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Histogenetic and neoplastic potential of different regions of the mouse embryonic egg cylinder

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SUMMARY

The histogenetic and neoplastic potentials of defined regions of the 8th day mouse embryonic egg cylinder were examined following ectopic transfer to beneath the testis capsule. No differences in histogenetic potential were detected between anterior and posterior slices of the embryo, either when composed of all three germ layers or of embryonic ectoderm alone. Small anterior and distal fragments of embryonic ectoderm also produced similar histogenetic profiles, although posterior fragments failed to grow in this ectopic site. The histogenetic potential of anterior and distal fragments exceeded the developmental fate ascribed to these two regions in the embryo (Beddington, 1981). There was some evidence for regionalization with respect to neoplastic potential, anterior slices of the embryo giving rise to a higher incidence of embryonal carcinoma cells than posterior slices.

INTRODUCTION

It is now well established that all the definitive foetal tissues in the rodent are derived from a single epithelial sheet, the embryonic ectoderm or epiblast (Gardner & Rossant, 1976, 1979; Levak-Svajger & Svajger, 1974; Diwan & Stevens, 1976). Recent experiments in the mouse using *in vitro* chimaeras have shown that a fate map of the epiblast can be constructed during gastrulation (Beddington, 1981, 1982) and heterotopic grafts have indicated that this regular pattern of tissue allocation at the late-primitive-streak-stage cannot be attributed to rigid mosaicism within the epiblast (Beddington, 1982). However, experiments on cultured embryos are necessarily short term. In order to conduct a more rigorous test of developmental potential the differentiation of different regions of the epiblast should be studied over a longer period.

This paper describes the development in ectopic sites of different fractions of the 8th day embryonic egg cylinder, consisting either of all three germ layers or of the epiblast alone. The production of experimental teratomas by this method provides a means of studying regional differences in developmental potential over a prolonged period. Furthermore, since the ectopic transfer of late-primitive-streak-stage embryos results in the highest incidence of teratomas containing embryonal carcinoma (EC) cells (Damjanov, Solter & Skreb, 1971;

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Damjanov, Solter, Belicza & Skreb, 1971) it is also of interest to determine whether the progenitors of these malignant stem cells have a generalized distribution in the embryo or whether they are restricted to specific regions, possibly associated with the location of the primordial germ cells.

MATERIALS AND METHODS

Recovery and dissection of embryos

All embryos were recovered on the morning of the 8th day of gestation and were derived from matings between either 129J Sv or CBA/H-T6 inbred mice. The decidua were removed from the uterus and the embryos dissected out in PB1 medium containing 10% FCS (Whittingham & Wales, 1969) instead of bovine serum albumin. The trophoblast, Reichert's membrane and the extraembryonic part of the conceptuses were removed with glass needles (Fig. 1). The remaining embryonic portions were divided into the following fractions for ectopic transfer:

i) Anterior and posterior slices of the egg cylinder

Using the foregut invagination as a marker for the anterior extreme of the embryo, two longitudinal incisions were made on each side of the distal tip of the cylinder. The anterior and posterior slices, composed of all three germ layers, were isolated and the central portion of the egg cylinder was discarded (Fig. 1).

ii) Anterior and posterior slices of isolated embryonic ectoderm

Anterior and posterior slices of the egg cylinder were prepared as described above. These slices were incubated in a mixture of 0·5% trypsin and 2·5% pancreatin (Difco) in calcium-magnesium-free Tyrode saline at pH 7·7 for 10 min at 4 °C (Levak-Svajger, Svajger & Skreb, 1969). Following incubation the endoderm and mesoderm were removed by gentle pipetting. The posterior slice was further cleaned by removing as many individual adherent mesoderm cells as possible with glass needles. However, it was never possible to remove all mesoderm-like cells from the primitive streak region (Fig. 2A).

iii) Anterior, distal and posterior fragments of isolated embryonic ectoderm

Small fragments of isolated embryonic ectoderm from three defined regions of the embryo were also prepared. Anterior embryonic ectoderm was removed with glass needles from beneath the foregut invagination and following removal of excess lateral ectoderm consisted of a small rectangular piece of tissue (approximately 70 × 50 μm). Distal embryonic ectoderm was removed from the tip of the cylinder and again most of the lateral ectoderm was cut away to leave a small square piece of tissue (approximately 70 × 70 μm). Posterior embryonic

Fig. 1. A diagram illustrating the dissection procedure for isolating anterior and posterior slices of the egg cylinder and for isolating anterior and posterior slices of embryonic ectoderm. A, site of anterior embryonic ectoderm fragment. D, site of distal embryonic ectoderm fragment. P, site of posterior embryonic ectoderm fragment.

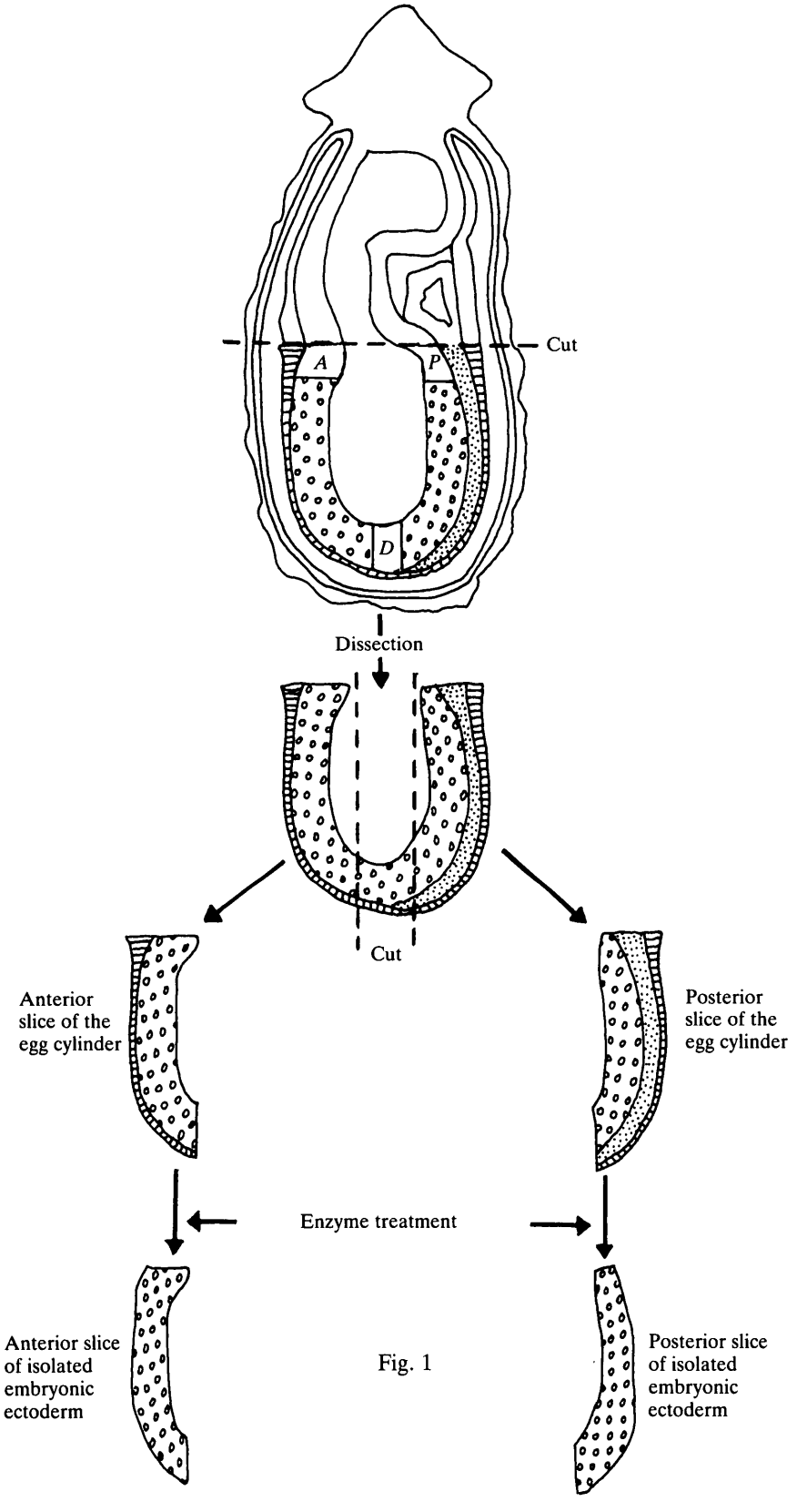
Cut
A
P
D
Dissection
Cut
Anterior slice of the egg cylinder
Posterior slice of the egg cylinder
Enzyme treatment
Anterior slice of isolated embryonic ectoderm
Posterior slice of isolated embryonic ectoderm

Fig. 1

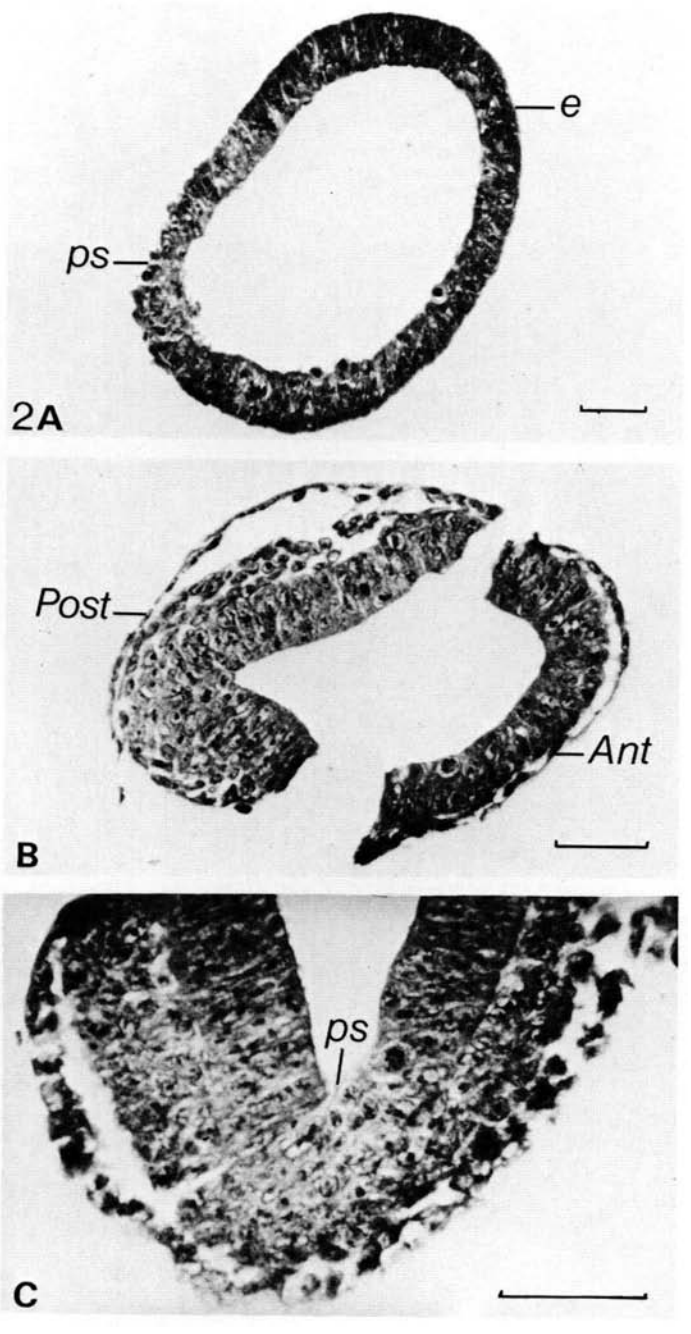

Fig. 2. A. Control for germlayer separation. A transverse section through the isolated embryonic ectoderm of an 8th day mouse egg cylinder. e, embryonic ectoderm; ps, primitive streak. Bar 40 μm. B. Transverse sections through anterior and posterior slices of the egg cylinder. Ant, anterior slice; Post, posterior slice; ps, primitive streak. Bar 40 μm. C. Transverse section through a posterior slice of the egg cylinder. ps, primitive streak. Bar 40 μm.

ectoderm was dissected from the caudal end of the primitive streak, just beneath the origin of the amnion, and trimmed to a size similar to that of anterior embryonic ectoderm. Usually, contaminating endoderm and mesoderm could be removed with glass needles but in some cases it was necessary to subject the fragments to enzyme treatment, incubating them for 10 min at 4 °C in a mixture of 0·5 % trypsin and 2·5 % pancreatin (Difco) in calcium-magnesium-free Tyrode saline at pH 7·7 (Levak-Svajger *et al.*, 1969). These fragments were similar in size to those which were used as a source for donor cells in orthotopic and heterotopic grafting experiments *in vitro* (Beddington, 1981, 1982).

Several anterior and posterior slices, both before and after the removal of endoderm and mesoderm, were fixed in formol-acetic-alcohol and prepared for routine histology. Serial transverse sections, 7 μm thick, were made and stained with haemalum and eosin. These sections demonstrated that the embryos had been cut reliably into anterior and posterior fractions, the primitive streak being a distinct landmark of the posterior slices (Figs 2B & 2C). Furthermore, it was clear that all endoderm and mesoderm had been removed from the anterior slices of embryonic ectoderm and only a few cells of intermediate ectomesodermal morphology were detected in the posterior ectoderm slices.

Ectopic transfer of embryonic material

The isolated fractions of embryonic tissues were transferred individually beneath the testis capsule of anaesthetized syngeneic male mice. All recipients were more than 4 weeks old. The grafts were made using a fine hand-drawn Pasteur pipette and each piece of embryo was transferred in a small quantity of PB1 medium. In most animals both testes received a graft but in two recipients, where one testis appeared abnormally small, only unilateral transfers were made. After 30 to 40 days the recipients were killed by cervical dislocation and the testes recovered and placed in warm PBS.

Serial transplantation of tumours

Primary tumours which were to be tested for transplantability were washed in PBS and any enveloping seminiferous tubules were removed with watchmakers' forceps. In some experiments the whole tumour was prepared for transplantation but in others approximately one third of the tumour was sliced off and fixed in formol-acetic-alcohol for subsequent histological analysis. The remainder of the tumour was minced with scissors and watchmakers' forceps before being injected subcutaneously into the dorsal upper thoracic region of adult syngeneic male mice (Auerbach, Morrissey & Sidky, 1978). Wherever possible, the homogenate of a single tumour was transferred to two recipients but when the tumour was very small only one recipient was used. All the recipients were monitored for the appearance of tumours on the dorsum for at least 8 weeks and in those animals showing no sign of secondary growth for up to a year.

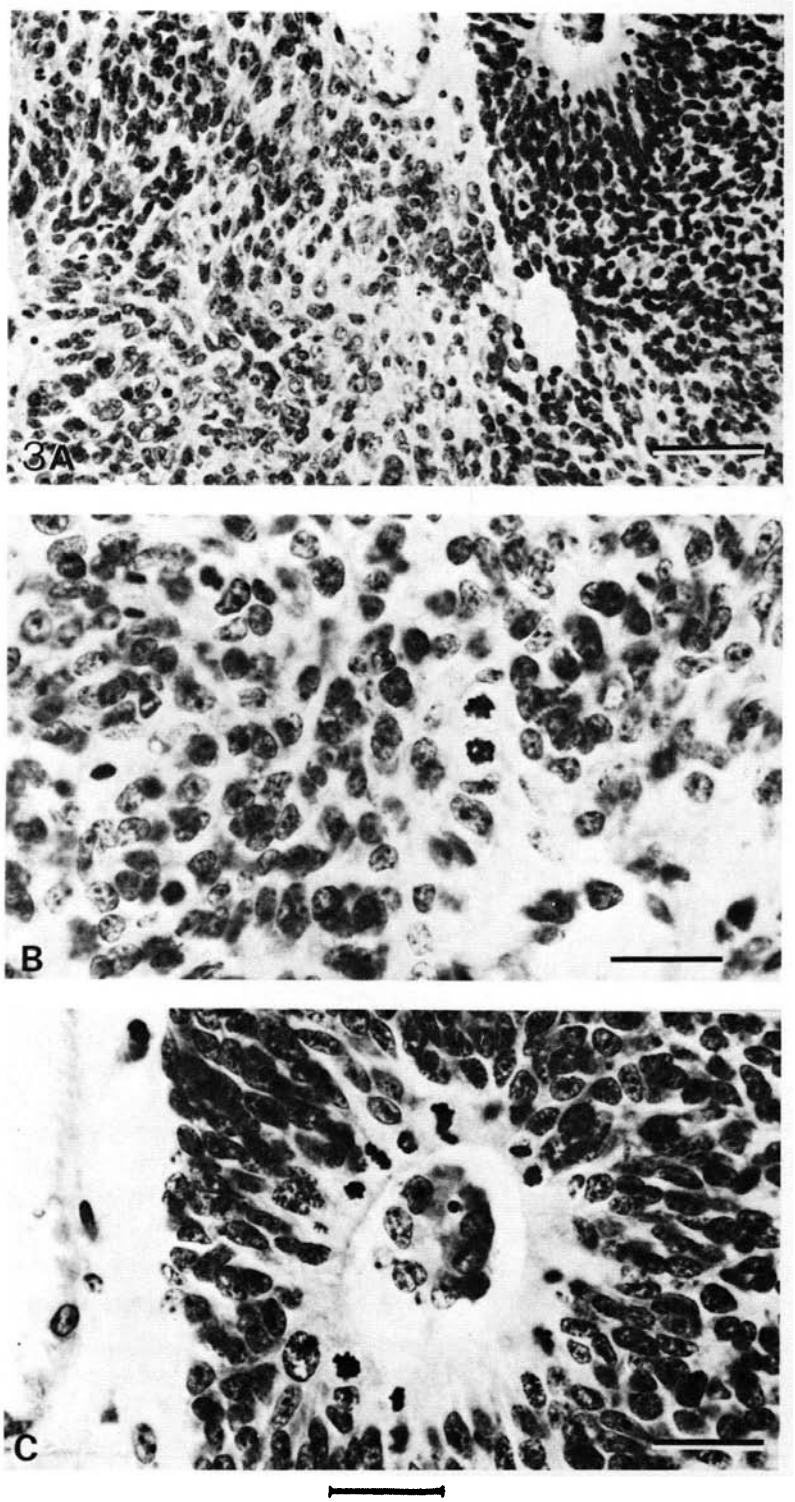

Fig. 3. A. Section through a teratocarcinoma showing undifferentiated, proliferating cells classified as EC cells. Bar 200 μm. B. EC cells. Bar 100 μm. C. Pseudoepithelial organization of EC cells. Bar 100 μm.

Histological examination of tumours

Intact primary tumours which were not tested for transplantability, and samples from primary tumours which were transplanted, were fixed in formol-acetic-alcohol. Subsequently, they were dehydrated, cleared and embedded in paraffin wax (m.p. 52 °C) and serially sectioned at 7 μm. The sections were stained either with Masson's Trichrome or with haemalum, eosin and Alcian Blue. For some tumours every tenth section was placed on a separate slide and stained by Holmes' silver nitrate method for nerve fibres (McManus & Mowry, 1960). Every tenth section of each tumour was scanned in a light microscope (Zeiss) and the assortment of differentiated tissues, and the presence or absence of EC cells was recorded. However, no quantitation of the relative amounts of the various differentiated tissues was attempted. In one series of experiments (the transfer of anterior and posterior slices of the egg cylinder), where the whole tumour was processed for histology, the number of serial sections was recorded for each tumour. In addition, the cross-sectional areas of central sections from each tumour were compared after making camera-lucida drawings onto graph paper.

The unequivocal recognition of EC cells in histological preparations is difficult

Table 1. *The tissues identified in experimental teratomas derived from anterior and posterior slices of the egg cylinder and from anterior and posterior slices of isolated embryonic ectoderm*

	Anterior egg cylinder	Anterior* ectoderm	Posterior egg cylinder	Posterior* ectoderm
Number transferred	8	11	10	12
Number of tumours	8	10	9	11
Number of tumours containing:				
Skin	8	9	6	8
Neural tissue	8	8	6	8
Respiratory tube	8	10	6	9
Glands	8	9	8	8
Intestine	8	7	8	6
Adipose tissue	6	2	2	2
Cartilage	8	2	3	1
Bone	7	1	4	2
Smooth muscle	8	6	8	8
Striated muscle	8	7	5	9
Pigment	8	6	3	5
Embryonal carcinoma cells	7	4	3	1

Assessment based on serial sections of the whole tumour.
* Assessment based on serial sections of one third of the tumour.

but in this study only cells with a relatively undifferentiated phenotype, often arranged in a pseudoepithelial organization, and showing a high incidence of mitoses were classified as EC cells (Fig. 3). It should be pointed out that although these cells bore a close resemblance to embryonic ectoderm or neurectoderm cells of the early embryo they were not morphologically identical to those EC cells seen in tumours derived from well-established teratocarcinoma lines (compare Fig. 3 with Kleinsmith & Pierce, 1964; Figs 3–5). It seems that EC cells in primary tumours tend to retain a more pronounced epithelial organization.

RESULTS

Anterior and posterior slices of the egg cylinder

Eight anterior slices were transferred to the testes and each graft developed into a tumour. The variety of tissue types found in these teratomas is shown in Table 1. It is clear that tissues representing derivatives of all three germ layers (Fig. 4) are formed in all tumours and there was no tendency for the grafts to differentiate into predominantly anterior adult structures such as foregut derivatives or brain. However, two teratomas showed no trace of adipose tissue and one tumour was deficient in bone. All but one of these tumours contained

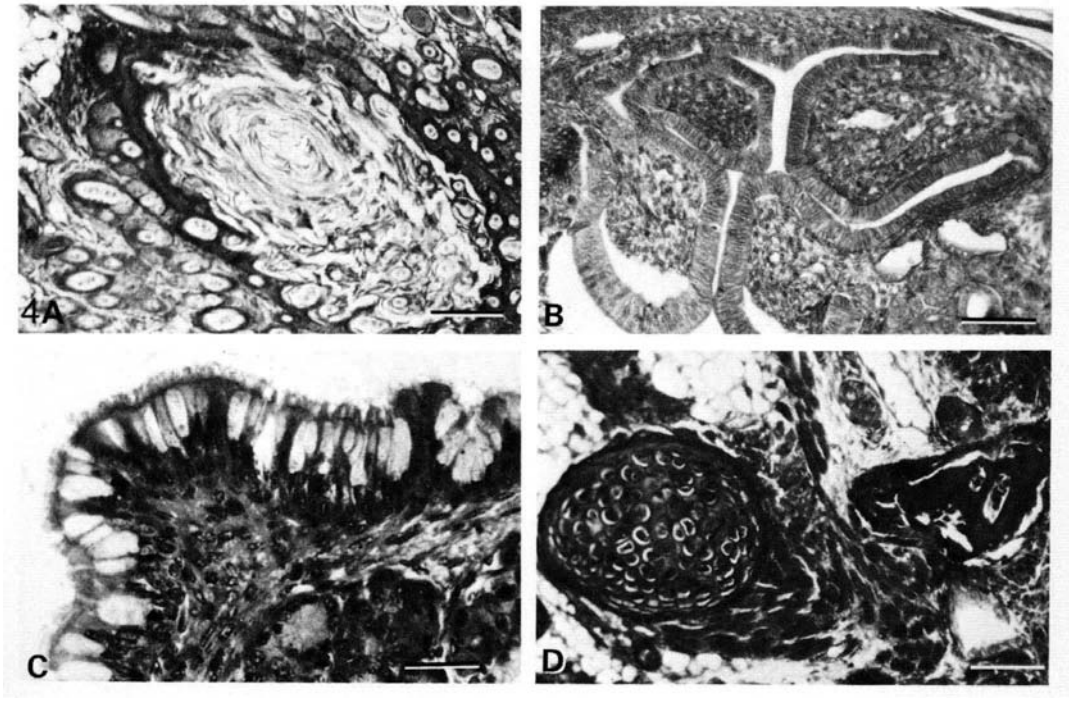

Fig. 4. Representative tissues found in teratomas. A. Well-differentiated keratinizing skin. Bar 125 μm. B. Gut epithelium. Bar 125 μm. C. Ciliated glandular epithelium. Bar 50 μm. D. Areas of bone, cartilage, adipose tissue and striated muscle. Bar 50 μm.

Table 2. *A comparison of the size of tumours derived from anterior and posterior slices of the egg cylinder*

Anterior slice of egg cylinder			Posterior slice of egg cylinder		
Tumour no.	‡No. sections	Cross-sectional†§ area	Tumour no.	‡No. sections	Cross-sectional†§ area
1	960	136	1	720	79
2	832	183	2	600	51
3	764	251	3	504	47
4	736	146	4	480	51
5	684	126	5	480	67
6	644	113	6	324	32
7	524	84	7	56	8
8*	650	80	8	54	3
			9	43	5

* This tumour was cryptorchid on recovery.

† The area is measured in arbitrary units.

‡ The number of several sections obtained from anterior-derived tumours is significantly higher than that from posterior derived tumours (t-test; $p \geq 0{\cdot}001$).

§ The cross sectional area of central sections from anterior-derived tumours is significantly greater than that from posterior derived tumours (t-test; $p \geq 0{\cdot}001$).

large patches of EC cells, evident in over 90 % of the sections scanned (Fig. 3). The testis which contained differentiated tissue but no EC cells was cryptorchid at the time of recovery. The seven tumours from anterior grafts which contained EC cells were larger than tumours derived from posterior slices as judged by the number of serial sections cut from each tumour and the cross-sectional area of the central sections of the teratomas (Table 2). A separate series of 18 anterior slices was transferred to the testes and 16 tumours (88·9 %) were recovered. After subcutaneous injection into secondary hosts nine of these tumours generated secondary growths (Table 3). Therefore, by histological classification 87·5 % of grafted anterior slices retain a population of malignant stem cells whereas by the more functional transplantation assay 56·3 % of such grafts qualify as malignant teratocarcinomas.

Nine tumours were formed from ten grafts of posterior slices of the egg cylinder (90 %). These slices tended to show a more limited range of differentiated products compared with their anterior counterparts (Table 1). Indeed, some teratomas from posterior slices contained only small endodermal cysts surrounded by loose connective tissue. However, if this series is considered as a whole the posterior slices also show the ability to form derivatives of all three germ layers and once again the differentiated tissues were not restricted to typically posterior adult structures but included anterior organs such as respiratory tube and pigmented neuroepithelium, presumed to represent a brain derivative. Embryonal carcinoma cells were present in three tumours (33·3 %) but in two of

these their distribution was limited to small patches in less than a third of the sections scanned. In the transplantation assay 16 grafts of posterior slices produced 15 tumours (93·8 %) but none of these teratomas generated secondary tumours following transplantation (Table 3).

Anterior and posterior slices of isolated embryonic ectoderm

Eleven slices of anterior ectoderm were transplanted to the testis and ten tumours were recovered (90·9 %). These teratomas showed some reduction in their differentiation compared with grafts of anterior slices composed of all three germ layers (Table 1). It is conceivable that this is an artifact since the histological assessment of the tumours derived from slices of the egg cylinder was based on the analysis of the whole tumour whereas the same assessment of the embryonic ectoderm slices was made using only a third of the tumour. Nonetheless, derivatives of all three germ layers, including definitive endoderm structures, were formed in the majority of cases. The infrequent occurrence of bone, cartilage and adipose tissue marks the greatest discrepancy between these teratomas and those derived from anterior slices of the egg cylinder. In addition, the incidence of EC cells is reduced although they were still detected in 40 % of the tumours. However, transplantation of these teratomas did not result in any secondary growths (Table 3).

Twelve slices of posterior embryonic ectoderm were transferred to the testis and eleven of these generated tumours (91·7 %). Once again derivatives of all three germ layers were evident in these teratomas including characteristically anterior adult structures such as respiratory epithelium (Table 1). Only one tumour contained recognizable EC cells and transplantation of these teratomas also failed to generate any secondary tumours (Table 3).

Anterior, distal and posterior fragments of embryonic ectoderm

Twelve out of 21 anterior fragments developed into tumours (57·1 %). The tissues found in the 12 tumours are shown in Table 4. It is clear that derivatives of all three germ layers are present and also that these fragments are capable of generating EC cells. Twenty-one fragments of distal embryonic ectoderm were

Table 3. *The incidence of transplantable tumours derived from anterior and posterior slices of the egg cylinder and from anterior and posterior slices of isolated embryonic ectoderm*

	Anterior egg cylinder	Anterior ectoderm	Posterior egg cylinder	Posterior ectoderm
No. transferred	18	11	16	12
No. of tumours	16	10	15	11
No. of transplantable tumours	9	0	0	0

Table 4. *The tissues identified in experimental teratomas derived from fragments of isolated embryonic ectoderm*

Type of graft	Anterior	Distal	Posterior
No. transferred	21	21	10
No. tumours	12	19	0
No. analysed histologically	11	19	
No. containing:			
Skin	8	15	
Nervous tissue	10	19	
Gut	9	15	
Respiratory tube	11	17	
Glands	9	12	
Adipose tissue	7	12	
Cartilage	5	3	
Bone	6	6	
Smooth muscle	11	16	
Striated muscle	6	10	
Pigment	2	4	
EC cells	5	5	

transferred and 19 tumours were recovered (90·5 %). Despite variation between tumours in the range and character of the differentiated tissues formed, the series as a whole demonstrated that distal ectoderm can also develop into derivatives of all three germ layers (Table 4). EC cells were also evident in some of these tumours. However, no transplantations were undertaken on this series of teratomas and so there is no experimental evidence for the malignancy of these tumours.

Ten posterior fragments of ectoderm were grafted but none of these transfers resulted in a teratoma. It is unlikely that this can be explained by a technical failure as the incidence of tumours from the other grafts is consistently high. Furthermore, posterior ectoderm prepared in an identical fashion provides a ready source of colonizing cells for *in vitro* chimaeras (Beddington, 1982) which excludes the possibility that this part of the embryo is particularly susceptible to damage during isolation. Therefore, it seems that small pieces of posterior ectoderm are unable to grow and differentiate beneath the testis capsule.

DISCUSSION

i) *Regional differences in histogenetic potential*

Previous work on the development of primitive-streak-stage embryos, in the rat and the mouse, has demonstrated that both the intact egg cylinder and its

isolated embryonic ectodermal component are capable of generating derivatives of all three germ layers when transferred to ectopic sites (Diwan & Stevens, 1976; Skreb & Svajger, 1975; Solter, Skreb & Damjanov, 1970). The possibility of axial regionalization in the rat embryo, with respect to histogenetic potential, has been tested directly only at the headfold stage (Svajger & Levak-Svajger, 1974) where differences were found in the type of gut derivatives formed depending on whether anterior or posterior fractions were grafted. However, using both wild-type and homozygous T/T mutant embryos in the mouse it was found that the posterior third of the embryo at the headfold stage could generate respiratory tube structures in an ectopic site (Bennett, Artzt, Magnusson & Spiegelman, 1977). More recent studies have attempted to look for regionalization in pre- and early primitive streak stage rat embryonic ectoderm (Svajger, Levak-Svajger, Kostovic-Knezevic & Bradamante, 1981). However, the longitudinal division of embryos was haphazard and posterior and anterior halves were not identified prior to grafting, nor were the mesodermal wings removed from the primitive streak. Furthermore, it is not clear in how many instances halves from the same embryo both generated teratomas. Therefore, although every teratoma recovered contained tissue derivatives of all three germ layers this cannot be taken as formal proof for a lack of regionalization in developmental potential.

The experiments described in this paper, where the origin of each fragment was known before grafting, indicate that neither the intact egg cylinder nor the embryonic ectoderm constituent of it are regionalized in their histogenetic capacity at the primitive streak stage in a way that might reflect the eventual body plan of the foetus. Anterior and posterior slices both gave rise to ciliated pseudostratified columnar epithelium characteristic of the respiratory tube; posterior slices were capable of generating heavily pigmented epithelium presumed to be a derivative of the brain; no consistent deficiency in one mature tissue was found associated with a particular type of graft. Even when small fragments of anterior or distal embryonic ectoderm were transferred no qualitative difference could be detected in the mature tissues that they formed. Therefore, these results support the notion (Beddington, 1982) that, during gastrulation, the embryonic ectoderm is not a mosaic tissue subdivided into large patches of cells committed to follow mutually exclusive developmental pathways.

ii) *Comparison of histogenetic potential with developmental fate and potency tested in the embryo*

The differentiation of anterior and distal fragments of embryonic ectoderm in ectopic sites may be compared with their normal fate, as judged from orthotopic injections, in the embryo (Beddington, 1981, 1982) and with their behaviour following heterotopic grafting in the embryo (Beddington, 1982). The fate of anterior ectoderm appears to be restricted largely to the formation of neurectoderm and surface ectoderm and even after heterotopic grafting, despite being able to colonize mesodermal tissue it retains a strong tendency to colonize skin

and neural precursors rather than to conform completely with the differentiation characteristic of its new surroundings (Beddington, 1982). In ectopic sites this same tissue shows the ability to generate definitive endoderm structures, such as respiratory tube and intestine, as well as a wide range of mesodermal derivatives (Table 5). It should be pointed out that some of these 'mesodermal tissues' could be derived from the neural crest rather than mesoderm (see Morriss & Thorogood, 1978). Nonetheless, it seems clear that the differentiation observed in these anterior grafts exceeds its normal fate in the embryo.

Distal ectoderm shows a similar range of differentiation in teratomas to that seen in grafts of anterior fragments and, once more, this exceeds the normal fate ascribed to this tissue (Beddington, 1981). For example, distal ectoderm readily forms skin in ectopic grafts while in its normal location in the embryo it never colonizes surface ectoderm. However, in heterotopic grafts distal ectoderm shows more lability than anterior ectoderm and converts readily to a pattern of colonization appropriate to its new surroundings (Beddington, 1982).

Thus, the present analysis of the developmental potential of anterior and distal fragments again demonstrates the pluripotent nature of embryonic ectoderm tissue, such that the predictable pattern of development observed in these two regions in the late-primitive-streak-stage embryo cannot be explained on the basis of a prior restriction in potency.

The failure of posterior ectoderm to grow in the testis is not readily explicable. Apart from a reduced proliferation rate (Pasteels, 1943) and the tendency to invaginate there are no known features which distinguish it from the rest of the embryonic ectoderm. In the chick embryo the posterior part of the primitive streak tends to form only blood in tissue culture (Murray, 1932) or following intrablastodermal grafting (Waddington & Schmidt, 1933). It is possible that posterior ectoderm from the mouse egg cylinder behaves in a similar way under the testis capsule. Certainly, a small patch of graft-derived blood cells would have been overlooked when recovering tumours from the testis.

iii) *Regionalization in neoplastic potential*

With regard to the formation of EC cells, it is clear from the histological analysis that tumours resulting from grafts of anterior slices of the egg cylinder show a greater incidence of these cells than do those from posterior slices (Table 1). Indeed, this incidence is considerably higher than that obtained following the transfer of whole embryonic egg cylinders from C3H embryos of an identical age (Solter *et al.* 1970; Damjanov *et al.* 1971). Therefore, it is possible that the isolation of the anterior half of the egg cylinder enhances neoplastic conversion. The difference between anterior and posterior grafts, in their ability to generate EC cells, is even more marked in the transplantation assay where 56·3% of anterior-egg-cylinder-derived tumours proved transplantable compared with none from posterior transfers (Table 3). Although there is an overall drop in

the frequency of EC cells when embryonic ectoderm alone is transferred, something which has not been seen in parallel studies on intact egg cylinders and their embryonic ectoderm component (Stevens, 1970; Diwan & Stevens, 1976), histological analysis again reveals a clear difference between the incidence of EC cells in anterior derived tumours and those from posterior grafts (Table 1).

The failure of any tumours from isolated ectoderm grafts to continue growth after transplantation does not support the histological assessment and confuses the interpretation of the results. As portions of the same tumour were scanned histologically and tested for transplantability in this series the discrepancy highlights the problem of classifying malignant tumours. Even where transplantable tumours were obtained from anterior slices of the egg cylinder their incidence was well below that of tumours showing the presence of undifferentiated, proliferating cells (56·3 % compared with 87·5 %). Disparities between histological classification of EC cells in primary tumours and transplantation assays have been encountered before (e.g. Stevens, 1970; Artzt & Bennett, 1972) although no rigorous analysis has ever been published. Such discrepancies suggest that the presence of EC cells may not always guarantee continued growth in a secondary host. This may be because EC cells are not present in sufficient quantity to generate secondary tumours. Certainly, the most convincing correlation between the morphological identification of EC cells in primary tumours and transplantability was obtained after selecting particularly large tumours for transplantation (Damjanov *et al.* 1971). Alternatively, it may be that morphologically similar cells are heterogeneous in other respects. For example, different EC cell lines in culture show considerable variation in their capacity to differentiate either *in vivo* or *in vitro* (see Graham, 1977), and also differ in their antigenic profiles (Heath, 1978). Therefore, while it is clear that all transplantable teratocarcinomas contain what are recognized morphologically as EC cells, primary tumours containing these cells are not necessarily transplantable.

With this reservation in mind only tentative conclusions on neoplastic potential can be drawn from the data presented in this paper. It seems that the anterior part of the egg cylinder serves as a better source of morphologically recognizable EC cells than does the posterior part (Tables 1 & 4) and that this regionalization does not coincide with the distribution of primordial germ cells at this stage. Studies on the localization of alkaline phosphatase activity in 8th day embryos (Ozdenski, 1967) and the autonomous differentiation of isolated fractions of primitive-streak-stage embryos (Snow, 1981) indicate that the primordial germ cells are situated at the posterior end of the primitive streak. As the posterior region of the primitive streak fails to generate teratomas beneath the testis capsule and EC cells predominate only in more anterior grafts it would appear that the ability of embryonic ectoderm to form EC cells may be distinct from that of primordial germ cells.

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