On the pars distalis hormone activities involved in spleen development in chicken embryos

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SUMMARY

Spleen development was studied in 11 groups of chicken embryos: (1) intact, I; (2) ‘hypophysectomized’, H, by partial decapitation, and nine groups of ‘hypophysectomized’ embryos in which each host received a specific type of pars distalis graft; (3) one cephalic region, HCe; (4) one middle region, HM; (5) one caudal region, HCa; (6) the entire pars distalis, HE; (7) two cephalic regions, H2Ce; (8) two middle regions, H2M; (9) two caudal regions, H2Ca; (10) one cephalic and one caudal region, HCeCa; or (11) one cephalic, one middle and one caudal region, HCeMCa. Embryos were killed at 20.5 days of incubation. Levels of spleen development were assessed by changes in mean weight and histogenesis.

Spleen development in ‘hypophysectomized’ embryos was normal only with cephalic and middle region pars distalis grafts but not with caudal grafts. Grafts of entire pars distalis or of two cephalic regions were no more effective than single cephalic regions in correcting development. Double caudal region grafts were as ineffective as single caudal region grafts. Apparently there are qualitative differences between pars distalis regions. Cephalic and middle grafts probably produced sufficient ACTH and TSH activities to stimulate adequate levels of adrenocorticoids and thyroid hormone and perhaps prolactin to repair spleen development. These may be the only hormones important for spleen development, because caudal graft ineffectiveness suggests that STH is unnecessary and may not be involved.

INTRODUCTION

Inhibition of red pulp by the pars distalis is essential for spleen histogenesis in chicken embryos (Betz, 1970; Goldberg, 1971; Goldberg & Betz, 1973). The pars distalis–adrenal axis seems to be involved (see Goldberg, 1971), and adrenocorticoids may act alone or perhaps in conjunction with other hormones in spleen development.

We used the regional grafting procedure of Brasch & Betz (1971) to analyse more precisely the pars distalis hormone activities important for spleen development in chicken embryos. This paper reports those results.

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MATERIALS AND METHODS

Fertilized White Leghorn eggs (Hyline 934F) were incubated at 39 ± 0.5 °C and 60 ± 5% relative humidity and some embryos were 'hypophysectomized' by partial decapitation as described before (Hart & Betz, 1972a). There were 11 experimental groups of embryos: (1) intact, I; (2) 'hypophysectomized', H; and nine groups of 'hypophysectomized' embryos in which each host received one kind of pars distalis graft – (3) one cephalic region, HCe; (4) one middle region, HM; (5) one caudal region, HCa; (6) the entire pars distalis, HE; (7) two cephalic regions, H2Ce; (8) two middle regions, H2M; (9) two caudal regions, H2Ca; (10) one cephalic and one caudal region, HCeCa; or (11) one cephalic, one middle and one caudal region, HCeMCA. Pars distales were dissected from 19-day-old donor embryos. Glands were either crushed (for entire pars distalis grafts) or cut into cephalic, middle and caudal region pieces just as described by Brasch & Betz (1971). Each piece was washed in two changes of balanced salt solution (Rugh, 1962). For composite grafts, separate washed regional pieces were crushed together and then placed as single grafts. Blotted tissues were transferred to the chorioallantois of 10-5-day-old 'hypophysectomized' hosts.

Grafts, collected and fixed at the end of 20-5 days of incubation had normal appearing cells (Hart & Betz, 1972b). All embryos were killed after 20-5 days of incubation (= 492 h + 3 for thermal equilibration). We used only viable embryos. In operated embryos with patent and non-patent esophagi (Betz, 1968; Hart & Betz, 1972b), absence of the pars distalis was insured by using specimens without upper beak rudiments. The yolk sac was removed. Embryos were decapitated between cervical vertebrae 2 and 3 and fresh, headless body weights were determined (nearest 0.1 g). The abdominal wall was slit, the bodies were fixed in Bouin's fluid, decolorized and stored in 70% ethanol. Fixed spleens were dissected, blotted and weighed (nearest 0.1 mg). Group means ± standard errors were calculated for absolute and relative spleen weights and compared by Duncan's (1955) new multiple range test at the 0.05 level of significance. Representative spleens were prepared with an ordinary paraffin technique, sectioned at approx. 8 μm and stained with hematoxylin. We photographed representative sections with Adox KB 14 film.

RESULTS

There was a significant splenomegaly both absolute and relative to body weight (Table 1) in groups without grafts (2. H), and with single or double caudal region grafts (5. HCa, and 9. H2Ca); this was accompanied by hyperplasia of red pulp and reduction in number and definition of white-pulp follicles (Figs. 1–4). Spleens from all other grafted groups receiving some cephalic region cells were indistinguishable from those of intact embryos (1. I).
Table 1. Weight (mg) of fixed spleens from 20-5-day-old intact and 'hypophysectomized' chicken embryos with and without single or composite, entire or regional pars distalis grafts

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen weight</th>
<th>Spleen weight</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (H)</td>
<td>5.8 ± 0.4 (15)*</td>
<td>0.3 ± 0.02 (15)</td>
<td>(a)**</td>
</tr>
<tr>
<td>2. (H)</td>
<td>11.5 ± 0.8 (11)</td>
<td>0.8 ± 0.05 (11)</td>
<td>(cd)</td>
</tr>
<tr>
<td>3. (H_{Ce})</td>
<td>7.3 ± 0.8 (10)</td>
<td>0.5 ± 0.06 (10)</td>
<td>(ab)</td>
</tr>
<tr>
<td>4. (H_{M})</td>
<td>9.2 ± 1.0 (12)</td>
<td>0.6 ± 0.07 (12)</td>
<td>(bc)</td>
</tr>
<tr>
<td>5. (H_{Ca})</td>
<td>12.8 ± 1.1 (9)</td>
<td>1.1 ± 0.09 (9)</td>
<td>(c)</td>
</tr>
<tr>
<td>6. (H_{E})</td>
<td>6.4 ± 0.7 (9)</td>
<td>0.5 ± 0.07 (9)</td>
<td>(ab)</td>
</tr>
<tr>
<td>7. (H_{2Ce})</td>
<td>8.3 ± 1.3 (9)</td>
<td>0.6 ± 0.08 (9)</td>
<td>(bc)</td>
</tr>
<tr>
<td>8. (H_{2M})</td>
<td>8.9 ± 1.5 (7)</td>
<td>0.6 ± 0.06 (7)</td>
<td>(bc)</td>
</tr>
<tr>
<td>9. (H_{2Ca})</td>
<td>12.3 ± 1.7 (8)</td>
<td>1.0 ± 0.17 (8)</td>
<td>(de)</td>
</tr>
<tr>
<td>10. (H_{CeCa})</td>
<td>7.6 ± 1.1 (5)</td>
<td>0.6 ± 0.07 (5)</td>
<td>(bc)</td>
</tr>
<tr>
<td>11. (-H_{CeMCa})</td>
<td>8.8 ± 1.5 (5)</td>
<td>0.7 ± 0.09 (5)</td>
<td>(bc)</td>
</tr>
</tbody>
</table>

* Mean ± standard error; (number of samples).
** Values with same letters are not significantly different at 0.05 level in Duncan’s (1955) multiple range test.
† See Materials and Methods section for treatments.

The results suggest that the cephalic region may inhibit red-pulp proliferation and support differentiation of white-pulp follicles. Also, the cephalic and caudal regions appear to have qualitatively different activities in influencing spleen development.

**DISCUSSION**

Most effects of partial decapitation seem equivalent to adenohypophysectomy (Betz, 1971; Brasch & Betz, 1971; Hart & Betz, 1972a). Intact pars distalis chorioallantoic grafts from 10-day donors to 9-day 'hypophysectomized' hosts correct spleen development (Betz, 1970). These kinds of grafts begin to be effective at least by 17.5 days of incubation (Goldberg & Betz, 1973), and near normal development is attained by 20-5 days. Thus, grafts as sources of hormones may duplicate conditions in intact embryos and are probably more appropriate, biologically (Hart & Betz, 1972a), than heterospecific exogenous trophic hormone treatments. Cephalic and caudal-region grafts contain normal-appearing cells associated with different trophic hormone activities – the cephalic with ACTH and TSH; the caudal with STH; and perhaps both regions with prolactin (Brasch & Betz, 1971; Hart & Betz, 1972b). We have not considered MSH or the gonadotrophins as there is no evidence concerning their participation in spleen development except for the greater sensitivity of female spleens in graft-versus-host reactions and in splenomegaly in response to 'hypophysectomy' (see Goldberg & Betz, 1973). We assume cephalic cells are responsible for middle-region-graft effectiveness.
Splenic histogenesis in 'hypophysectomized' embryos was modified and splenomegaly developed in both absolute or relative terms in agreement with previous data (Betz, 1970). Evidence suggesting that splenic changes in 'hypophysectomized' chicken embryos occur because of the absence of ACTH stimulation of sufficient levels of adrenocorticoids to inhibit splenomegaly is reviewed by Goldberg (1971) and Goldberg & Betz (1973). Cephalic and middle-region pars distalis grafts repair adrenal and thyroid gland histogenesis but caudal region grafts do not (Brasch & Betz, 1971). In our results cephalic and middle-region grafts repaired spleen development in operated embryos. Perhaps spleen development in chicken embryos is controlled by adrenocorticoids, but our results cannot exclude the participation of ACTH, TSH or thyroid hormone. Embryos with higher levels of duodenal alkaline phosphatase specific activity (Hart & Betz, 1972a, b) usually had smaller spleens and improved white-pulp follicular development. If enzyme activity levels reflect adrenocorticoid titers then this may also implicate adrenocorticoids in spleen development.

A presumably specific tadpole bioassay for STH (Enemar, 1967) showed confinement of a growth-promoting activity by 15 days of incubation to the caudal region of the chicken-embryo pars distalis. This suggests STH production by caudal cells only. If so, the ineffectiveness of caudal region grafts in supporting splenic development indicates the non-essentiality of STH. Betz (1971), Brasch & Betz (1971) and Hart & Betz (1972a, b) discuss some problems of STH ontogenesis in chicken embryos. Prolactin has some STH-like activities in birds (see Hart & Betz, 1972a) but its production in either region of the embryonic chicken pars distalis has not been verified. In post-hatching stages both gland regions may secrete prolactin. Participation of this hormone in spleen development has not been settled because our results cannot exclude the possibility of prolactin contributing to cephalic region graft effectiveness in correcting spleen development.

**Figures 1-4**

Spleen histogenesis in 11 groups of chicken embryos at 20-5 days of incubation. Bouin's fixed, paraffin sections approx. 8 µm, stain hematoxylin. Scale line (Fig. 1) equals 125 µm, but 588 µm in lower magnification insets.

Fig. 1. Representative of spleens from intact embryos (group 1). Light grey foci are white-pulp follicles surrounded by dark grey red pulp.

Fig. 2. Representative of spleens from 'hypophysectomized' embryos which were ungrafted (group 2) or given single or double caudal region pars distalis chorioallantoic grafts (groups 5, 9). Enlargement due to red-pulp hyperplasia. No obvious white-pulp follicles.

Figs. 3, 4. Representative of spleens from 'hypophysectomized' embryos given pars distalis grafts containing cephalic region cells (groups 3, 4, 6-8, 10, 11), showing range of variation. Note repair of white-pulp follicles.
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REFERENCES


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