The Effects of Chorioallantoic Grafts on the Developing Chick Embryo

I. Studies on Weight and Histology of Homologous and Heterologous Tissues

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WITH ONE PLATE

INTRODUCTION

The grafting of tissues to the chorioallantoic membrane (CAM) of the chick embryo has been widely used for study of organ-specific growth stimulation. Murphy (1916) and Danchakoff (1916) first observed that chorioallantoic grafts of adult chicken spleen induced enlargement of the spleens of host embryos. The former attributed spleen hypertrophy to an increase in the number of small lymphocytes while the latter attributed it to an intense proliferation of lymphoid haemocytoblasts which ultimately differentiated into granulocytes. In a subsequent study Danchakoff (1918) observed that transformation of mesenchyme into granuloblastic cells was not confined to the spleen but extended throughout the whole mesenchyme of the host.

An extensive investigation of the problem of the effect of CAM grafts of adult chicken tissue on homologous tissues of the host embryo was carried out by Ebert (1955). He observed a very marked enlargement of spleens in host chicks following grafts of adult chicken spleen (Ebert, 1951). In other experiments using CAM grafts labelled with radioactive methionine, Ebert (1954) observed that enlarged spleens had an increased nitrogen content and a higher specific radioactivity than the kidney and liver. The DNA content of enlarged spleens did not differ significantly from control spleens. On the basis of these observations he suggested that enlargement of the spleen was due to increased protein content; that transfer from the graft to the host was tissue-specific and there was a selective incorporation of tissue-specific proteins from grafts to homologous tissues rather than transfer of whole cells. A transfer of such specific protein moieties was shown by Walter et al. (1956). Following injection of either a clear super-

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natant of $^{35}$S-labelled homogenized liver or heart into 9-day-old embryos, a higher specific activity was observed in the respective host tissues of liver and heart. Similar studies have also been conducted by Ebert (1957; 1958 a, b). This investigator was able to demonstrate the localization of tissue-specific fractions in homologous organs following intravenous injections of cytoplasmic materials. Selective localization was obtained following the injection of microsomal and supernatant fractions, but not with nuclear and mitochondrial fractions. On the other hand, Van Haeften (1958) observed significant enlargement of the host spleen following grafting of cell-free homogenates of spleen to the CAM.

An alternative hypothesis, that of a 'graft-versus-host reaction', has been advanced to explain splenic enlargement following introduction of adult spleen or blood-cells into the chick embryo. Simonsen (1957) observed that 18-day chick embryos, injected intravenously with adult chicken spleen-cells or blood-cells, manifested symptoms of severe haemolytic anaemia, and that marked hypertrophy occurred in the spleen, liver, and thymus about 2 weeks after hatching. He further observed that reticuloendothelial cells replaced erythropoietic and myelopoietic cells in the bone-marrow. The enlargement of the spleen, the haemolytic anaemia, and the histological changes in the bone-marrow he attributed to an immune reaction of the injected cells against the host and colonization of the host's tissues by injected cells. Recently, Cock & Simonsen (1958) have shown that when blood from one inbred line of adult chickens was injected into newly hatched chicks of a cross between two highly inbred lines, gross enlargement of the spleen and liver occurred. On the other hand, when $F_1$ blood was injected into $F_1$ chicks only a relatively slight enlargement occurred, and this they attributed to the antigenic diversity within one of the inbred lines. Terasaki (1959) has shown that when adult chicken lymphocytes were injected into chick embryos there was a marked splenic enlargement in the host. On the other hand, adult monocytes and thymocytes did not cause significant enlargement of the spleen. Billingham & Brent (1957) also attribute the production of 'runt disease' in mice and the mortality observed following injections of A-strain mice with C57 spleen-cells to the immunological reactions produced by the inoculated adult spleen-cells against the tissue antigens present in their young hosts.

Evidence of a cellular immune reaction on the part of adult spleen-cells in an embryonic environment has been demonstrated in the chick by Ebert (1957; 1958 a, b) and in the larval salamander by De Lanney (1958). The former investigator observed that following intracoelomic grafts of adult spleen on the 4th day of incubation, a profound effect was produced on the vascular system, leading to death in 30 per cent. of the host embryos. The latter investigator observed that when adult salamander spleen was grafted into a pocket in the dorsal fin or in the coelom of the larval salamander, Taricha torosa, growth of the host spleen was suppressed.

From this review of the literature it would seem that in order to ascertain if
organ-specific growth stimulation exists some organ should be studied which is
not known to be engaged in haematopoiesis. The histogenesis of the duodenum
and histochemistry of the mucopolysaccharides in the connective tissues of this
organ have been described by Van Alten & Fennell (1957). The objectives of the
present investigation were to study: (1) the effects of chorioallantoic grafts of
embryonic duodenum and various adult organs (spleen, liver, heart, skin, brain,
and duodenum) upon the weight of homologous and heterologous organs in the
developing chick embryo; (2) the effects of soluble extracts of adult and em-
bryonic chicken organs on the host embryo when injected into the yolk sac; and
(3) the histology of the various embryonic organs following CAM transplants.

MATERIALS AND METHODS

Chickens of both sexes, of different breeds and of ages ranging from 3 months
to about one year after hatching were used as donors of tissues for grafting.
Embryonic duodena were obtained from embryos incubated 15, 16, 17, 18, and
20 days.

The procedure of the chorioallantoic transplantations is given in detail by
Van Alten (1958). The approach has been to compare weight differences of the
total host embryo and various host organs (spleen, liver, heart, intestine, and
duodenum) following: (1) chorioallantoic transplants of fresh adult organs;
(2) grafts of adult chicken duodenum treated with 95 per cent. ethanol at
—20° C. for 24 hours, lyophilization, or heat (80° C. for 20 minutes); (3) sham
operations in which the complete chorioallantoic transplantation procedure
was carried out, but only a drop of sterile Ringer's solution, or a small piece of
2 per cent. agar, was put on the CAM; (4) transplants of adult rat duodenum; and
(5) grafts of 15-, 16-, 17-, 18-, and 20-day embryonic duodena.

The chicken was killed by decapitation and various tissues (duodenum,
spleen, liver, heart, skin, and brain) were quickly removed and cut into small
(2—3 mm.3) pieces in chick Ringer's solution. In order to sterilize the duodenum
it was placed in 200 ml. of chick Ringer's solution containing 1,000 mg. of
chloromycetin for 10 minutes. In order to determine if the tissue was sterilized
an occasional piece of tissue was streaked on a blood-agar plate. In no case was
contamination found. One of the prepared pieces of tissue was then placed at the
bifurcation point of blood-vessels on the CAM. The above procedure was also
used for adult rat duodenum.

In order to ascertain the effect of soluble cell-free material of the duodenum,
liver, and heart of the adult chicken on the chick embryo, the following pro-
cEDURE was carried out. Tissues, either adult or 20-day embryonic chick, were
homogenized in a Waring blendor with a 1 to 5 ratio of 0·15 M saline (buffered
to pH 7·4 with 0·005 M phosphate buffer) for 20 minutes at 4° C., after which the
homogenate was centrifuged at about 500 g. for 30 minutes at 4° C. The super-
natant after Seitz filtration was used for injection into the yolk sac.
The statistics used in comparing the weight of the chick, spleen, liver, heart, intestine, and duodenum following various treatments to the CAM, were an analysis of variance (Dixon & Massey, 1951) and the multiple range test for heteroscedastic means (Duncan, 1957).

Following various CAM grafts, host tissues of spleen, liver, heart, duodenum, and CAM were fixed in Bouin’s fixative for 24 hours and embedded in paraffin by routine methods of tissue preparation. Tissue sections were cut at 5 μ. All tissues were routinely stained with haematoxylin and eosin, Gomori’s trichrome stain (Gomori, 1950), the triple stain of Himes & Moriber (1956), and the Azure II eosin procedure (Lillie, 1954). For elastic tissue Weigert’s resorcin fuchsin was used and Van Giesen’s picro-acid fuchsin was used for collagenous tissue.

RESULTS

The effect of chorioallantoic transplants of adult and embryonic chicken tissues on the weight of homologous and heterologous tissues of the host embryo

It is evident from Table 1 that, following adult duodenal grafts, there was a significant reduction in weight of the whole embryo and a marked enlargement of the spleen, liver, and heart, and that there was no significant weight difference in the intestine or duodenum. It can also be seen that after liver grafts the liver and heart showed a significant enlargement over those of controls (sham operation). However, the liver and heart were significantly larger after grafts of duodenum than after liver grafts. When alcohol-inactivated adult chicken duodenum was transplanted to the CAM it was observed that there was a decrease in weight of both the whole embryo and the duodenum.

TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Whole chick (g.)</th>
<th>Spleen (mg.)</th>
<th>Liver (g.)</th>
<th>Heart (g.)</th>
<th>Intestine (g.)</th>
<th>Duodenum (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Grafts of adult chicken duodenum</td>
<td>33</td>
<td>16.25</td>
<td>36.41</td>
<td>0.6609</td>
<td>0.2115</td>
<td>0.3963</td>
<td>67.08</td>
</tr>
<tr>
<td>B. Grafts of adult chicken liver</td>
<td>29</td>
<td>18.13</td>
<td>16.97</td>
<td>0.5421</td>
<td>0.1786</td>
<td>0.4148</td>
<td>67.08</td>
</tr>
<tr>
<td>C. Sham operation</td>
<td>25</td>
<td>18.86</td>
<td>12.00</td>
<td>0.4716</td>
<td>0.1592</td>
<td>0.3924</td>
<td>61.96</td>
</tr>
<tr>
<td>D. Grafts of inactivated adult chicken duodenum</td>
<td>21</td>
<td>17.60</td>
<td>13.19</td>
<td>0.4514</td>
<td>0.1448</td>
<td>0.3757</td>
<td>55.37</td>
</tr>
<tr>
<td>Statistical F test</td>
<td></td>
<td>9.41*</td>
<td>22.9*</td>
<td>30.77*</td>
<td>28.75*</td>
<td>0.75</td>
<td>5.72*</td>
</tr>
<tr>
<td>Statistical multiple range test1.</td>
<td></td>
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</tbody>
</table>

1 Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.
In Table 2 it is evident that the spleen and heart were significantly larger when brain was grafted to the CAM than when control procedures were used. The duodenum was larger, but other significant growth changes were not observed after grafts of rat duodenum.

**Table 2**

**Fresh weights of whole embryos and of homologous and heterologous organs following chorioallantoic transplants**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Whole chick (g.)</th>
<th>Spleen (mg.)</th>
<th>Liver (g.)</th>
<th>Heart (g.)</th>
<th>Intestine (g.)</th>
<th>Duodenum (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Grafts of adult chicken duodenum</td>
<td>19</td>
<td>20-63</td>
<td>54-66</td>
<td>0-7158</td>
<td>0-2121</td>
<td>0-5778</td>
<td>58-73</td>
</tr>
<tr>
<td>B. Grafts of adult chicken brain</td>
<td>28</td>
<td>23-26</td>
<td>17-49</td>
<td>0-5681</td>
<td>0-2026</td>
<td>0-6143</td>
<td>58-60</td>
</tr>
<tr>
<td>C. Grafts of adult rat duodenum</td>
<td>21</td>
<td>22-58</td>
<td>9-89</td>
<td>0-5267</td>
<td>0-1590</td>
<td>0-5700</td>
<td>62-93</td>
</tr>
<tr>
<td>D. Sham operation</td>
<td>26</td>
<td>22-53</td>
<td>9-83</td>
<td>0-5142</td>
<td>0-1546</td>
<td>0-5692</td>
<td>55-77</td>
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<tr>
<td>Statistical F test</td>
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<tr>
<td>Statistical multiple range test</td>
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<tr>
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<td>(BC) (CD)</td>
<td>(AB) (CD)</td>
<td>(ABCD) (ABC) (ABD)</td>
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</tr>
</tbody>
</table>

† Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.

**Table 3**

**Fresh weights of whole embryos and of homologous and heterologous organs following chorioallantoic transplants**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Whole chick (g.)</th>
<th>Spleen (mg.)</th>
<th>Liver (g.)</th>
<th>Heart (g.)</th>
<th>Intestine (g.)</th>
<th>Duodenum (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Grafts of adult chicken duodenum</td>
<td>20</td>
<td>21-36</td>
<td>43-31</td>
<td>0-7280</td>
<td>0-2405</td>
<td>0-6285</td>
<td>64-72</td>
</tr>
<tr>
<td>B. Grafts of adult chicken lyophilized duodenum</td>
<td>38</td>
<td>23-16</td>
<td>13-89</td>
<td>0-5253</td>
<td>0-1861</td>
<td>0-5737</td>
<td>54-19</td>
</tr>
<tr>
<td>C. Sham operation</td>
<td>28</td>
<td>23-35</td>
<td>13-10</td>
<td>0-5393</td>
<td>0-1636</td>
<td>0-5925</td>
<td>64-06</td>
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<tr>
<td>Statistical F test</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Statistical multiple range test</td>
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<tr>
<td>(BC)</td>
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<td>(BC)</td>
</tr>
</tbody>
</table>

† Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.

It is evident on the basis of the multiple range test (Table 3) that, following adult duodenal grafts, the weight of the whole embryo was significantly less and
the weights of the spleen, liver, and heart were significantly more than after lyophilized duodenal grafts, as well as (in agreement with Table 1) more than in controls. On the other hand, when lyophilized duodenum was placed on the CAM the weight of the host heart was significantly higher, while the duodenum was significantly lower than in controls.

Table 4 again confirms the effects of a graft of adult chicken duodenum. Following grafts of either heated or alcohol-extracted chicken duodenum there were no significant weight differences from controls in either the whole embryo or the individual organs.

**Table 4**

_Fresh weights of whole embryos and of homologous and heterologous organs following chorioallantoic transplants_

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean weight of host and host organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole chick</td>
</tr>
<tr>
<td>A. Grafts of adult chicken duodenum</td>
<td>34</td>
</tr>
<tr>
<td>B. Grafts of heated adult chicken duodenum</td>
<td>30</td>
</tr>
<tr>
<td>(80°C for 20 minutes)</td>
<td></td>
</tr>
<tr>
<td>C. Grafts of alcohol extract of adult chicken duodenum</td>
<td>36</td>
</tr>
<tr>
<td>D. Sham operation</td>
<td>27</td>
</tr>
<tr>
<td>Statistical F test</td>
<td></td>
</tr>
<tr>
<td>Statistical multiple range test1</td>
<td></td>
</tr>
</tbody>
</table>

1 Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.

**Table 5**

_Fresh weights of whole embryos and of homologous and heterologous organs following chorioallantoic transplants_

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean weight of host and host organs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole chick</td>
</tr>
<tr>
<td>A. Grafts of adult chicken heart</td>
<td>19</td>
</tr>
<tr>
<td>B. Grafts of adult chicken skin</td>
<td>25</td>
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<tr>
<td>C. Sham operation</td>
<td>35</td>
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<td>Statistical F test</td>
<td></td>
</tr>
<tr>
<td>Statistical multiple range test1</td>
<td></td>
</tr>
</tbody>
</table>

1 Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.
Table 5 shows that there was no significant difference from controls in the weights of whole chicks or of individual organs following heart grafts. Grafting of skin significantly increases weight of spleen, liver, and duodenum as compared with controls.

The question naturally arises as to whether enlargement of host embryonic organs following grafts of adult tissue was dependent on an increase in protoplasm or was merely a manifestation of oedema. The analysis in Table 6 indicates that the enlargement was due to an increase in dry weight, both fresh and dry weights of the spleen and heart being significantly larger following spleen grafts.

Table 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean weight of host organs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Spleen fresh (mg.)</td>
</tr>
<tr>
<td>A. Gifts of adult chicken duodenum</td>
<td>25</td>
<td>32-65</td>
</tr>
<tr>
<td>B. Gifts of adult chicken spleen</td>
<td>25</td>
<td>24-04</td>
</tr>
<tr>
<td>C. Sham operation Statistical F test</td>
<td>28</td>
<td>13-37</td>
</tr>
<tr>
<td>Statistical multiple range test1</td>
<td></td>
<td>21-69*</td>
</tr>
</tbody>
</table>

1 Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.

and duodenal grafts. Moreover, following duodenal grafts the fresh and dry weights of the liver were significantly increased. The dry weight of the duodenum was not significantly different from the controls after either duodenal or spleen grafts. Duodenal grafts produced a greater weight increase of host organs than did spleen grafts.

Tables 1–3 show that the weight of the whole chick following duodenal grafts was lower than that of control embryos, and that the weight of the duodenum after duodenal grafts was not significantly different from that of controls. The question arises as to whether the relative weights of duodena following duodenal grafts are significantly higher than those of the controls. This was tested by covariance analysis of the data in Table 1. An $F$ value, which measures the difference between the two sample regression coefficients, was found to be 6·6 with df. = 1, 51, which, at the 95 per cent. level, is significant. This clearly indicates that the weight of the duodenum following duodenal grafts is significantly larger than in the controls when allowance is made for the difference in body-weight.
Since transplantation of adult tissue to the CAM induces profound changes in various organs of the embryo, the question arises as to whether or not grafts of embryonic tissue would have the same effect. Since the adult duodenum was the most effective stimulant for host tissues, duodenal from various ages of embryos were transplanted to the CAM.

The multiple range test in Table 7 shows that the weight of the whole chick following grafts of 16-day embryonic duodenum is significantly higher than the weight of embryos following grafts of 18- and 20-day duodena but is not signi-

**Table 7**

*Fresh weights of whole embryos and of homologous and heterologous organs following chorioallantoic transplants*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Whole chick (g.)</th>
<th>Spleen (mg.)</th>
<th>Liver (g.)</th>
<th>Heart (g.)</th>
<th>Intestine (g.)</th>
<th>Duodenum (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Grafts of 16-day chick embryo duodenum</td>
<td>24</td>
<td>26-13</td>
<td>13-47</td>
<td>0-5908</td>
<td>0-1817</td>
<td>0-6613</td>
<td>74-65</td>
</tr>
<tr>
<td>B. Grafts of 18-day chick embryo duodenum</td>
<td>24</td>
<td>24-66</td>
<td>13-05</td>
<td>0-5717</td>
<td>0-1796</td>
<td>0-6658</td>
<td>67-70</td>
</tr>
<tr>
<td>C. Grafts of 20-day chick embryo duodenum</td>
<td>37</td>
<td>24-86</td>
<td>12-72</td>
<td>0-5630</td>
<td>0-1768</td>
<td>0-6286</td>
<td>60-88</td>
</tr>
<tr>
<td>D. Sham operation</td>
<td>22</td>
<td>25-56</td>
<td>11-62</td>
<td>0-5577</td>
<td>0-1709</td>
<td>0-6541</td>
<td>71-39</td>
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<td>Statistical F test</td>
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<td>Statistical multiple range test</td>
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</table>

1 Any two means appearing together within the same parentheses are not significantly different at the 5 per cent. level.

significantly different from control experiments. However, the weight of the whole chick following 18-day embryonic duodenal grafts is significantly less than that of the controls. Further, compared with the controls, the weights of the spleen, liver, heart, and intestine are not significantly higher following grafts of 16-, 18-, or 20-day embryonic duodena. However, after grafting 20-day chick embryonic duodenum the weight of the host duodenum is significantly decreased, but it is not significantly different from controls after grafting 18- and 16-day duodena.

*The effect of extracts of adult and embryonic chicken organs on the host embryo when injected into the yolk sac*

The supernatant of homogenates of 0.1 ml. of adult chicken duodenum, liver, and heart were injected into the yolk sac of 96-hour embryos; controls were injected with 0.1 ml. of 0.15 M saline. A mortality rate of 100 per cent. followed the injection of supernatant of homogenized duodena. The time of death varied
with the amount of the material injected. The results of this experiment are summarized in Table 8.

Table 8

The effect of soluble antigens on the host embryo when injected into the yolk sac

<table>
<thead>
<tr>
<th>Amount (mg. wet wt.)</th>
<th>Time of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>36-48</td>
</tr>
<tr>
<td>7.7</td>
<td>48-60</td>
</tr>
<tr>
<td>6.7</td>
<td>56-72</td>
</tr>
</tbody>
</table>

Similar results, i.e. the death of all embryos, were observed following the injection of adult chicken liver and heart and 20-day embryonic duodenal supernatant. This mortality could not be attributed to pressure because the control embryos survived until the 20th day of incubation, at which time they were harvested. The supernatant and yolk of eggs which had just died were streaked on blood-agar plates. No bacterial colonies were found on these plates so death could not be attributed to infection.

Morphology of chick embryos and various homologous and heterologous organs after chorioallantoic transplantation of adult and embryonic chicken tissues

Whole embryo. It was observed that embryos harvested on the 18th day of incubation after grafting of adult chicken duodenum were small and appeared pale. When adult chicken spleen had been transplanted a few chicks appeared pale, but none as pale as after duodenal transplants. All embryos appeared normal in the controls and after grafts of adult chicken liver, heart, brain, skin, the variously treated duodena, and embryonic duodenum.

Duodenum. The gross morphology of the duodenum appeared essentially the same following transplants of adult and embryonic chick tissue and after control procedures, but the microscopic structure of the duodenum was somewhat altered following grafting of adult duodenal tissues (Plate, fig. A). Following grafting of adult duodenum, the connective tissue within the villi consisted of a compact mesenchymal layer until the 15th day of incubation, and this was succeeded by the lamina propria mucosae on the 16th day. Van Alten & Fennell (1957) identified the lamina propria mucosae on the 17th day under normal conditions. Further, Van Alten (1955) found goblet cells first on the 17th day of incubation in untreated embryos; however, following adult duodenal grafts, goblet cells were identified in great abundance on the 16th day. These observations indicate that duodenal grafts accelerated differentiation of the duodenal tissues of the host embryo.

After grafting either adult chicken duodenum or spleen there was an infiltra-
tion of lymphocytes into the duodenal tissue, but this was not observed following liver, heart, brain, skin, or embryonic duodenal grafts (Plate, fig. B).

It is evident from the results summarized in Table 9 that transplantation of adult duodenum to the CAM accelerates the histochemical differentiation of both connective tissues and goblet cells in the embryonic duodenum.

**Table 9**

*The differentiation of polysaccharides in duodenal tissues following CAM adult duodenal grafts*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age of embryo in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle connective tissue</td>
<td>Lamina propria mucosae</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
</tr>
<tr>
<td>Adult chicken duodenum</td>
<td>13</td>
</tr>
</tbody>
</table>

All tissues were stained by the Hotchkiss–McManus PAS reaction.

*Spleen.* Spleens of host organs following duodenal grafts were markedly enlarged with a diameter of about 10 mm., the maximum in control embryos being 2 mm. The surface of enlarged spleens exhibited numerous white tumour-like protuberances. Essentially the same type of reaction occurred following spleen and skin grafts. On the other hand, spleens of control chicks, and host spleens after grafts of heart, liver, inactivated duodenum, and embryonic duodena (15-, 16-, 17-, 18-, and 20-day embryos), were spherical and dark reddish brown in colour.

In sections of enlarged spleens of 18-day embryos which had received CAM grafts of adult chicken duodenum, spleen or skin, marked changes were observed (Plate, fig. D). There were fewer venous sinuses than in the control spleens (Plate, fig. C). Within the pulpa there were few reticular cells, i.e. mesenchymal cells, and many haemocytoblasts were observed. Large nodular areas (as many as six in a single section) filled with granulocytes were walled off by reticuloendothelial cells, and multinucleated giant cells were found to be quite numerous and uniformly distributed throughout these enlarged spleens. In contrast to control spleens, which contained many erythrocytes within the venous sinuses, very few erythrocytes were observed in enlarged spleens.

*Liver.* On the 18th day of incubation the liver exhibited what Kingsbury *et al.* (1956) described as a deep sulphur-yellow colour. This was essentially the appearance of the liver following CAM transplants of adult chicken spleen, liver, heart, brain, inactivated (by heat, lyophilization, or alcohol) duodenum, and embryonic duodenum. Following grafts of adult skin, the liver of host embryos (18th day of incubation) was buff coloured, with small red spots of about 2 mm. in diameter uniformly distributed over the entire surface. Following
CAM grafts of adult chicken duodenum the host livers were a bile-green colour and friable, and usually exhibited several relatively large grey areas.

By the 18th day of incubation, in both control and treated embryos, lymphocytes existed as small nodules in the connective tissues. Microscopic examination of the enlarged green livers revealed that from a histological point of view they were essentially similar to those of control embryos. This appears rather striking in the light of the gross changes which were observed.

Heart. The heart of 18-day embryos following CAM grafts of adult duodenum was pale pinkish-grey in colour and had a length in excess of 10 mm. Hearts of control embryos of the same age had a maximum length of approximately 10 mm. and a bright pink colour. Following grafts of various tissues (adult duodenum, spleen, liver, heart, skin, brain, and embryonic duodenum) the microscopic structure of the hearts of host embryos appeared to be essentially similar to that of control embryos.

Chorioallantoic membrane. The grafting of small pieces of either adult duodenum or spleen to the CAM induced the development of numerous small tumour-like masses which were sufficiently extensive to cover most of the CAM. Following the grafting of heart tissues to the CAM haemorrhagic regions were frequently observed adjacent to or near the grafted tissue on the 18th day of incubation.

A microscopic examination of the CAM adjacent to the site of grafts of adult duodenum, spleen, liver, heart, and skin, exhibited an abundance of granuloblasts and granulocytes. Many basophilic cells were also observed there and these cells were identified as haemocytoblasts. In almost every instance there were foci of grafted tissue which had not survived and these appeared as an acidophilic mass with numerous basophilic granules (Plate, fig. F).

When embryonic duodenum from 15-, 16-, 17-, 18-, and 20-day chicks was transplanted to the CAM the integrity of the graft tissue was not destroyed; the mucosa, lamina propria, submucosa, and tunica muscularis were identifiable. The epithelium of the mucosa overlying the villi consisted of simple columnar epithelium with numerous functional goblet cells (Plate, fig. E).

**DISCUSSION**

The data presented above demonstrate that profound changes occurred in the chick embryo after grafting adult chicken tissues to the CAM. The host spleen was enlarged following grafts of adult spleen, duodenum, brain, and skin. The host liver was enlarged following transplants of adult liver, spleen, and duodenum, while the heart was enlarged following spleen, liver, and duodenal grafts. In all cases the adult duodenum produced the most pronounced enlargement of the embryonic organs studied. Enlargement of the host duodenum following grafts of adult duodenum could be demonstrated only on a relative basis; the weight of the whole embryo was less than that of the controls, while the weight of the duodenum was about the same.
Histological studies of the enlarged spleen showed that the enlargement was primarily caused by granulopoiesis regardless of which adult organ (spleen, duodenum, skin, or brain) was used to stimulate it. Although the gross appearance of the host liver (it was bile-green in colour) and of the heart (it was enlarged and pale) was altered following CAM grafts of adult chicken duodenum, the microscopic anatomy was similar to that of the controls.

The hypothesis of organ-specific growth stimulation as proposed by Weiss (1947) and Ebert (1955) has been based largely on the observations of Murphy (1916), Danchakoff (1916), Willier (1924), Weiss (1947), and Ebert (1951). These workers found that when a small fragment of an adult chicken organ was transplanted to the vascular bed of an embryo (either CAM or vascular area of the blastoderm) it greatly stimulated growth of the homologous embryonic organs. Weiss & Andres (1952) observed an increase in the mitotic rate of the embryonic kidney after injections of kidney brei into the CAM blood-vessels, and also in the mitotic rate of the kidney and liver following injections of mesonephric brei (Andres, 1955).

Observations made during the course of this study do not entirely support the hypothesis of organ-specific growth stimulation. Van Alten (1959) also observed that the antigens prepared from spleens stimulated by adult duodenal grafts exhibited a different antigenic pattern than control spleens, and that antigens prepared from duodena stimulated by adult spleen grafts exhibited a different antigenic pattern from control duodena. Danchakoff (1918) observed that enlargement after CAM transplants of adult spleen was not confined to the spleen but extended throughout the whole mesenchyme of the host. Andres's (1955) studies were not organ-specific after injection of mesonephric brei. He observed that mitotic indices of the liver increased 23 per cent, while the mesonephros increased 46 per cent. However, Levy (1956) was unable to demonstrate the retrogression of the mesonephros after CAM grafts of 18-day embryonic mesonephros or metanephros which would be expected if organ-specific growth stimulation had occurred. Also, Wilson & Leduc (1947) have demonstrated that a number of agents (pulped liver of mice and guinea-pigs, boiled and autolysed liver, pulped kidney, and boiled egg-yolk) produced an increase in the mitotic rates in mouse livers on the 5th day after injection. Further, Saetren (1956) has observed that after injections of macerated homologous tissue into the peritoneal cavity of rats following partial nephrectomy or removal of a portion of the liver, there was a marked inhibition of mitoses in regenerating portions of kidney and liver. Steuart (see Ebert, 1958b) has confirmed this observation on the kidney and, further, has shown that liver homogenate suppresses the mitotic activity in the remaining kidney stump but that kidney homogenate is more specific. However, it should be kept in mind that the systems studied by Andres and those of Wilson & Leduc, Saetren, and Steuart are quite different.

The tracer studies of Ebert (1954) showed that when labelled tissues were placed on the CAM, homologous organs had a higher specific activity than
heterologous organs. However, Horn & House (1955) observed that when they injected tagged homogenates of liver, kidney, spleen, and thymus into young mice the uptake value of the spleen was consistently higher than other organs. They suggested that the spleen was the most effective organ of the reticulo-endothelial system for removing foreign protein from the circulation.

An alternative hypothesis of graft-versus-host reaction was proposed by Simonsen (1957) to account for the enlargement of embryonic spleens following grafts of adult spleen. Billingham et al. (1956) observed a 95 per cent. mortality of chick embryos following injection of adult blood. Death occurred toward the end of incubation and was attributed to an infective agent. However, Simonsen (1957) and Cock & Simonsen (1958) believe that adult spleen or blood-cells can colonize host lymphoid organs, in which they multiply and react immunologically against the host. Recently, Terasaki (1959) has shown that when adult chicken lymphocytes were injected into chick embryos there was a marked splenic enlargement in the host. On the other hand, adult monocytes and thymocytes did not cause significant enlargement of the spleen. The generalized effects, i.e. enlargement of spleen, liver, and heart, observed in these studies following grafting of adult chicken duodenum may be accounted for on the basis that this organ contains much lymphocytic tissue and thus produces a graft-versus-host reaction. However, this hypothesis fails to give a satisfactory explanation as to why brain grafts, which contain very few lymphocytes, produced splenic enlargement while adult liver transplants, which contain many lymphocytes, did not elicit splenic enlargement. Further, the present study also shows that adult duodenal grafts, which contain fewer lymphocytes than spleen, elicited a greater enlargement of the host spleen than did splenic grafts. However, these results are in contrast to Ebert’s (1959), who obtained no splenic enlargement in the embryo after grafting of adult brain tissue and observed that adult liver grafts produced a marked stimulation (about 40 per cent. as much as adult spleen) of the embryonic spleen. The graft-versus-host hypothesis also fails to explain why the microscopic morphology of the adult grafted tissue was destroyed and replaced by a myeloid metaplastic centre, a type of reaction to the CAM which has also been observed by Van Haeften (1958) with cell-free material. Simonsen (1957), on the basis of direct injections of adult cells, postulates that enlargement of host spleens was due to colonization of the host organ by injected cells. Ebert (1954), on the basis of DNA content of the enlarged spleens, ruled out the transfer of whole cells. Recently, however, Ebert (1958a, b) has re-evaluated this question in the light of experiments involving serial transplantation of stimulated spleens; he observed no dilution of growth-promoting activity by serial passage. On the basis of this observation he proffers as the simplest explanation the colonization of the host spleen by whole cells from the donor, these cells being capable of reproduction. Nevertheless, he was not able to identify large populations of adult cells in the host spleen by histological analysis and thus is unable to rule out the possibility of subcellular material as the causative agent.
Recently, Van Haeften (1958) grafted a cell-free homogenate of adult spleen to
the CAM and observed hypertrophy of the host homologous organ. However,
Ebert (1958a) did not observe stimulation of the homologous organ with cell-free
material but only 'predominant localization'. By placing spleen grafts in mem-
brane filters, which prevent the passage of cells, Ebert (1958b) observed a
modest stimulation of the host spleen.

It was shown in the preceding paragraphs that both the organ-specific growth
stimulation and the graft-versus-host hypothesis fail to adequately explain the
results observed following grafts of adult duodenal tissue. It may be postulated
that the duodenum contains substances which are non-specific and cause various
organs to enlarge. Levi-Montalcini (1952) has observed enlargement of the
spleen and liver of the chick after transplantation of mouse sarcoma 37 or 180 to
the allantoic vesicle. The enlarged liver was described as 'deeply suffused with
bile'. Van Alten (1959) observed an increase in the number of antigens in both
the duodenum and spleen after grafts of adult duodenum. This would further
indicate that some sort of non-specific effect was being produced.

Heart enlargement was probably due to a compensatory reaction related to
the anaemic condition of the chicks. This anaemic condition probably was due
to the fact that the spleen was almost totally given over to the production of
granulocytes rather than erythrocytes. The grafts were made on the 9th day of
incubation, at which time Fennell (1947) observed that the definitive erythro-
cytes had replaced the primitive erythrocytes to become the most numerous type
in peripheral blood. Thus, spleen and liver enlargement may be due in part to
removal of primitive generation of blood-cells and foreign substances from the
blood vascular system. On the other hand, heart enlargement may be due, in
part, to compensation.

It was also observed in the course of this study that when either normal duo-
denum, liver, or heart were injected into the yolk sac on the 4th day of incuba-
tion, they caused all the embryos to die within 72 hours. This observation is in
keeping with that of Fennell (1947) who, using a much more dilute inoculum of
minced normal liver, observed that 67 per cent. of the embryos died within 5 days
after inoculation. He further observed that injections of normal liver-mince pro-
duced blood changes which meet the requirements for haemocytoblastosis.

SUMMARY

1. It was observed that following CAM grafts of adult chicken duodenum
there was a marked decrease in the absolute weight of the host, a marked
increase in the weight of the spleen, liver, and heart, and a relative weight in-
crease in the duodenum. Further, following grafts of adult skin and brain the
spleen and liver were significantly heavier. Following liver grafts the liver and
heart showed a significant increase in weight. Adult chicken spleen grafts caused
a marked increase in the weight of the spleen and heart. Further, it was observed
that, regardless of what tissue was used for grafting, 9 days later the morphological integrity of the graft was destroyed and the area was replaced by a myeloid metaplastic centre. On the other hand, embryonic duodena retained their integrity and continued to differentiate.

2. Grafting of adult duodenum caused acceleration of tissue differentiation of the host duodenum. The polysaccharides in the connective tissue and goblet cells of the duodenum differentiated at least 24 hours earlier than in control chicks. Following grafting of duodenum, spleen, and skin, the host spleen exhibited a marked increase in granuloblasts and granulocytes. The heart and liver following grafts were essentially like those in control embryos.

3. Treatment of the adult duodenum prior to grafting with either 95 per cent. alcohol for 24 hours at \(-20^\circ\text{C.}\), lyophilization, or heating at \(80^\circ\text{C.}\) for 20 minutes, resulted in inactivation, the host not being affected.

4. When duodena of 15-, 16-, 17-, 18-, and 20-day embryos were grafted to the CAM, no changes in the weight of host spleen, liver, or heart were found. Following 20-day duodenal grafts the weight of the duodenum was significantly decreased.

5. All embryos receiving adult duodenum, liver, and heart extracts into the yolk sac at 4 days of incubation, died within 72 hours after inoculation.

6. The results are discussed in the light of organ-specific growth stimulation and graft-versus-host reaction, both of which fail to adequately explain all the results observed.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Drs. A. S. Fox and J. R. Shaver for their help and many valuable suggestions, and to Dr. P. J. Clark for his help with the statistical analysis. We are also grateful to Dr. J. D. Ebert, Department of Embryology, Carnegie Institution of Washington, for a critical discussion of the manuscript. We also wish to thank Mr. P. G. Coleman, photographer, Agricultural Experiment Station, Michigan State University, for the photomicrographs.

The material reported in this paper is part of a thesis submitted by P. J. Van Alten to the Department of Zoology, Michigan State University, in partial fulfillment of the requirements for the Ph.D. degree, and was carried out during the tenure of a pre-doctoral fellowship (CF 6731), Cancer Division, Public Health Service.

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EXPLANATION OF PLATE

FIG. A. Photomicrograph of a cross-section through the duodenum of a control 18-day embryo. 1. epithelium; 2. goblet cell; 3. lamina propria; 4. tunica muscularis; 5. blood-vessel.

FIG. B. Photomicrograph of a cross-section through the duodenum of an 18-day embryo following adult chicken duodenal grafts. 1. epithelium; 2. goblet cell; 3. lamina propria; 4. tunica muscularis; 5. blood-vessel; 6. lymphocytes.

FIG. C. Photomicrograph of a cross-section through a spleen of a control 18-day embryo showing the reticular structure.

FIG. D. Photomicrograph of a cross-section through a spleen of an 18-day embryo following adult duodenal grafts. 7. granulocytic nodule surrounded by multinucleated giant cells.

FIG. E. Photomicrograph of a cross-section of the CAM through a 15-day embryonic duodenal graft on the 18th day of incubation. 2. goblet cell; 3. lamina propria; 4. tunica muscularis; 10. chorioallantoic membrane.

FIG. F. Photomicrograph of a cross-section of the CAM through an adult duodenal graft on the 18th day of incubation. 8. foci of degenerating adult duodenal tissue surrounded by invading lymphocytes and granulocytes.

All sections were stained by the Himes Moriber triple stain. Micrometer scale insert: 1 division = 0.01 mm.

(Manuscript received 23:ii:59)