Behaviour of mouse primordial germ cells in the chick embryo

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SUMMARY

Hind guts of 9½-day mouse embryos were transplanted into the posterior part of the coelomic cavity of 2½-day chick embryos. The hosts were sacrificed after 1–7 days and the mouse primordial germ cells (PGCs) in the graft and in the surrounding host tissues were searched for by means of the histochemical technique for alkaline phosphatase. Altogether 94 grafts were examined.

During the first 3 days of intracoelomic development of the graft accumulations of mouse PGCs close to the mesonephros, the mesentery or the gonad of a chick embryo were observed in 26 out of 51 cases. In 12 grafts single PGCs crossed the boundary between the host and the graft and settled in host tissues such as the mesonephros, the mesentery or the gonad.

After 3 days mouse PGCs are no longer visible in the chick tissues. However, the number of PGCs in the grafts also gradually decreases and from the 4th day onwards many of the grafts contain no PGCs. The ability of mouse PGCs to survive extragonadally, even in the embryonic hind gut, is thus limited.

In some of the 4-to 7-day-old grafts PGCs occur on the periphery of the graft in the form of single aggregations. From the 6th day the only PGCs which survive are those in aggregations. The experiments indicate that the gonads, together with adjacent tissues (mesonephros, mesentery) of a chick embryo are attractive to mouse primordial germ cells and that the hypothetical attractive substance is not species specific.

INTRODUCTION

During the development of Amniota primordial germ cells (PGCs) appear extra-embryonically and reach the embryo either by the vascular route or by interstitial migration (for review, see Simon, 1960; Franchi, Mandl & Zuckerman 1962; Pasteels, 1962). If primordial germ cells are originally located anteriorly (which is characteristic of birds and some reptiles) their migration is accomplished passively through the blood vessels. The second type of migration is characteristic of mammals and of some other reptiles, in which PGCs originate in the vicinity of the hind region of the embryo. In the mouse embryo PGCs appear in the posterior region of the embryo, then pass to the hind gut and through the mesentery to reach the genital ridges (Chiquoine, 1954; Bennett, 1956; Mintz, 1971).

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Migration of PGCs is probably due to their amoeboid movements (Blandau, White & Rumery, 1963).

There is general agreement that in birds the genital ridge attracts primordial germ cells and that this attraction is probably of a chemotactic character (Simon, 1960; Dubois, 1968). Under experimental conditions the genital ridges of the chick embryo can attract germ cells directly from the germinal crescent (Dubois, 1968; Rogulska, 1969) as well as from undifferentiated or even differentiated gonad (Dubois, 1968). Moreover, the germinal epithelium of the young embryo can attract germ cells of a different species of bird: Simon (1960) and Reynaud (1969) obtained colonization of chick genital ridges by PGCs of the duck and the turkey respectively. Although no definite information about the nature of the ‘attractive factor’ responsible for settlement of PGCs is as yet available, the experiments mentioned above indicate that this attraction is not species specific. It seemed to us interesting to examine the behaviour of mammalian primordial germ cells submitted to the influence of the genital ridge of an avian embryo. The experiment, which is described in this report, consisted of introducing the hind gut of a mouse embryo into the coelomic cavity of a chick embryo. Numerous primordial germ cells, present in the hind gut, are thus placed in the vicinity of the gonadal Anlage of the chick. The high concentration of alkaline phosphatase in mouse PGCs (Chiquoine, 1954) makes it possible to recognize them easily both in the graft and in the host tissues.

MATERIALS AND METHODS

Hind guts from 9½-day mouse embryos of the A strain (13–23 somites) were excised in Ringer's solution and transplanted into posterior parts of the coelomic cavity of chick embryos of the Leghorn strain (about 66 h of incubation, 21–30 somites). Transplantations were carried out unilaterally or bilaterally, by means of Hamburger's technique of intracoelomic grafting (1938), as modified by Hara (1961). Hosts were sacrificed after 1–7 days and the grafts, together with the surrounding host tissues, were fixed in 75% ethyl alcohol, embedded in paraffin wax and sectioned serially at 10 μ. The sections were stained with fast red TR Salt (G. Gurr) according to the azo dye coupling method of Gomori for displaying alkaline phosphatase activity. Primordial germ cells were identified by virtue of the dense staining for the enzyme. Some of the sections were subsequently restained with haematoxylin and eosin. A total number of 94 transplants was examined.

RESULTS

Grafts were recovered in the abdominal region, attached to the host tissues such as mesonephros, mesentery, gonad or body wall. Individual variations in the developmental stage of donors and of hosts at the time of operation had no influence on the further fate of PGCs and will not be taken into account.
Controls

In order to establish the number of PGCs present in the grafts at the time of operation, five hind guts were excised from 15–20 somite embryos and examined for the presence of PGCs. They contained 90, 116, 140, 160 and 167 PGCs, with a mean number of 135.

Table 1. Numbers of primordial germ cells in grafts

<table>
<thead>
<tr>
<th>Age of graft (days)</th>
<th>No. of grafts</th>
<th>No. of PGCs</th>
<th>Mean no. of PGCs</th>
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</thead>
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<tr>
<td>0 (controls)</td>
<td>5</td>
<td>90, 116, 140, 160, 167</td>
<td>135</td>
</tr>
<tr>
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<td>36, 103, 149, 170, 190, 196, 207, 290, 298, 308, 346, 354, 362, 363, 370, 481</td>
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<td>3</td>
<td>11</td>
<td>1, 5, 23, 37, 40, 49, 61, 148, 166, 203, 345</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>0, 0, 8, 16, 90, 108, 109, 112, 147</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>2, 4, 4, 11, 14, 16, 18, 20, 21, 22, 23, 24, 29, 30, 31, 34, 36, 47, 86, (747*)</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>0, 0, 0, 0, 0, 0, 20, 70, 112</td>
<td>20</td>
</tr>
</tbody>
</table>

* Not included in calculated mean

One-day-old grafts (16 grafts)

The number of PGCs varied from 36 to 481, with a mean number of 264 (Table 1). In nine cases PGCs tended to occupy those parts of grafts which faced the mesentery, the mesonephros or the genital ridge (Figs. 1–3). In six out of these nine grafts single PGCs penetrated into the tissues of the host embryo and were seen in the region of mesonephros, gonad and mesentery (Figs. 4–6). In the remaining seven grafts distribution of PGCs within the graft was fairly uniform and migration of PGCs into chick tissues was not observed.

Two-day-old grafts (24 grafts)

The number of PGCs varied from 4 to 490, with a mean number of 153 (Table 1). Thirteen grafts contained accumulations of PGCs in those parts which faced the mesentery, the mesonephros or the genital ridge (Fig. 7). In four out of these thirteen grafts single PGCs penetrated into the chick mesentery or mesonephros. In the other grafts neither accumulations of PGCs close to these organs nor passage of germ cells to the host tissues were observed.
Mouse germ cells in chick embryo

(4) Three-day-old grafts (11 grafts)

The number of PGCs varied from 1 to 345, with a mean number of 98 (Table 1). Accumulations of PGCs close to the gonad and mesonephros were observed in four grafts only. In two of these grafts single PGCs penetrated into the mesenchyme of the mesonephros (Fig. 8).

In all 1- to 3-day-old grafts the localization of PGCs was examined with respect to the site of attachment of the grafts. It was found that accumulation of PGCs close to the mesonephros, the mesentery or the gonad (and migration of single PGCs into these tissues) occurs in grafts attached either directly to these organs or in close proximity to them. It should be stressed, however, that this directional movement does not always occur, even in grafts attached in the most favourable way.

(5) Four-day-old grafts (9 grafts)

The number of PGCs varied from 0 to 147, with a mean number of 65 (Table 1). PGCs were observed exclusively in the grafts. In six out of seven transplants containing PGCs, the germ cells formed a single small aggregation, composed of densely packed cells. These aggregations were always situated on the periphery of the grafts, and three of them were found close to the mesentery and mesonephros. One of the aggregations was restained with haematoxylin and eosin; the germ cells did not display any changes characteristic of meiosis.

(6) Five-day-old grafts (20 grafts)

The number of PGCs varied from 2 to 86, with a mean number of 25 (Table 1). PGCs were present only within the grafts. Aggregations of PGCs, similar to those described in 4-day-old grafts, were found in six grafts (Figs. 9–12). All of them were situated close to the mesonephros. The aggregations were restained with haematoxylin and eosin. In five of them germ cells had not initiated

Abbreviations on figures: g = gonadal rudiment; m = mesentery; mn = mesonephros. The dotted line shows the boundary between the graft and the host.

Figs. 1–3. Accumulations of PGCs in 1-day-old grafts. Accumulations are always situated on the periphery of the graft, in the proximity of the 'attractive sites' of the host embryo (gonadal rudiment, mesonephros) and contain a majority of the PGCs of the graft. × 175, × 65, × 175.

Fig. 4. One-day-old graft. Two mouse PGCs are located in chick mesenchyme, close to the place of attachment of the graft. × 175.

Fig. 5. One-day-old graft. Several mouse PGCs have invaded the gonad of the chick embryo (arrow); note that they are located in the mesenchyme, and not in the germinal epithelium. Other PGCs have accumulated close to the site of attachment. × 175.

Fig. 6. One-day-old graft. A chain of mouse PGCs is located beneath the coelomic epithelium of the host. Other PGCs, which are still present in the graft, are moving towards the place of attachment. × 65.
meiotic prophase, but the sixth aggregation developed into a small ovary-like body containing about 750 germ cells in meiotic prophase (Figs. 11, 12).

Four 5-day-old grafts (not included in Table 1) were examined in haematoxylin and eosin preparations but the identification of germ cells was unsuccessful.

(7) Seven-day-old grafts (10 grafts)

Only three grafts contained germ cells (20, 70 and 112) and there were no germ cells outside the transplant. PGCs which survived in the graft were always collected in one group. These aggregations were observed in different parts of the graft, without special relationship to the axial organs of the host. When restained with haematoxylin, the phosphatase-positive cells displayed features of degeneration, their chromatin was very compact and no obvious changes characteristic of meiotic prophase were visible.

DISCUSSION

Distribution of primordial germ cells within the grafts seems to be considerably changed under the influence of the host tissues. The fact that PGCs translocate themselves within the graft is not surprising in itself, as primordial germ cells of mammals are known to have amoeboid properties (Blandau et al. 1963). However, the interesting point is that their movement seems often to be of a directional character, bringing the mouse PGCs close to the mesonephros, the gonad or the mesentery of the chick host. This phenomenon cannot be explained simply by the movement of primordial germ cells towards the place of attachment of the graft. Ożdżeński (1969) studied the behaviour of primordial germ cells of the mouse in hind guts transplanted to the anterior chamber of the eye or to the chorio-allantoic membrane and never observed PGCs leaving the graft or accumulated at the site of attachment. In those of our grafts which were attached to the body wall (which occurs in many cases) accumulations of

Fig. 7. Two-day-old graft. Mouse PGCs, arranged in a chain, approach the gonadal rudiment, but do not invade it. × 175.

Fig. 8. Three-day-old graft. This graft was firmly attached to the mesonephros. Single mouse PGCs entered the mesonephros but have not moved far away from the graft (arrows); other PGCs are grouped together on the periphery of the graft, in the proximity of the gonad. × 65.

Fig. 9. Five-day-old graft. The picture shows a section through a small finger-like process, which protrudes from the graft, on the surface facing the mesonephros. The main part of the graft is completely deprived of PGCs. × 65.

Fig. 10. The same process as in Fig. 9, under higher magnification. It is covered by flattened epithelium and is almost completely filled with PGCs. × 400.

Fig. 11. Five-day-old graft. Another example of an accumulation of mouse germ cells within a small process-like fragment of the graft (the preparation restained with haematoxylin and eosin). The remaining part of the graft was free of PGCs. × 100.

Fig. 12. A fragment of Fig. 11, showing three mouse germ cells in meiotic prophase. × 1000.
PGCs close to the place of attachment of the graft were never observed. Accumulation of PGCs close to the mesonephros, the gonad or mesentery was observed in 20 out of 34 1- to 3-day-old grafts attached to these organs and even in 6 out of 17 1- to 3-day-old grafts attached only to the body wall but not far from the attractive region. These observations suggest that the attractive region includes mesonephros, genital ridge and mesentery, and that the attractive factor operates over a rather short distance, attracting perhaps only those PGCs which were situated initially very close to this region. It may be that in each graft only a small number of PGCs was in such an advantageous situation from the very beginning of intracoelomic development. This could explain the fact that not all grafts attached in the 'optimal position' showed PGCs tending to accumulate close to the attractive organs of the host.

It is known from in vitro experiments (Dubois, 1968) that the attractive factor, emanating from the genital ridge of a chick embryo, can attract germ cells from the chick germinal crescent, undifferentiated and even differentiated gonad. This attraction leads to the settlement of PGCs in the germinal epithelium. These observations were confirmed in vivo by Rogulska (1969), who made intracoelomic transplants of germinal crescents in chicks. In our present experiments proper colonization of the chick genital ridge by mouse PGCs was not obtained. Only single PGCs succeeded in crossing the boundary between the host and the graft, and even in grafts attached firmly to the attractive organs PGCs were often observed to stop at this boundary (Fig. 7). It is interesting that although PGCs are sometimes present in the mesenchyme of, or close to, the genital ridge of the chick, they never settle in the germinal epithelium, where native PGCs finish their migration.

From the 4th day onwards no alkaline phosphatase-positive cells of mouse origin were observed in the chick tissues. Primordial germ cells which, at least in some cases, were probably previously present in the host tissues, must have degenerated or have lost their phosphatase-positive reaction and become undetectable. The former possibility seems to us more likely. Whatever the final fate of these cells, the present observations provide evidence that the chick genital ridge, together with the neighbouring tissues, attracts mouse primordial germ cells, and suggest that the attractive factor in Amniota is of rather general character.

Relatively little is known about the fate of primordial germ cells which have been prevented from completing their migration to the genital ridges. According to Simon (1960) and Dubois (1968) chick PGCs degenerate fairly rapidly under such conditions. When germinal crescents are transplanted into the coelomic cavity of 4-day-old chick embryos (Komar, 1969), primordial germ cells can survive for a few days but their number decreases steadily; in 3-day-old grafts the number of PGCs is already low and the cells that remain display a tendency to form small aggregations. Mouse PGCs in the embryonic hind guts grafted to the anterior chamber of the eye or on the chorio-allantoic membrane also gradually
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disappear and after 7 days they are no longer present in the grafts; aggregations of PGCs have not been observed (Ozdzeńsksi, 1969).

In our material a gradual decrease in the number of primordial germ cells in the transplanted hind gut is also evident, although it is not as distinct as in Ozdzeński’s experiments. This decrease is most probably caused by degeneration of PGCs—in fact some degenerating PGCs were observed after restaining the grafts with haematoxylin. The ability of mouse PGCs to survive in non-gonadal tissue thus appears to be rather limited. However, one cannot exclude the possibility that the extragonadal survival of PGCs requires a specific environment which in the above experiments has not been provided by the host, either the embryo (chick) or the adult (mouse).

It is noteworthy that the decrease in the number of PGCs is accompanied by formation of aggregates which are first observed on the 4th day. After 6 days the only germ cells which survived were those in aggregates. The large number of PGCs in some of the aggregates suggests that some of the germ cells had been undergoing mitotic divisions and were not degenerating. The most interesting is a case of one 5-day-old graft in which germ cells forming an aggregation entered into meiotic prophase. Since the total age of the graft was 15 days, it means that the meiotic prophase began at the same time as in normal development (Brambell, 1927; Borum, 1961). This observation could be of great interest in pointing to the ability of PGCs to begin differentiation into definite germ cells independently of the gonad. However, one cannot exclude the possibility that during the preparation of the transplant some of the genital ridge material, adjacent to the hind gut, had been by chance included in the graft, and consequently the aggregation was, in fact, formed in its ‘own’ gonadal territory, which might provide nearly normal conditions for survival and differentiation of primordial germ cells. Whether the occurrence of aggregates of PGCs in other grafts could be explained in a similar way remains unknown.

RÉSUMÉ

Comportement des cellules germinales primordiales de souris dans l’embryon de poulet

Des intestins postérieurs d’embryons de souris de 9 jours ½ ont été transplantés dans la partie postérieure de la cavité coelomique d’embryons de poulet de 2 jours ½. Les hôtes ont été sacrifiés un à 7 jours plus tard, et les cellules germinales primordiales de souris (CGPs) ont été recherchées dans les greffons et dans les tissus environnants de l’hôte au moyen de techniques histochimiques révélant la phosphatase alcaline. En tout 94 greffons ont été examinés.

Pendant les 3 premiers jours du développement intra-coelomique du greffon, on observe des accumulations de CGPs de souris près du mesonephros, du mésetère ou de la gonade de l’embryon de poulet, dans 26 cas sur 51. Dans 12 greffons des CGPs isolés ont traversé la frontière entre l’hôte et le greffon et se sont établies dans des tissus de l’hôte, tels que le mésonephros, le mésetère ou la gonade.

Après 3 jours les CGPs de souris ne sont plus visibles dans les tissus de poulet. Cependant le nombre de CGPs décroît progressivement dans les greffons, et à partir du 4ème jour, de nombreux greffons ne contiennent plus de CGPs. La faculté qu’ont les CGPs de souris de
survivre en dehors des gonades, même dans l'intestin postérieur embryonnaire, est donc limitée.

Dans quelques uns des greffons de 4 à 7 jours, on trouve des CGPs à la périphérie du greffon sous la forme d'agrégrats isolés. A partir du sixième jour, seules les CGPs qui sont groupées peuvent survivre.

Les expériences indiquent que les gonades ainsi que des tissus adjacents (mésonephros, mésentère) de l'embryon de poulet montrent une attraction pour les cellules germinales primordiales de souris et que la substance attractive hypothétique n'est pas spécifique de classe.

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REFERENCES


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