Cancer and Super-Regeneration in *Triturus viridescens* Limbs

Waddington[1] and Needham[2] have suggested that cells should be less susceptible to cancer-inducing influences if they are part of an individuation field, a somatic region which exerts as yet uncharacterized morphogenic controls to maintain the cellular components within it as an integrated structure. These authors also suggested that the individuation field might be able to control a cancer placed within it. The regeneration-competent amphibian limb represents a persistent individuation field in a post-embryonic organism, and has been widely used in attempts to test both the above hypotheses[3–6]. The present authors have demonstrated[7] that normal adult *Xenopus laevis* kidney implanted in the non-amputated forelimbs of recently-metamorphosed members of the same species will elicit the development of both lymphosarcomas and accessory limb structures (super-regeneration). We have also shown[8] that mounds of 3-methylcholanthrene crystals, which augment the incidence of lymphosarcoma in *Xenopus*, when implanted within amputated or non-amputated forelimbs of *Xenopus*, will also initiate this dual response of lymphoid cancer from lymphoid cells attracted to the site and accessory limb structures from regeneration-competent cells freed from the limb tissue architecture. While *Xenopus* is an unusual anuran in that it does regenerate forelimbs amputated after metamorphosis[9], it is not as efficient at the process as postmetamorphic urodeles, such as *Triturus viridescens*. Thus, it might be argued that the *Xenopus* limb failed to control the development of lymphosarcoma within it, because it represents only a weak regenerating system.

The present report concerns an experiment using lymphosarcoma and the urodele limb. A population of *T. viridescens* was chosen for the experiment after two of its members had been killed and found to contain extensive lymphosarcomas. 20 newts from the infected colony were implanted with freshly biopsied kidney from normal, adult *Rana pipiens*. The implants were made subcutaneously in the dorsal portion of the radio-ulnar region of the right forelimb, a site used in previous research[10] which had demonstrated that *R. pipiens* xenogenic kidney grafts can serve as powerful inducers of super-regeneration in urodele limbs. Some hosts died early in the experiment, and only the 12 recovered between 41 and 68 days after implantation will be discussed in this analysis, since grossly visible, accessory limb structures became prevalent at this time and the purpose of the experiment was to look into the interaction of cancer and super-regeneration. 9 of the 12 right forelimbs had developed accessory limb structures, and 6 of these 9 had also developed lymphosarcomas which were integrated with the regenerating systems. Figure 1 shows a grossly visible super-regenerate, the lymphsarcoma generally distributed within it and the differentiating skeleton of the accessory limb. Figure 2 shows that muscle as well as cartilage differentiates within an accessory structure, although it is infiltrated by the lymphoid tumor cells. 3 of the limbs developed accessory limbs but had no lymphosarcoma, while the remaining three limbs analysed bore lymphosarcomas but failed to respond to the implants by producing super-regenerates. 11 of the 12 hosts possessed generalized visceral lymphosarcomas involving the liver, spleen and kidneys, the sites typically affected by this cancer in *Xenopus* and the other amphibian species previously tested[11]. The high incidence of cancer in the hosts cannot be attributed to the implantation procedure itself, since unoperated control animals taken from the same infected population showed a similarly extensive

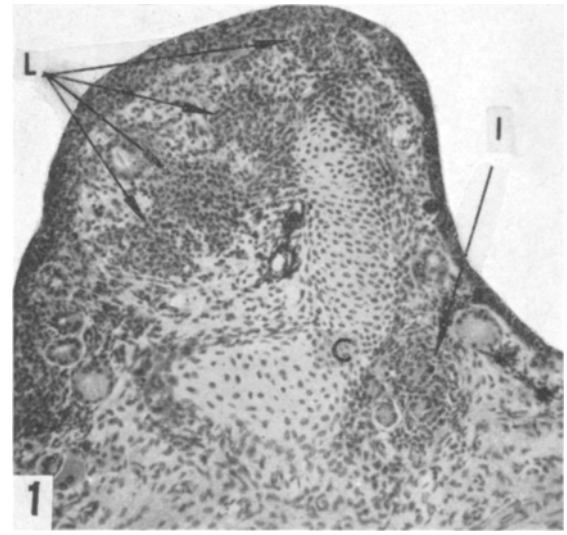

Fig. 1. A longitudinal section through a dorsally directed (top) super-regenerate. The remains of the xenogenic kidney implant (I), newly-developed cartilaginous skeleton (C) and multifocal lymphosarcoma (L) within the accessory structure may be observed. About × 100.

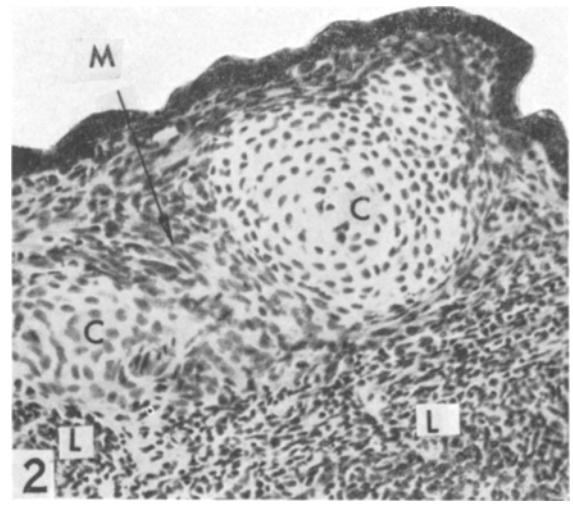

Fig. 2. A longitudinal section through super-regenerate cartilage (C) and muscle (M). The normal accessory limb tissues were effectively separated from the remainder of the host limb by extensive lymphosarcoma (L). About × 120.

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occurrence of lymphosarcoma (13 of 16). Two separate control series were also run, involving the implantation of *R. pipiens* kidney into the right forelimbs of *T. viridescens* from uninfected populations, but no lymphosarcomas were obtained.

We consider these results to support the view that we have expressed previously[8], namely, that an individuation field, as represented by a regeneration-competent amphibian limb, is not capable of controlling cancer developing within it, if the cells forming the cancer do not possess limb tissue-forming capacities. That circulating and wandering cells find themselves within the limb does not necessarily mean that they are subject to control by the individuation field[12].

Résumé. Du rein de *Rana pipiens* adultes a été transplanté dans les membres antérieurs de vingt *Triturus viridescens* qui étaient en train de développer des lymphosarcomes. Parmi les 12 pattes analysées, six étaient infiltrées par le cancer et avaient répondu à la présence des greffes xénogéniques en formant des structures accessoires de la patte. Nous en avons conclu que le champ de régénération n'est pas capable de contrôler le développement de ce cancer.

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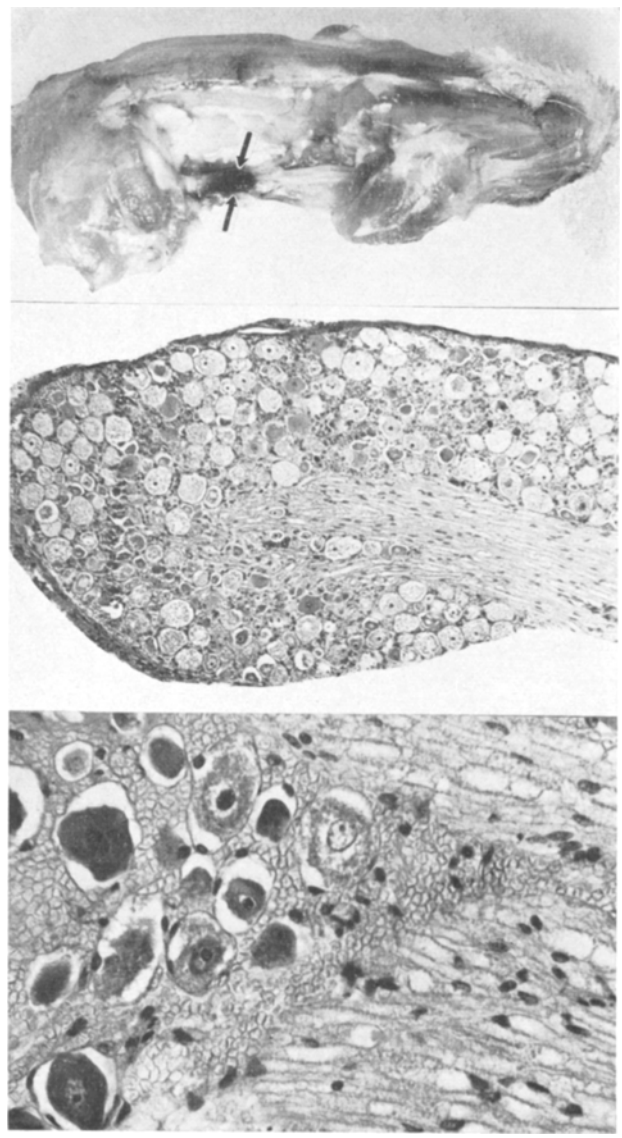
Action of Cadmium Chloride on Sensory Ganglia[1]

Cadmium is known as a highly toxic metal which is employed for several industrial purposes. While studying the pharmacology of certain metallic compounds, we noted that, in the rat, the subcutaneous or intravenous administration of cadmium chloride produced severe lesions of the Gasserian ganglion and of the spinal sensory ganglia. Here we would like to report the work performed with a view to investigating this phenomenon more thoroughly.

In the first experiment 80 Sprague-Dawley rats (40 males and 40 females) with a mean body weight of 189 g (range 180–198 g) were divided into 4 equal groups and given different amounts of cadmium chloride ($CdCl_2$, Fisher Scientific Co., Fairlawn, N.Y., U.S.A.) subcutaneously in the back, always in 1 ml of water at the doses indicated in the Table. All animals were killed three days after the injections. For the second experiment, we injected 25 female rats of 190 g (range 182–201 g) with 3.5 mg of cadmium chloride in 1 ml of water subcutaneously in the back and killed 5 animals 30 min, 1, 3, 5, and 24 h respectively after the injections to study the chronological development of the lesion.

As can be seen in the Table, ganglionic lesions were found in all the groups treated with the different doses of cadmium chloride. The percentage of incidence was similar in males and females. Macroscopically, the first signs were observed 5 h after the injection and consisted of hemorrhagic spots under the capsule; 24 h later the affected organs appeared dark red because of massive hemorrhages sharply localized in the ganglionic tissue (Figure). Oc-



Hemorrhagic necrosis of sensory ganglia induced by cadmium chloride. Top: Macroscopic aspect of the Gasserian ganglion. The necrosis is sharply limited to the ganglionic tissue (arrows). Middle: Necrosis of the nerve cells in a spinal ganglion (Susa, PAS, × 120). Bottom: Pycnosis of nuclei and lysis of cytoplasm in the Gasserian ganglion cells which appear surrounded by hemorrhages (Susa, PAS, × 460).

[1] This work was supported by the John A. Hartford Foundation and the Medical Research Council of Canada.