examination of representative tumors from groups II and IV revealed the presence of polypoid adenomas, well-differentiated adenocarcinomas and undifferentiated mucinous adenocarcinomas, in keeping with observations made by Ward[12]. The ear tumor found in group IV was identified as a well-differentiated squamous cell carcinoma. The livers of animals given BHT were enlarged, but microscopically they were normal. Multiple metastatic nodules from an intestinal adenocarcinoma were observed throughout the liver in 1 rat from group IV.

Discussion. This study demonstrated that the simultaneous administration of a phenolic antioxidant (BHT) and a potent colon carcinogen (DMH) had no effect on the incidence and number of colon tumors, an observation in agreement with that of Wattenberg[6]. On the other hand, Weisburger et al.[7] claim that BHT inhibited intestinal carcinogenesis induced by a metabolite of DMH, azoxymethane (AOM). In their experiment, BHT was given to F344 rats 2 weeks prior to weekly s.c. injections of AOM. The observed differences between the 2 studies are perhaps due to the use of different strains of rats, the timing of the initiation of BHT feeding and the use of a metabolite of DMH.

BHT stimulates hepatic microsomal enzymes[8,9] and would therefore be expected to alter the metabolism of DMH by enhancing its oxidation to AOM and by increasing the rate of hydroxylation to form the proximate carcinogen, methylazoxymethanol (MAM). Increased formation of MAM-β-glucuronide could decrease the amount of active carcinogen available to react with cellular macromolecules[13]. From the morphologic results presented in this study, it appears that BHT does not alter the metabolism of DMH to decrease its carcinogenicity. Further, if BHT had any effect on the oxidative reactions of DMH metabolism, it would have been more apparent in the present study where DMH was used instead of AOM as in the work of Weisburger et al.[7], since AOM is already in an oxidized state[14]. The reason for the lack of inhibition of BHT on colon carcinogenesis in our study is not clear, but it seems likely that the rate limiting step may be the deconjugation of MAM-β-glucuronide, and this is not affected by BHT.

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Course of development of isolated rat embryonic ectoderm as renal homograft[1]

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Summary. When the isolated head-fold stage rat embryonic ectoderm is grafted under the kidney capsule, it gives rise to a new mesenchyme with the capacity to differentiate into mesodermal tissues.

The rat embryonic ectoderm can be cleanly separated from the mesoderm and tested for the capacity of differentiation in renal homografts[2,3]. We have previously shown that, in these experimental conditions, mesodermal tissues (cartilage, bone, muscle) developed even from isolated pieces of the head-fold stage ectoderm other than the primitive streak and the Hensen's node regions[4]. The possible explanations for this observation are: a) contamination of the ectoderm with adherent mesodermal cells, b) induction of mesodermal tissues in the connective tissue of the host renal capsule, c) neural crest origin of mesenchymal tissues, d) presence of undifferentiated stem cells in the ectoderm, and e) formation of a new ectodermal region of morphogenetic cell movements ('regeneration' of the primitive streak).

In this preliminary experiment, the histology of rat ectodermal grafts was studied at short time intervals in order to establish the course of events leading to the development of the final teratoma.

Material and methods. The head-fold stage rat embryos (9 days) of the inbred Fischer strain were used. The embryonic ectoderm was cleanly separated from the underlying mesoderm by the combined treatment with enzymes

Development of the isolated ectoderm as renal homograft

Time after grafting	Histological structure of grafts
2 days	Thickening of the ectoderm. Numerous mitoses. Differentiation and beginning invagination of the neuroepithelium (fig. 2, a). Immigration of loosely arranged cell groups (mesenchyme) beneath the ectoderm (figure 2, a, b).
5 days	Enlarged, vascularized graft. Predominantly immature neural tissue; massive cell necrosis (figure 2, c). 2-layered epidermis (cysts). Onset of myotube formation. The rest of the mesenchyme undifferentiated.
7 days	Advanced differentiation of the neural tissue (ependyma, choroid plexus) and epidermis (multilayered). Onset of chondrogenesis (figure 2, d).
9 days	Abundant neural tissue, muscle and cartilage. Osteogenesis. Epidermis: onset of keratinization, hair buds.
12 days	Onset of adipose tissue differentiation. Maturation of other tissues.

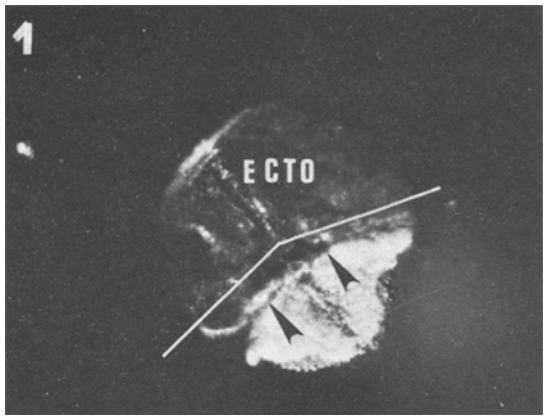

Fig. 1. Last step of ectoderm (ECTO) separation from the mesoderm. The white line indicates the cut by which the primitive streak region (arrows) is separated from the rest of the ectoderm.

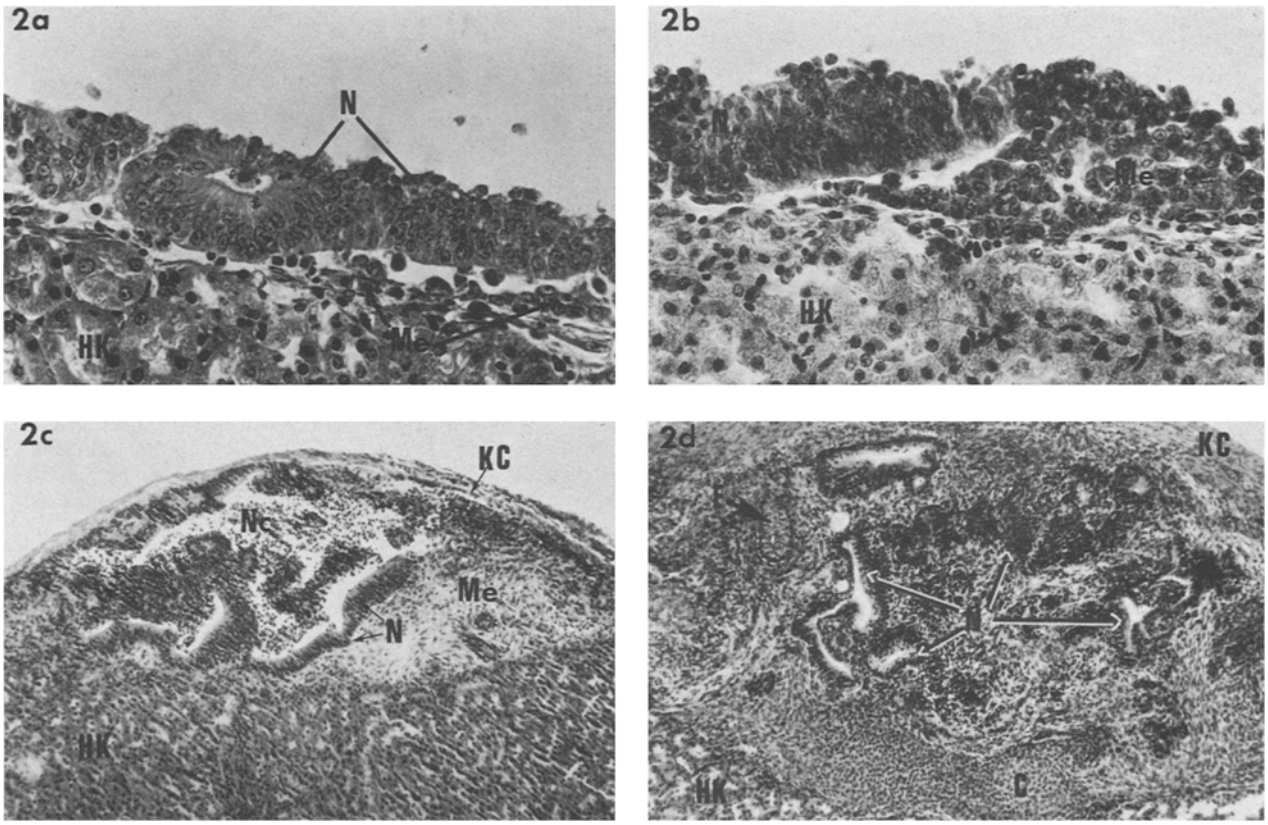

Fig. 2. Histological structure of the grafted ectoderm after 2 days (*a* and *b*, ×400), after 5 days (*c*, ×100) and after 7 days (*d*, ×100). C: cartilage, E: epidermis, HK: host kidney, KC: kidney capsule, Me: mesenchyme, N: neural tissue, NC: necrotic cells.

and microsurgery[2]. The primitive streak and the Hensen's node regions were cut off (figure 1). The rest of the ectoderm was grafted under the kidney capsule of syngeneic adult male rats. The course of its development as homograft was analyzed by routine histology after 2, 5, 7, 9 and 12 days. This report is based on a total of 58 grafts.

Results and discussion. The consecutive developmental events in the grafted ectoderm are summarized in the table and partly illustrated in figure 2, a–d. It is obvious that the development of ectodermal grafts proceeds in 2 major steps: a) morphogenetic displacement of cell groups resulting in the invagination of the neuroepithelium and the formation of a new mesenchyme, and b) histological differentiation of both the ectoderm (neural tissue, epidermis) and the mesenchyme (muscle, cartilage, bone). The differentiation of the neural tissue is accompanied by massive cell necrosis probably due to insufficient vascularization. This observation may explain our earlier findings of fibrous scars in large embryo-derived teratomas[5].

The formation of a new mesenchyme by cell immigration from the grafted ectoderm deserves special attention. 2 days after grafting, groups of loosely arranged cells were displaced in the space between the ectoderm and the cortex of the host kidney (figure 2, a, b). Occasionally these cell groups were continuous with the ectoderm which, in this region, assumed the characteristics of the primitive streak (figure 2, b). It is evident that, although the grafted ectoderm did not contain the primitive streak and the Hensen's node, some cells continued to leave their original intraectodermal position and to form a separate mesenchymal layer beneath the ectoderm. One may therefore suppose that the apparent regionalization of the embryonic ectoderm in situ[6] is a prerequisite for the organization of the bilaterally symmetrical embryo, but not for the forma-

tion of germ layers with different developmental fates. In experimental conditions, the primitive streak can be functionally replaced by other (any?) parts of the ectoderm. These findings seem to explain the appearance of mesodermal tissues in teratomas derived from the head-fold stage ectoderm. However, it is not possible to decide whether the newly formed mesenchyme in this experiment originates from prospective mesodermal or neural crest cells, or from any other undetermined cells which have assumed unexpected morphogenetic and differentiative capacities in response to atypical environmental conditions in the experiment.

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Alteration of thoracic macrochaet development in *Drosophila*

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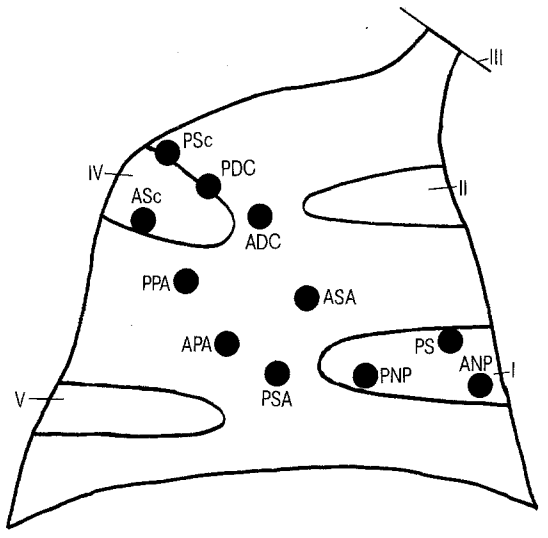
Summary. Incisions into 5 areas of the right dorsal mesothoracic disc in mature *Drosophila* larvae produced 2 types of alteration of macrochaet development. Macrochaets tended to be lost most commonly in the notopleural areas, and duplicated in the scutellum.

Methods. The 11 macrochaets of each side of the dorsal mesonotum of *Drosophila melanogaster* have been mapped with increasing accuracy on the dorsal mesothoracic imaginal disc in recent years[1,2]. Their locations were determined by transplantation of parts of the disc taken from mature third instar larvae into hosts of the same age[3]. Metamorphosis of the implanted tissue coincident with that of the tissues of the host reveals which macrochaet is produced in each case. Since removal and surgical cutting of the disc necessarily place stress on its constituent cells, it is possible that alterations could be caused in both the number and location of macrochaets destined to develop from the tissue[4]. Therefore surgical incisions in situ could reveal the extent of such changes without the additional trauma of transplantation of the whole disc[5,6]. This report summarizes a series of such operations whose effects were evaluated by examining the macrochaets of the operated animal after its eclosion.

All operations were performed on mature third instar larvae of the Oregon-S wild type strain. Larvae were grown on standard medium from eggs collected over a 6-h period. The operations were performed within the last 2 h before pupariation. Ether was applied for 1 min[7], and the anesthetized animals were attached to a glass slide by allowing saline solution to evaporate to dryness beneath them[8]. Tungsten needles sharpened in molten sodium nitrite were inserted through the posterior dorsolateral margin of the third annulus in the anterior direction toward the right disc in operations on the lateral side of the disc. For operations on the medial side, the needle was inserted posteriorly through the posterior edge of annulus one slightly to the right of the midline. The incisions could then be made in the disc and the needle withdrawn quickly with minimal damage to other tissues. An average of 69.8% of all operations performed were followed by successful metamorphosis and eclosion of the operated animal. One incision was made in the right disc of every operated animal. 5 areas were chosen for the incisions in the thoracic portion of the disc, as shown in the figure. A total of 301 animals survived the operations.

Results. Table 1 summarizes the results of the 5 types of operations. In each of the 5 areas, the most common result of the operation was the deletion of a macrochaet together with its socket, and multiple deletions were not uncommon. Duplication of certain bristles and their sockets was also observed, and in 2 cases extra macrochaets appeared in positions not parts of the normal complement. Results for each macrochaet are summarized in table 2.

Area I. Incisions into area I produced the highest incidence of macrochaet anomalies of any of the 5 areas tested, and



Location of areas of incision in the thoracic portion of the right dorsal mesothoracic disc, viewed from the dorsal aspect. Fate map positions of macrochaets after Bryant[2]. Abbreviations: ANP: anterior notopleural; PNP: posterior notopleural; PS: presutural; ASA: anterior supraalar; PSA: posterior supraalar; APA: anterior postalar; PPA: posterior postalar; ADC: anterior dorsocentral; PDC: posterior dorsocentral; ASc: anterior scutellar; PSc: posterior scutellar.