Phosphate for 15 min at pH 9.4, and then mounted in glycerine jelly[2]. The PGCs of alternate sections were counted and their locations recorded.

Excluding resorptions, 84 embryos were studied, 1/4 of which were expected to carry the genes *S1/S1^d* and thus express the germ cell defect. Besides the gestational age, each embryo was staged by somite number. Somite counts ranged from 8–17 on day 9, 25–33 on day 10 and 35–43 on day 11. Two embryos of 8 and 11 somites were among the day 10 embryos and thus were classified with the day 9 group for analysis.

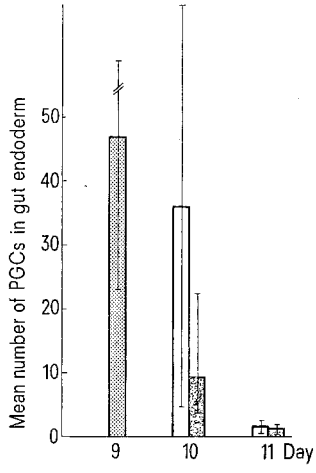

Fig. 3. The decrease in PGCs from the gut endoderm in both mutant and normal embryos indicates an exodus of germ cells from this region after 9 days.

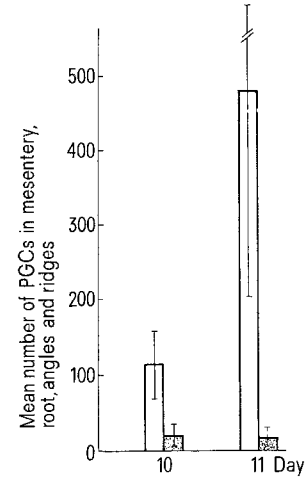

Fig. 4. The infiltration and proliferation of normal germ cells into the dorsal mesentery, mesenteric root, coelomic angles, and gonadal ridges at 10 and 11 days. Mutant PGCs are in these same locations. Few PGCs are in the gonadal ridges on day 10 whereas most are there on day 11.

On day 9, the percent frequency of total PGC counts, as arranged on a log scale (Figure 1), shows that the *S1/S1^d* embryos cannot be distinguished from their heterozygous and wild type littermates. On days 10 and 11, bimodal distributions are evident indicating a segregation within the germ cell population. The number of embryos in the two smaller groups on these days represents 28.8% of the total, which is well within an expected 25% frequency range ($\chi^2 = 0.44$, 1 d.f., $0.5 < p < 0.7$). The germ cell counts found in the smaller groups are presumed to be from the *S1/S1^d* mutants and include all day 10 and 11 embryos having a PGC total of 104 or less.

The means of the day 9 total counts (Figure 2) and the mutant day 10 and 11 counts are not significantly different. However, a dramatic difference exists between the day 9 mean and the means of the day 10 and 11 normal embryos, the two latter representing a 3- and an 8-fold increase respectively.

A reduction in the PGC population from the gut endoderm (Figure 3) is an indication that the mutant germ cells do migrate. In fact, mutant PGCs, although few in number, appear in the same locations on the same days as do normal PGCs (Figure 4). Less than 2% of the normal germ cells reached the gonadal ridges by day 10, whereas after 11 days over 70% populate the ridges. In the mutant embryos, 23% of the germ cells reached the ridges by day 11 and an additional 12% were found in the adjacent mesenteric root and coelomic angles. 44% of the germ cells are located in sites ectopic to the normal path of migration and the remaining 21% are found in the gut mesoderm. Considering that the mutant germ cells do not proliferate it would appear that their rate of migration is comparable to that of normal PGCs. In other words, at least 63% of the PGCs in the normal migratory path will reach the gonadal ridges.

Except for a paucity of germ cells, there was no obvious developmental difference between the mutants and their litter mates from the 9th to the 11th day of development.

The reduced numbers of PGCs in mutant embryos may be due to a failure in proliferation or an excessive rate of cell death. There does not, however, seem to be a deficiency in the capacity of the PGCs of mutant embryos to migrate towards the gonadal ridges[10].

Résumé. Les souris qui sont homozygotes pour la mutation Steel (*S1*) sont stériles, due à l'absence des cellules germinales dans des gonades adultes. Les gonocytes mutants, malgré que leurs nombres sont faibles, émigrent par la voie normale.

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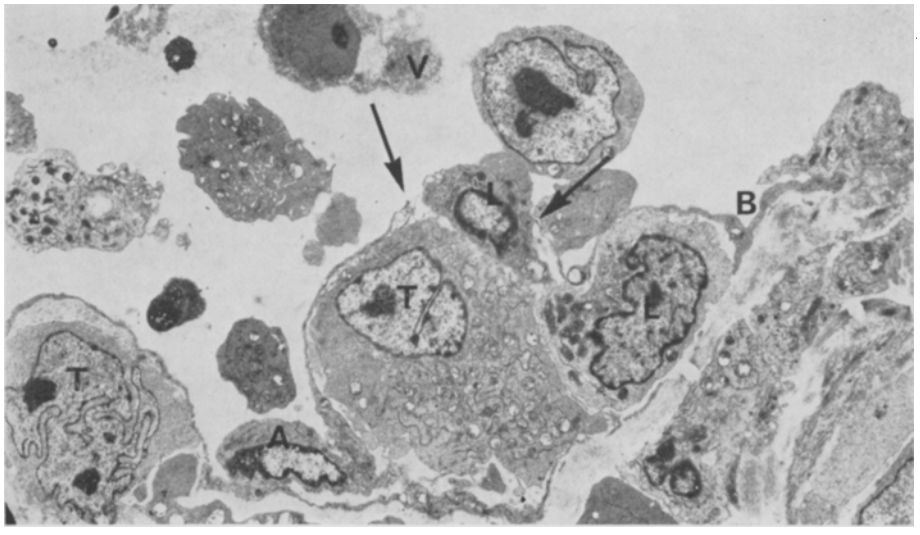
Reverse Diapedesis; the Mechanism of Invasion of Lymphatic Vessels by Neoplastic Cells

The common human cancers kill by metastasis, initially by lymphatic metastasis. Because experimental animal tumours rarely metastasize to lymph nodes the phenomenon of lymphatic metastasis has been little studied. The way in which tumour cells initially penetrate lymphatic vessels is not known. Possible mechanisms include access through major deficiencies in the lymphatic wall or open ended lymphatics, enzymatic destruction of lymphatic endothelium and active cellular movement (diapedesis) between lymphatic endothelial cells.

In a model of lymphatic metastasis described elsewhere[1,2], Rd/3 tumour cells are injected into the rat



Electron micrograph of the wall of a lymphatic vessel at the edge of the tumour mass present in a rat footpad 7 days after injection of 20 million Rd/3 cells into the footpad. Tumour cells (T) and lymphoid cells (L) are migrating through the lymphatic wall, into the lumen of the lymphatic vessel (V). The endothelium is invaginated between points (A) and (B) and there is an open gap between endothelial cells at the points arrowed. ×3200.

footpad and metastasize to the ipsilateral popliteal lymph node; direct intralymphatic injection has been excluded. The model is a fair, though accelerated model of natural tumour metastasis. In this model continued recruitment of tumour cells from the primary site to the lymph node occurs. The present report describes the ultrastructure of the lymphatic vessels at the edge of the footpad tumour 7 days after injection of 20 million tumour cells into the footpad using glutaraldehyde-osmium fixation, araldite embedding and transmission electron microscopy of thin sections.

The lymphatic endothelial junctions are normally closed; beneath the lymphatic endothelium, there may be seen sometimes a basement membrane and usually an interlacing network of collagen fibres through which neoplastic cells have to penetrate to gain access to the lymphatic. Rd/3 tumour cells in suspension or in non-metastasizing situations are usually of circular or ovoid profile. Around lymphatic vessels on the other hand they are elongated with organelle-free pseudopodia at both ends.

Tumour cell processes and tumour cells lie in patent inter-endothelial gaps with endothelial cytoplasm applied closely to tumour cell cytoplasm (Figure). Collagen fibres very close to the tumour cells show no significant changes; on morphological grounds it seems unlikely that the tumour cells secrete a collagenase which acts to destroy the collagen barrier. However short-range collagenolytic activity cannot be excluded. The direction of movement of the neoplastic cells is virtually certainly from without inwards because experiments have shown consistent metastasis in this direction. Macrophages and lymphocytes migrate between endothelial cells in a similar way either alone, or with tumour cells, so that the lymphatics come to contain as many lymphoreticular cells as tumour cells. It seems likely that tumour cells and lymphoreticular cells gain access to the vessels independently and in the same way. Blood vessels are not similarly invaded by tumour cells in this model, possibly because their walls are thicker or not so well held open by collagen bands.

These findings support the view that in this model of lymphatic metastasis, tumour cells gain access to lymphatic vessels, in significant numbers, by a process of reverse diapedesis.

Résumé. Dans un modèle animal de métastasie lymphatique, on a pu démontrer que les cellules tumorales migraient entre les cellules endothéliales pour entrer dans les vaisseaux lymphatiques (diapédèse inversée). Les cellules endothéliales des vaisseaux sanguins ne laissent pas passer les cellules tumorales.

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Macrophage Activation in Mice Lacking Thymus-Derived (T) Cells

T-cell-mediated immunity (CMI) plays a major role in protection from *Listeria monocytogenes* infection in mice. Sensitized T lymphocytes trigger protection by activating macrophages[1–5], which then kill the bacteria. Spleen cells from mice which were adult-thymectomized, lethally irradiated and bone marrow reconstituted (ATx-BM) before immunization do not transfer protection[4], but surprisingly, ATx-BM mice survive primary infection. Evidence is presented here that ATx-BM mice possess activated macrophages before infection, thus explaining this paradox. Macrophages of athymic (nude) mice also show some evidence of activation.

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