nuclei of which were previously labelled by tritiated thymidine. In 4 cases, blastoderms healed completely. They were fixed earlier with Carnoy, already at the head fold stage. Serial sections were prepared for the autoradiographic analysis according to the method of FICQ[7]. Labelled cells were found in the endoblast layer as well as in the mesoblast, but the largest amount went into the endoblast (about 80%). As for the mesoblast, labelled cells took part in the formation of the lateral and paraxial mesoblast, especially the head mesenchyme and the heart primordia (Figure 3B).

All these results show conspicuously that the post-nodal area of the full grown streak did not contribute to the notochord formation even after the node removal. Therefore, the notochord formed under these conditions at the expense of cells of the perinodal upper layer, which were still able to invaginate. Though the origin of the notochord in nodeless chick embryos has been established, it does not mean that all questions relative to the regulation are solved, since the node of the full grown streak yields more foregut cells than mesodermal cells (NICOLET[8]). As regards this aspect of regulation, the contribution of the post-nodal area to the endoblast formation was abnormally great after the node excision: 80% instead of 40%. The anterior tip of the foregut also contained labelled cells, while this area does not normally contribute to the building of the anteriormost part of the foregut (NICOLET[8]). Hence the node excision had elicited a noticeable loss of presumptive foregut cells. In any event this lack was partially filled up by cells coming from the post-nodal area[9].

Résumé. Le nœud de Hensen excisé de la ligne primitive achevée peut être reconstitué par les cellules chordales présomptives encore non invaginées au moment de l'operation et par les cellules endoblastiques provenant du segment postnodal de la ligne primitive.

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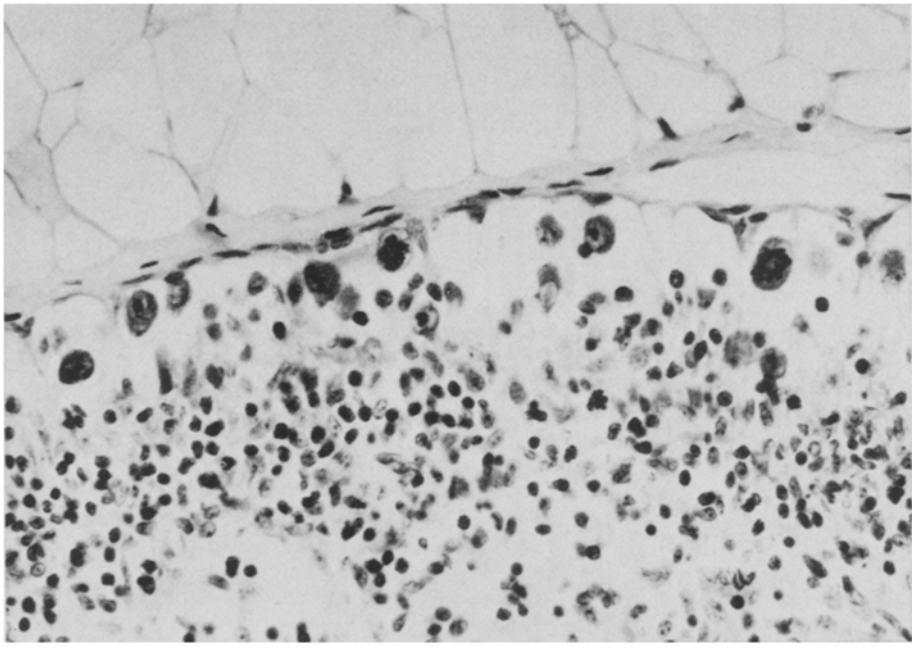
7 A. FICQ, in: *The Cell* (Academic Press, New York 1959), p. 67.
8 G. NICOLET, J. Embryol. exp. Morph. *23*, 79 (1970).
9 This work was generously supported by the Swiss National Foundation for Scientific Research, Bern (Switzerland).

Lymphatic Metastasis of Transplantable Animal Neoplasms

Experimental metastasis of neoplasms to lymph nodes is not easy to produce. The relatively few published reports of experimental lymph node metastasis often involve direct intralymphatic injection of tumour; even in those which do not this possibility has not been excluded (review by FISHER and FISHER[1]). This report describes a simple reproducible model of lymphatic metastasis, in which intralymphatic injection has been shown not to occur.

The RD3 tumour is a dibenzanthracene induced neoplasm carried in a close-bred strain of rats in the University of Sheffield for over 35 years. It is histologically undifferentiated and highly malignant, and is normally carried by implantation of a tumour mush into the flank. Under these circumstances a small metastasis occasionally appears in the axillary lymph node. We have obtained consistent lymph node metastasis by injecting it into the footpad using the following technique.

1 B. FISHER and E. R. FISHER, in *Methods in Cancer Research*, vol. 1 (Ed. H. BUSCH; Academic Press, New York, London 1967), vol. 1.



Popliteal lymph node from rat 24 h after injection of 5 million RD3 tumour cells into the ipsilateral footpad. Tumour cells are present in the subcapsular sinus.

Rats 200–250 g body weight of a closed sub-colony of the above strain were used. The tumour used was harvested 8 days after flank implantation and a tumour cell suspension prepared in Hank's solution at a concentration of 5 million viable cells/100 µl. The viability assessed by dye exclusion was considered satisfactory if above 95%. Tumour cell suspensions were prepared by mincing in a plastic petri dish, in Hank's solution. The fluid was pipetted off, filtered through a coarse filter, and allowed to stand for 5 min to allow fragments to settle. The supernatant was centrifuged at 200 *g* for 5 min and the pellet resuspended in Hank's solution; centrifugation and resuspension were repeated twice. The final suspension was adjusted to give the required concentration.

When a dose of 5 million tumour cells in 100 µl is injected into the footpad, a few tumour cells are present in the subcapsular sinus of the ipsilateral popliteal lymph node 24 h afterwards (Figure), the subcapsular sinus is crowded with tumour cells by 48 h, the medullary sinuses contain tumour cells by 3 days and by 5 days there is subtotal replacement of the node. Further metastasis occurs to the para-aortic nodes by 7 days and, in animals which are allowed to survive long enough, often to the lungs. The incidence of popliteal lymph node metastasis at this dose is over 95%. Complete serial section of 3 ipsilateral popliteal lymph nodes immediately after injection of tumour and 3 lymph nodes 6 h after injection, showed that no tumour cells were directly injected into lymphatics. The presence of tumour cells in the afferent lymphatics of the popliteal node throughout the experiment gave evidence of continuous recruitment of tumour cells. The experiment has been repeated using varying doses of tumour cells and several hundreds of animals. When dead tumour cells are used they do not appear in the lymph node.

The same technique has been tried with other tumours. In a group of 4 hamsters given 6 million cells of an SV40 induced tumour, there was metastasis in the popliteal node in 3 animals; in a group of 10 CBA mice given cells of spontaneous mouse carcinoma, probably of skin appendage origin, there was metastasis in the popliteal node in 5 animals. Both of these tumours showed marked malignancy histologically.

Similar experiments carried out with transplantable spontaneous mammary tumours in 12 C3H mice and 8 Swiss mice, and with an adenovirus induced tumour in 8 CBA mice were unsuccessful. These tumours are histologically less malignant. It appears therefore that some histologically malignant animal tumours will metastasize when this technique is used. Other histologically more benign lesions do not. It seems likely that this technique will be of some value in the study of an important but poorly understood aspect of neoplasia[2].

Résumé. L'injection de 5 millions de cellules tumorales RD3 dans la patte du rat, fait apparaître des dépôts métastasique sous forme de ganglions lymphatiques poplitéaux. L'examen histologique détaillé montre qu'ils sont vraies métastases. Des résultats pareils sont apparus dans 2 autres tumeurs qui étaient histologiquement malignes, mais non dans celles qui étaient bénignes. Cette technique semble convenir à étude de la métastase lymphatique.

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[2] This work was supported by the Cancer Research Campaign (Yorkshire Branch).

On the Determination of Intestinal Epithelial Cell Generation Time from Labeling Index and DNA Synthesis Duration

Two methods commonly used for determination of the generation time of the intestinal epithelium involve 1. construction of a percent labeled mitoses (PLM) curve, or 2. use of the relation Tg = Ts/LI, where Tg is the generation time in hours, Ts is the duration of the S phase (DNA synthetic phase) in hours and LI represents the fraction of labeled cells following a pulse exposure to tritiated thymidine[1].

Sample	Tg (PLM)	Ts (PLM)	LI (%)	Tg(Calc.)
Normal mouse jejunum	13.1 h	7.6 h	56.6	13.4 h
Post-irradiation mouse jejunum	10.3 h	5.6 h	51.7	10.8 h
Normal mouse ascending colon	—	8.5 h	39.0	21.8 h
Normal mouse descending colon	—	7.5 h	39.0	19.2 h
Post-irradiation mouse ascending colon	11.4 h	5.9 h	40.6	11.8 h
Human jejunum	0 h	13.0 h[7]	34.5	37.1 h
Human ascending colon	1–2 days[3,9]	14–20[3,9]	40.6	34.5–49.2

The second method is commonly used with human tissue, where repeated sampling is not possible. However, use of this relationship with the intestinal mucosa results in an overestimate of Tg, primarily because LI, expressed as labeled cells/total crypt cell is artificially low. The low labeling index is the result of substantial non-proliferative compartments in the crypt, primarily goblet cells and maturing columnar cells. A more precise estimate of Tg can be obtained by using the relationship, labeled cell/epithelial cells in the proliferative compartment, excluding differentiated cells. However, PELC[2] has estimated that 30 to 40% of labeled cells in jejunal crypts are not in cycle and do not proceed to mitosis. He conjectured that labeling of these cells is due to metabolic DNA. If this was true, the entire relationship, Tg = Ts/LI, would be invalid as applied to the intestinal mucosa.

Materials and methods. Male C57 BL/J mice, 100–200 days old, were used. One group was injected i. p. with 50 µCi of tritiated thymidine (0.36 Ci/m*M*, Schwarz/Mann), and sacrificed by cervical dislocation at regular intervals. Samples were taken from jejunum, ascending

[1] J. E. CLEAVER, *Thymidine Metabolism and Cell Kinetics* (North Holland Publishing Co., Amsterdam 1967), p. 111.
[2] S. R. PELC, Cell Tissue Kinet. *4*, 577 (1971).