical analysis indicates that collagen should be present and our E.M. work demonstrates it conclusively. Elastic fibres and their ultrastructure were also discussed.

STROMA

Histochemically the light cells with ovoid nuclei possess small quantities of protein, R.N.A., phospholipid and neutral mucopolysaccharides while electron microscopy indicates extensive cisternae of rough endoplasmic reticula. The latter indicates a glandular-type cell elaborating a protein-rich product, a conclusion only partially supported by the histochemical data. The dark cells with pleomorphic nuclei possess numerous dark bodies with crystalloids that may correspond to the protein and non-sulphated mucopolysaccharide reactions. The possibility that two types of dark cell may exist was indicated by the aldehyde fuchsin reaction in a small percentage of these cells. This is a hypothesis that is supported by the discovery of an "intermediate dark cell" (Figure 37). The lacunae border cells were found to be of an endothelial type similar to those lining capillary walls.

THECAL CELLS

The ultrastructure of these cells indicates they are involved in
protein and steroid synthesis, however, our histochemical observations only corroborate protein synthesis while the steroid activity of these cells was noted by Lance and Callard (1969). Ultrastructurally, the thecal cells of *Squalus* are thought to contribute to the formation of the surrounding granular and collagenous matrix as well as the basal lamella. The presence of acidic and sulphated mucopolysaccharides, which are often associated with collagen (Swanson, 1965; Pearse, 1968), in the thecal cells supports this hypothesis.

**BASEMENT LAMINA**

The basement lamina consists predominantly of acid and sulphated mucopolysaccharides and is formed by the follicle cells. Ultrastructurally, the vesicles in the basal region of the follicle cells represent the mucopolysaccharide precursors that form the basement lamina. Hyaluronic acid, one of the glycosaminoglycans present in the basement lamina is a highly hydrated molecule and is very viscous (Schubert & Hamerman, 1956). This viscosity is reflected in the electron density of the membrane and in turn provides structural rigidity to the oocyte as well as a means for controlling molecular diffusion.

**VITELLINE MEMBRANE**

Histochemically, the vitelline membrane is a protein-polysaccharide complex and the R.N.A. activity of the apical regions of the follicle cells reflected in the ribosomes and R.E.R. of this region is related to the development of the membrane. The dual chemical composition of the membrane is responsible for its two major functions—support and chemical selectivity.
FOLLICLE CELLS

Beyond the activity of the follicle cells in basement lamina and vitelline membrane formation little else can be stated conclusively. The ultrastructural evidence for steroid activity as noted by Lance and Callard (1969) in the follicle cells is purely circumstantial and is based only on the presence of quantities of smooth membrane bound vesicles and cisternae (Nicholls & Maple, 1972; Peel & Bellairs, 1972).

OOPLASM

The major constituents of the ooplasm are protein and lipid. The M.G.B.s, M.V.B.s, and G.V.B.s comprise the proteinaceous portion while the lipid yolk droplets account for the lipid portion of the ooplasm.

NUCLEOLI

The numerous nucleoli present are producing rRNA and ultrastructurally this is represented by their granular nature and the fragmentation of the peripheral regions of the body.

MITOCHONDRIAL MASS

Histochemically, this organelle system is rich in protein, RNA and phospholipids. Ultrastructurally, the cloud is constructed of mitochondria, smooth endoplasmic reticulum and ribosomes. Primarily the cloud is the centre for mitochondrial multiplication and possibly some packaging of yolk material. Such juxtanuclear oocytic bodies are often mistakenly referred to as yolk nuclei.
LIPID YOLK

The presence of lipid yolk in early dogfish oocytes is indicated by the histochemical analysis and ultrastructurally it was noted that lipid yolk arose de novo in the ooplasm from material of extra-ocytic origin.

PROTEIN YOLK

The amount of protein present in the ooplasm of early oocytes is reflected in the vesicles and granules found in the electron micrographs. In the smallest oocytes (< 0.4 mm), multigranular (M.G.B.) and multivesicular (M.V.B.) bodies form from Golgi, S.E.R., micropinocytotic vesicles and vesicles derived from the nuclear membrane. These fuse together forming granular-vesicular (G.V.B.) bodies. In larger oocytes (0.4 mm +), distended Golgi lamellae, S.E.R. cisternae and loops of S.E.R. vesicles form limiting membranes and take up M.G.B.s, M.V.B.s and small G.V.B.s. These large G.V.B.s undergo a dissolution of their contents with subsequent recondensation and crystallization of the core to form a mature protein yolk platelet.


Farquhar, M.G. 1964. Glomerular permeability investigated by electron microscopy; in, "Small Blood Vessel Involvement in Diabetes mellitus" pp. 31-38, A.I.B.S., Wash. D.C.


Guraya, S.S. 1965. A comparative histochemical study of fish (Channa maruleus) and amphibian (Bufo stomaticus) oogenesis. Z. Zellforsch. 65:662-700.


APPENDIX A

HISTOCHEMICAL TECHNIQUES EMPLOYED

STAINING

1. **DNFB (Tranzer & Pearse, 1964)**
   
   Incubate 10 hrs. at 22°C in 1% alcoholic DNFB alkalinized with 0.2 ml 1N NaOH, wash in 90% EtOH followed by DH2O and reduce for 30 minutes in titanous chloride. Wash in citrate buffer, DH2O, and diazotize 5 minutes in fresh nitrous acid. Wash in DH2O and couple with H-acid for 5 minutes at 4°C, wash, dehydrate, clear and mount.

2. **PAS (Hotchkiss, 1948)**
   
   Oxidize 5 minutes in alcoholic periodic acid, rinse in 70% EtOH, immerse in reducing bath for 1 minute, rinse in 70% EtOH, treat with Schiff's (Barger & DeLamater) for 20 minutes. Wash, stain in celestin blue 2 to 3 minutes, Mayer's haemalum 2 to 3 minutes, differentiate with 1% acid alcohol, wash, counterstain with Orange G, wash, dehydrate, clear and mount.

   
   Stain 5 to 10 minutes in methyl green-pyronin in acetate buffer (pH 4.8), rinse rapidly in DH2O, blot dry, dehydrate quickly in acetone, clear and mount.

4. **TOLUIDINE BLUE (Pearse, 1968)**
   
   Stain for 10 to 20 minutes in 0.5 to 1.0% aqueous Toluidine blue (G.T. Curr). Rinse, dehydrate, clear and mount in permount.

5. **ALCIAN BLUE (Steedman, 1950)**
   
   Stain 20 minutes in 1% alcian blue (Allied Chemical) in 3% acetic acid (pH 2.5). Wash, dehydrate, clear and mount.
6. **DIALYSED IRON (Hale, 1946)**

Flood with dialysed iron for 10 minutes, wash, flood with acid ferrocyanide for 10 minutes, wash, dehydrate, clear and mount.

7. **ALDEHYDE FUCHSIN (Halmi & Davies, 1953)**

Bring to 70% EtOH, stain 20 minutes with acidic AF, rinse in 70% EtOH, dehydrate, clear and mount.


Stain for 30 minutes in 0.2% Azure A (Fisher Scientific Co.) in phosphate buffer with the pH adjusted to 1.5 with 0.1N HCl. Dehydrate in EtOH and clear or dehydrate in air and mount in permount.

9. **COPPER PHTHALOCYANIN (Kluver & Barrera, 1953)**

Bring to absolute alcohol, stain in Luxol Fast Blue MBS (Hartman-Leddon Co.) for 12 hours at 60°C. Rinse in 70% EtOH and differentiate in 0.05% aqueous lithium carbonate for 2 hours, rinse in water. Counterstain for 20 minutes in 1% aqueous neutral red, rinse, dehydrate, clear and mount.

10. **SUDAN BLACK B (McManus, 1946)**

Cryostat. Stain 30 minutes with saturated Sudan black B (E. Gurr Ltd.) in 70% EtOH. Rinse quickly in 70% EtOH, wash in running water, mount in Farrant's medium.

**ENZYME TREATMENT**

1. **DIASTASE (Humanson, 1972)**

Digest sections in 1.0% diastase of malt (Nutritional Biochemicals Corp.) in disodium phosphate buffer for 1 hour at 37°C (pH 6.0) to remove glycogen. Control slides were incubated in DH2O at 37°C for 1 hour.
2. **PANCREATIC RIBONUCLEASE (Pearse, 1972)**

   Incubate sections for 1 hour at 37°C in 0.1% pancreatic Ribonuclease-A (Sigma Chemical Corp.) in DH$_2$O. Sections were then treated with 1.0% aqueous toluidine blue or methyl green-pyronin to demonstrate the loss of basophillic material which is considered to be RNA. Control slides were incubated in DH$_2$O at 37°C.

3. **TRICHLOROACETIC ACID (Humanson, 1972)**

   Sections were incubated in 5% TCA (Fisher Scientific Co.) for 30 minutes at 60°C to remove RNA. Control slides were incubated in DH$_2$O at 60°C.

4. **TESTICULAR HYALURONIDASE (Pearse, 1972)**

   Incubate sections for 3 hours at 37°C in 0.1% testicular hyaluronidase (Sigma Chemical Corp.) in 0.85% saline. Control slides were treated at 37°C in 0.85% saline only. Acidic mucopolysaccharides staining in the controls but not in enzyme treated slides may be either hyaluronic acid, chondroitin sulphate A and/or chondroitin sulphate C.

**SELECTIVE BLOCKING AND UNBLOCKING**

1. **METHYLATION-DEMETHYLATION (Culling, 1963)**

   Dehydrated sections were treated for 4 hours at room temperature in 1% HCl in methanol, rinsed in absolute EtOH, hydrated and stained.

   To saponify, methylated sections were rinsed in EtOH and treated with 0.1N KOH in DH$_2$O for 45 minutes at room temperature, washed in water and stained.