The developmental endocrinology of the spleen in chicken embryos

II. The pars distalis and splenomegaly

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\textbf{SUMMARY}

Pars distalis-extract treatment of intact or ‘hypophysectomized’ chicken embryos resulted in a splenomegaly, perhaps of a host-versus-graft nature, by 17 and 17.5 days of incubation. Pars distalis grafts in intact embryos reduced spleen size, encouraged white-pulp follicle formation, but ‘suppressed’ red-pulp development. Adult spleen extract and bovine serum albumin did not change spleen sizes or histology appreciably in intact embryos.

Perhaps pars distalis hormones inhibit red-pulp development or stimulate white-pulp follicles which may compete with red-pulp proliferation but encourage splenic vasculogenesis alone or by stimulating adrenocorticoid, thyroid, and other hormone levels while other extract factors cause splenomegaly.

\textbf{INTRODUCTION}

Partial decapitation of chicken embryos results in splenomegaly (82\% enlargement), with red-pulp hyperplasia but retarded white-pulp follicle development and vasculogenesis by 20 days of incubation (Betz, 1970). Single pars distalis grafts restore splenic histogenesis and size (growth?) in ‘hypophysectomized’ chicken embryos, but one or two additional grafts are not different in effect (Betz, 1970; Goldberg, 1971). Perhaps in reducing presumed adrenocorticoid inhibition ‘hypophysectomy’ amplifies splenomegaly (Betz, 1970).

Splenomegaly is also a response to chorioallantoic grafts of adult spleen with mesenchymal differentiation of lymphoid hemocytoblasts to form erythroblasts and inhibition of sinus, capillary and reticular development (see DeLanney, Ebert, Coffman & Mun, 1962). Follicle tissue differentiates as lymphoid hemocytoblasts forming lymphocytes but in red pulp these cells form granular leucocytes. Graft leucocytes produce antibodies against host proteins causing a graft-versus-host (GVH) response. Enlargement involves immunologically competent graft-cell colonization of host spleens, antibody damage, and a host-versus-graft (HVG) response of premature immunological competence and host spleen cell proliferation (Simonsen, 1957; DeLanney \textit{et al.} 1962; Mun & Burns, 1962).
Splenomegaly by ‘hypophysectomy’ and HVG are histologically distinct. Spleens of ‘hypophysectomized’ embryos still respond to adult spleen grafts (Ebert, 1957), showing the pars distalis is unnecessary for HVG responses.

We studied some effects of pars distalis extract and grafts, adult spleen extract and bovine serum albumin (BSA), on splenic development in chicken embryos to attempt separation of responses to protein hormones and other factors. Pars distalis extract should have hormonally active and inactive proteins. Pars distalis grafts should release only protein hormones. BSA should be only hormonally inactive protein and adult spleen extract should be a potent HVG inducer. Juvenile pars distalis extract was included for comparison.

MATERIALS AND METHODS

We analysed splenic responses to pars distalis extract and grafts in chicken embryos from previous studies (Goldberg, 1971), and in intact embryos treated with BSA and adult spleen extract.

Fertilized White Leghorn eggs (Hyline 934 F), selected at random, were incubated at 39 ± 0.5 °C and 60 ± 5% relative humidity and some embryos were ‘hypophysectomized’ by partial decapitation (see Goldberg, 1971) at stage 10 or 11 of Hamburger & Hamilton (1951). Shells were ‘windowed’ on day 2 of incubation for access to the chorioallantois for treatment as before.

There were four experimental groups. In group 1, killed at day 17-5 of incubation, intact embryos received either 0.2 ml saline or 2.5, 5, or 10 gland equivalents (GE) of adult pars distalis extract in 0.2 ml saline daily between days 12.5 and 16.5 of incubation. This group was repeated once. A small group similarly treated with 5 and 10 GE cockerel pars distalis extract responded identically so data were pooled. In group 2, killed at day 17, both intact and ‘hypophysectomized’ embryos received saline, or 5 or 10 GE of extract (same regime), between days 12 and 16. One GE was the extract from one adult or two cockerel partes distales. Unhealthy embryos were omitted before treatments. In group 3, each intact embryo received three chorioallantoic grafts of embryonic chicken partes distales – two 19-day-old glands at 9.5 days of incubation and one 20-day-old gland at 10.5 days as before (Betz, 1967). From one to three grafts were found at autopsy at 17.5 days of incubation. Data were pooled because responses to grafts were indistinguishable. Treatments did not appreciably affect mortality.

Pars distalis extract preparation from frozen broiler chickens and fresh 1-day-old cockerels is as described by Goldberg (1971). These extracts in equivalent concentrations were not different in effect. In group 4, killed at day 17 of incubation, spleens from 11.5-month-old White Leghorn hens (PB-58) were frozen, lyophilized, and saline extracted exactly as described for pars distalis tissue by Goldberg (1971). Spleen extract and BSA (fraction V, Nutritional Biochemicals) were given in amounts equal to the protein added in pars distalis extract treatment (7 mg), using a duplicate regime.
Developmental endocrinology of the spleen

In all embryos decapitated between cervical vertebrae 5 and 6 the abdomen was opened, sex recorded and bodies fixed in Bouin's fluid, decolorized in 70% ethanol saturated with lithium carbonate, and stored in 70% ethanol (Betz, 1967). Fixed spleens were dissected in 70% ethanol, blotted and weighed (nearest 0.1 mg), then dehydrated in tertiary butyl alcohol and ethanol, embedded in paraffin in vacuo, sectioned at approx. 8 µm, mounted on slides, deparaffinized in xylol, hydrated in ethanol, stained with H & E, PAS-hematoxylin, or eosin Y-orange G and toluidine blue (modified Dominici stain fide Gray, 1954). Sections stained with H & E or PAS-hematoxylin were dehydrated in ethanol, cleared in xylol, and mounted in Technicon. Sections stained in Dominici stain were mounted directly in Kaiser's glycerol jelly (Humason, 1967) to preserve metachromacy. Representative sections were photographed with Adox KB 14 film.

Means and standard deviations were calculated and means were compared by Duncan's (1955) test at the 1 and 5% levels of significance.

RESULTS

Spleens in pars distalis extract-treated intact embryos by 17.5 days of incubation tended to be significantly heavier and enlarged mostly by red-pulp proliferation. Perhaps a slight increase in the proportion of white pulp occurs (Table 1, Figs. 1–5). Pars distalis grafts in intact embryos significantly reduced spleen weight and increased the number of small diffuse white-pulp follicles proportionately (Fig. 6). Splenomegaly was significant in pars distalis extract-treated females (14.3, s.d. = ± 4.7 mg), but males (9.7, s.d. = ± 2.3 mg) did not significantly exceed controls of both sexes (8.2, s.d. = ± 2.3); a problem for future analysis.

Spleens of untreated 'hypophysectomized' embryos were enlarged insignificantly. With increased extract doses, weights became significantly greater. Histologically, 'hypophysectomized' embryos were deficient in white pulp and vasculogenesis (Figs. 3, 4). Extract treatment improved white-pulp development and vasculogenesis but these parameters did not reach control levels, perhaps because of splenomegaly (Fig. 5). BSA treatment did not increase spleen weight significantly or alter histology. Adult spleen extract was ineffective in both respects.

DISCUSSION

Splenomegaly may result from 'hypophysectomy' or foreign proteins. A secondary HVG response can be stimulated by a cell-free extract of a variety of tissues (DeLanney et al. 1962). Microsomal fractions, apparently RNA (Sharon & Schwartz, 1970), can stimulate splenomegaly by 17 days of incubation. The pattern of response at 17 days, even with adult spleen grafts, usually does not result in necrotic foci characteristic of full reaction. HVG splenomegaly is also greater in females (Solomon, 1961). Enlargement of pars distalis extract-
Table 1. Mean fixed spleen weights (± s.d.) of intact and ‘hypophysectomized’ chicken embryos with pars distalis extract and grafts at about 17 days of incubation

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of specimens</th>
<th>Treatment</th>
<th>Spleen weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>Saline</td>
<td>9.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.5 GE†</td>
<td>12.2 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5 GE</td>
<td>17.5 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10 GE</td>
<td>12.2 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Saline</td>
<td>7.8 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.5 GE</td>
<td>8.4 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5 GE</td>
<td>10.6 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10 GE</td>
<td>8.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Saline</td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5 and 10 GE (cockerel extract)</td>
<td>14.0 ± 3.4*</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>Saline</td>
<td>9.2 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5 GE</td>
<td>14.0 ± 2.7*</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>10 GE</td>
<td>19.4 ± 7.7**</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Saline</td>
<td>7.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5 GE</td>
<td>10.2 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>10 GE</td>
<td>13.6 ± 4.7**</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Sham control</td>
<td>8.7 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Pars distalis grafts (1–3)</td>
<td>5.6 ± 2.3**</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>Saline</td>
<td>7.9 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Adult spleen extract</td>
<td>7.6 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Bovine serum albumin</td>
<td>9.3 ± 2.4</td>
</tr>
</tbody>
</table>

* Significantly different from control at 5 % level.
** Significantly different from control at 1 % level.
† 2.5 GE = gland equivalents of extract from 2.5 partes distales. Splenomegaly was significant in extract-treated females but males did not exceed controls of both sexes.

Figures 1–6

Spleen histology in chicken embryos from experimental groups 1, 2 and 3. Bouin’s fixed, paraffin sections approx. 8 μm, stain Dominici’s. Scale line (Fig. 1) equals 100 μm.

Fig. 1. Spleen from saline control embryos. White-pulp (arrow) development more than usual.

Fig. 2. Spleen from intact embryo given ten gland equivalents (GE) of pars distalis extract. White pulp increased.

Figs. 3, 4. Spleens from untreated ‘hypophysectomized’ embryos to show range of variation.

Fig. 5. Spleen from ‘hypophysectomized’ embryo given ten GE of extract. Slightly more small white-pulp follicles and improved vasculogenesis.

Fig. 6. Spleen from pars distalis-grafted intact embryo. Number of small diffuse white-pulp follicles (arrow) increased and vasculogenesis improved.
treated spleens in our study suggests a HVG type of response to some part of the extract in agreement with DeLanney et al. (1962). Trophin (ACTH) stimulation of adrenal cortices should reduce spleen size as cortisone abolishes HVG splenomegaly in rats and adrenalectomy enhances the onset and extent of reaction (Kieffer & Ketchel, 1971). Adrenalectomy stimulates spleen ‘growth’ in fetal rabbits (Bearn, 1967), as does ‘hypophysectomy’ in chicken embryos (Betz, 1970), presumably by reducing adrenocorticoid levels. The pars distalis–adrenal axis is apparently established by 14-5 days of incubation in chicken embryos (Woods, De Vries & Thommes, 1971). STH increases spleen growth in rats (Fast, Garland, Thomson & Richards, 1970), which is coincident with general growth stimulation. In pars distalis extract-treatments, ACTH may mask STH effects. Thus, hormone activities of extract should decrease spleen weight as indicated by pars distalis graft effects. Grafts may release protein hormones but probably few if any other kinds of proteins. Apparently protein trophic hormones do not also stimulate a HVG reaction. Spleen weight with BSA treatment does not help to support the hypothesis that non-hormonal extract components caused splenomegaly. All extracts producing splenomegaly had some blood. Thus, leucocytic proteins might be effective agents, as Simonsen (1957) indicated. But apparently adult spleen extract was ineffective in this respect for unknown reasons.

Splenomegaly after 1-day-old cockerel pars distalis extract-treatment is anomalous if the extract-produced splenomegaly and HGV reaction are related. Day-old cockerels should be immunologically incompetent (Billingham, Brent & Medawar, 1956), so extracts from them should not perhaps elicit HGV responses. Although enlargement of these spleens was not less than that produced by adult gland extract, it may be important because pars distalis grafts decreased spleen weight.

Our interpretation of extract hormonal histological effects is subjective and tentative, but appears consistent with maintenance of red–white pulp balance by ‘suppression’ of red-pulp proliferation or stimulation of white pulp, presumably by adrenocorticoids and/or other hormones. Perhaps white pulp inhibits red-pulp development. White-pulp development appears improved proportionately in spleens of pars distalis extract-treated intact and ‘hypophysectomized’ embryos as does vasculogenesis, but the factors involved are not known. These problems require further analysis.

Reasons for: similar responses to 5 and 10 GE cockerel-extract; to 1–3 grafts; insignificant splenomegaly after ‘hypophysectomy’ in contradiction of other data (Case, 1951 fide Betz, 1970); and inconsistent responses, between and within treatment groups, to pars distalis extract are unknown.

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