

Reproducibility of female rats following irradiation of the head region at the age of 8 days with different doses of X-rays

Dose of X-rays (R)	At first pregnancy		When 10-17 months old					
	Animals tested (No.)	Fertile animals (%)	Mated animals (No.)	Pregnant animals (No.)	Ratio pregnant/mated	Littering ( $\bar{X}$ )	Litter size ( $\bar{X}$ )	Resorptions ( $\bar{X}$ )
600	15	87	13	7	0.54	1.7	6.9	1.3
700	19	95	18	8	0.44	1.9	7.7	0.7
800	22	91	20	9	0.45	1.4	5.4	1.0
Total irradiated	56	91	51	24	0.47	1.7	6.7	1.0
Controls	32	94	30	28	0.93	1.7	6.9	0.8

in comparison to 94% of nonirradiated controls. The picture becomes strikingly different when the reproducibility of experimental and control animals is followed during the period of 10 to 17 months of their life. Whereas 28 rats from a group of 30 normal animals produced progeny, approximately half of the irradiated animals never became pregnant during this period. However, the other half of the irradiated rats, regardless of the dose applied, reproduced at about the same rate as did the controls. Also, they did not differ significantly from the controls in respect to the litter size and to the number of resorptions.

We have shown earlier that a single whole-body exposure of 8-day-old female rats to 100 R of X-rays produced sterility in 86% of the animals<sup>5</sup>. It is also known that the irradiation of 1-, 2- or 3-week-old mice with a dose as low as 30 R decreases fertility<sup>6</sup>. In this experiment, almost all the irradiated animals proved to be fertile at the age of 4 months or so, and the differences in reproducibility, represented by an equal ratio of normally reproducible and nonreproducible animals, appeared later on. This suggests that the phenomenon of scattering, if any, might have been of minor importance.

None of the phenomena so far mentioned showed dose-dependence. Pronounced dose-dependence ( $r = 0.971$ ), however, was observed when mortality of the animals was followed. Compared with the 94% survival for the controls, the chances for animals that were irradiated with 600, 700 or 800 R to live longer than 17 months were 77, 65 and 36%, respectively. In animals which died prematurely, regardless of their capacity to reproduce, symptoms of cachexia were plainly visible.

The results presented suggest that the irradiation of the head region of 8-day-old female rats with doses of 600, 700 or 800 R of X-rays does not incapacitate the rat

to reproduce during early months of its reproductive life, but significantly reduces chances for such animal to produce progeny when older than 10 months. The results obtained further suggest that the irradiated animals, within the interval of life considered in this work, if reproducible at all, behave like nonirradiated animals in regard to the frequency of littering and to the litter size. In the population of irradiation animals, visible signs of a rapid process of aging were frequent and were accompanied by a high, statistically dose-dependent, mortality rate<sup>7</sup>.

*Résumé.* L'irradiation de la tête des rats femelles âgés de 8 jours à des doses de 600, 700 ou 800 R n'a pas d'influence sur la capacité de reproduction au cours des premiers mois du stade adulte, mais réduit celle-ci quand l'âge de 10 mois est dépassé. Parmi les animaux irradiés, on a souvent constaté des signes visibles de vieillissement accéléré et d'un taux élevé de mortalité dépendent statistiquement de la dose.

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<sup>5</sup> D. SLADIĆ-SIMIĆ, N. ZIVKOVIĆ, D. PAVIĆ and P. N. MARTINOVITCH, in: *Symposium on Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation* (Academic Press, London and New York 1962), p. 327.

<sup>6</sup> R. RUGH and M. WOHLFROMM, *Atompraxis* 10, 511 (1964).

<sup>7</sup> This work was supported by a grant from the Serbian Academy of Sciences and Arts.

## Regulation in Nodeless Chick Blasoderms

In the chick, GRABOWSKI<sup>1</sup> and GALLERA<sup>2</sup> have reported that a complete regulation may occur after node excision. Of course such a result implies that a new node is formed, and the main question is to know-how this reconstitution takes place. GRABOWSKI<sup>1</sup> and BUTROS<sup>3</sup> have assumed that the node reorganizes itself at the expense of presumptive notochordal cells still included in the upper layer all around the node at the stage of operation. Accordingly, GRABOWSKI<sup>1</sup>, as well as GALLERA<sup>2</sup>, have established that blatoderms did not succeed in regulating completely, if

the node was removed at the head process stage, a stage at which all notochordal cells are already invaginated. BELLAIRS<sup>4</sup> has suggested that the streak area of the full grown streak located behind the node may play this role

<sup>1</sup> C. T. GRABOWSKI, *J. exp. Zool.* 133, 301 (1956).

<sup>2</sup> J. GALLERA, *C. r. Ass. Anat.* 49, 632 (1964).

<sup>3</sup> J. BUTROS, *J. Embryol. exp. Morph.* 17, 119 (1967).

<sup>4</sup> R. BELLAIRS, in: *Developmental Processes in Higher Vertebrates* (Logos Press, London (1971)).

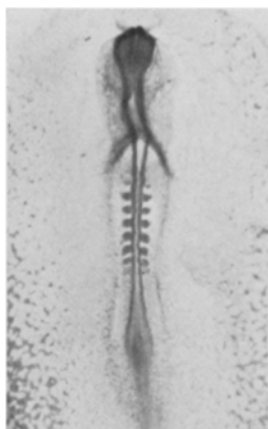


Fig. 1. After the node removal, the wound has healed completely and the blastoderm has given rise to a perfectly normal embryo.  $\times 14$ .



Fig. 2. Though the wound healing was incomplete in this case, a fairly normal embryo formed, but its trunk was divided in two parts by a long slit.  $\times 14$ .

after the node removal. For our purposes, it will be useful to call this area the post-nodal area of the streak. She puts forward this suggestion as a consequence of the older experiments of ABERCROMBIE<sup>5</sup>. He had cut the anterior third of the full grown streak, put it in place again after he had submitted its cephalo-caudal axis to a rotation of 180°. In many cases, a complete regulation was obtained. This hypothesis is the more attractive as the post-nodal fragment can differentiate into notochord when explanted into the area opaca (GALLERA<sup>2</sup>).

At present we have tried to test these 2 hypotheses. The experiments were performed on chick blastoderms cultured in vitro (NEW<sup>6</sup>). In 29 blastoderms, the node, included in a square with a side of 0.3 mm, was extirpated

at the full grown streak stage. Before describing, we should mention that the wound healing did not occur so easily in vitro as the peripheral outgrowth led to the enlargement of the hole. To slow down this tendency, blastoderms were maintained after operation for 4 or 5 h at 25°C. Meanwhile the wound became smaller and its edges thickened. The wound was not entirely closed when they were returned at the normal temperature of incubation. Nevertheless, the healing was sufficiently advanced to be achieved in 2 h at 38°C. Their development was observed step by step up to the head fold stage to follow the main events linked to the regulative processes. About 24 h later, all blastoderms were fixed with Bouin or Carnoy and studied later on serial sections.

The 29 blastoderms were divided into 3 series. In the first, nodes of 17 blastoderms were excised. Their development was studied with great care. The head process formation was clearly delayed: it appeared only 3 to 5 h after the complete healing. In 11, the wound succeeded in closing entirely and the subsequent development was perfectly normal (Figure 1). In 6 cases, the healing was incomplete. In such conditions, the head process always appeared in front of the wound. Later on the peripheral outgrowth, as well as the regression movement, elicited the enlargement of the wound. This incomplete healing did not prevent the formation of fairly well organized embryonic axis (Figure 2). At the wound level the notochord went along the right or the left side of the wound to reach finally the regressing node.

In the 8 blastoderms of the second series, a Nile blue mark was applied on the post-nodal area of the streak just prior to the node excision. At the beginning, the mark was square-shaped with a side of about 0.3 mm. At the head process stage, the new node reconstituted itself in front of the mark which meanwhile became V-shaped (Figure 3A). The area confined in the arms of the mark was too slightly coloured to ascertain that it was actually due to post-nodal cells which have crept forward.

To establish more accurately to what extent the post-nodal area contributes to the formation of axial structures, node excision preceded by exchange of the post-nodal fragment was performed in the third series. The area used for the exchange came from blastoderms, all

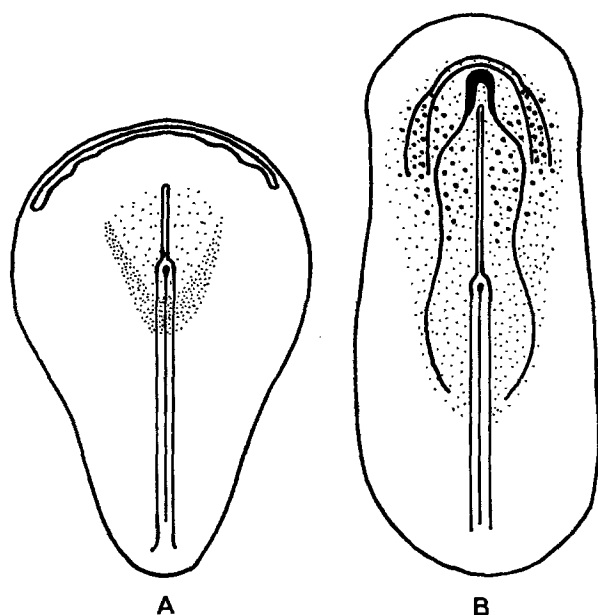


Fig. 3. The Figure 3A shows the localization and the shape of a Nile blue mark which was applied on the post-nodal area of the streak. At the head process stage, the mark became V-shaped. The Figure 3B summarizes the results of the third series. At the head fold stage, most of the labelled cells were found in the embryonic endoblast (light dots) and the other labelled cells took part in the formation of the head mesenchyme or the heart primordia (heavy dots).

<sup>5</sup> M. ABERCROMBIE, Phil. Trans. R. Soc. [B] 234, 317 (1950).

<sup>6</sup> D. A. T. NEW, J. Embryol. exp. Morph. 3, 327 (1955).

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<sup>7</sup>. 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<sup>8</sup>). 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<sup>8</sup>). 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<sup>9</sup>.

*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'opération et par les cellules endoblastiques provenant du segment postnodal de la ligne primitive.

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<sup>7</sup> A. FICQ, in: *The Cell* (Academic Press, New York 1959), p. 67.

<sup>8</sup> G. NICOLET, *J. Embryol. exp. Morph.* 23, 79 (1970).

<sup>9</sup> This work was generously supported by the Swiss National Foundation for Scientific Research, Bern (Switzerland).

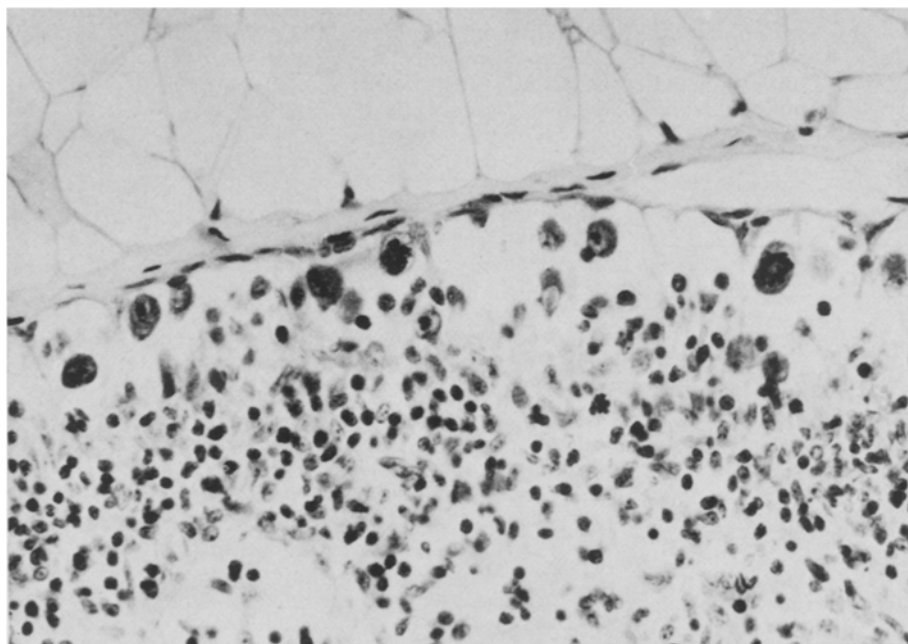
### Lymphatic Metastasis of Transplantable Animal Neoplasms

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<sup>1</sup>). 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.

<sup>1</sup> 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.