Stimulation of cell division in ectopic liver tissue following partial removal of the lung

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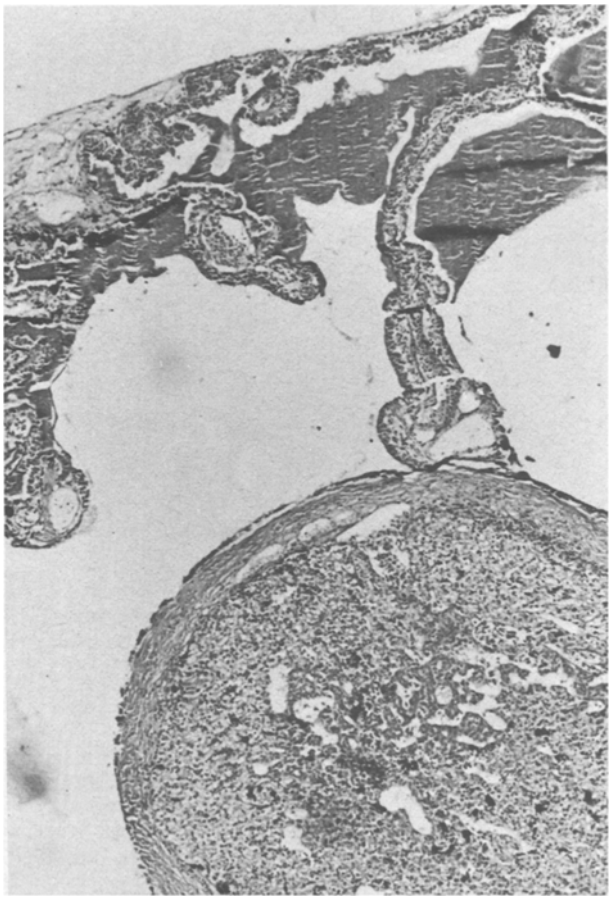
Summary. Autografts of liver were implanted into the left lung (*Xenopus laevis*). Subsequent removal of the right lung stimulated increased mitotic activity in the lung and in the liver graft.

Injury or partial removal of tissue is generally followed by increased cell production and compensatory growth in the remaining tissue of that organ[2]. Most evidence suggests that this response is tissue or organ specific: for example injury to the liver causes a response in the liver but in no other organ. While there is no general concensus as to the nature of the homeostatic mechanism controlling the response, most hypotheses make the following common assumptions[3]: 1. that the control is mediated by some form of chemical growth regulator, 2. that each organ or possibly tissue has its own specific growth regulator and 3. that, in cases where the site of response is distant from the site of injury (for example the hyperplasia of 1 kidney which follows damage of the contralateral kidney), these chemical regulators may circulate in the blood and form a 'systemic pool'.

As the result of a series of experiments on compensatory growth in the mammalian lung[4–6] we have questioned these assumptions[3]. In a further experiment we showed[7] that increased cell division in a piece of kidney tissue grafted into the lung could be stimulated by removal of the contralateral lung. This casts doubt on the accepted concept of the organ-specificity of the response. However, before this result can be considered relevant to general theories of compensatory growth regulation it must be shown that it is a more general phenomenon rather than a specific case pertinent only to that particular combination of grafted tissue and host organ.



The liver graft is a rounded compact mass, enclosed by a capsule of connective tissue, attached to the alveolar septa of the surrounding lung tissue.

Materials and methods. 2 autografts of liver tissue, each measuring 4 × 1 mm were implanted into the left lungs of each of 13 immature Xenopus laevis frogs (trunk length 25–35 mm) using a technique described elsewhere[7]. 28 days later 7 animals were anaesthetized and the right lung removed and fixed in Carnoy's fluid. At the same time the remaining 6 (control) animals were subjected to a 'sham operation' in which the right lung was exposed but not removed. After a further 2 days all 13 animals were sacrificed and the following tissues taken for fixation: right lung (present in control animals only), left lung, kidney and liver. Mitotic counts were made on 6 µm sections stained with haematoxylin and eosin, slides being randomized before counting. Accurate identification of each cell proved difficult and differential counts according to cell type were not attempted. The mitotic index (MI), expressed as the proportion of mitoses (prophase to telophase) per 10^4 cells, is therefore a composite value covering a number of cell types within each organ.

Results. All animals survived the grafting operations and unilateral pneumonectomy. Liver grafts, usually fused into 1 mass, were identified in the lungs of all but 1 of the 13 animals implanted (figure); they contained blood vessels and there were no areas of necrotic or degenerating tissue.

Removal of the right lung caused a 2.4 × increase in the MI (table) in the left lung and also a 90% increase in the MI of liver grafts situated in the left lung. There was no significant difference between the livers of unilaterally pneumonectomized and control animals but in the kidney the MI was 37% lower in the unilaterally pneumonectomized animals. The MI in liver grafts was lower than in the intact liver.

Discussion. The increased cell division observed in the lung following unilateral pneumonectomy is consistent with previous observations in Amphibia[7,8] and in mam-

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mals[5,9]. The increased cell division in the liver graft cannot be interpreted as a general nonspecific stimulatory effect: no such increase was observed in the liver in situ while in the kidney unilateral pneumonectomy seemed to produce a slight decrease in mitotic incidence, possibly due to the stress of the operation. The mitotic stimulation thus appears to be confined to the 'lung field' but within that field any tissue may respond. The fact that results previously obtained for kidney grafts[7] have now been repeated for liver grafts suggests that this may be a general phenomenon.

It is therefore suggested that the compensatory response may be 'field specific' rather than strictly organ- or tissue-specific. This has a bearing on hypotheses for the control of the compensatory response since these are largely based on the concept of organ specificity. Injury to tissue is almost always followed by an increase in the rate of blood flow through homologous tissue and we have suggested that this may stimulate mitosis by causing a change in the local concentration of mitotic regulatory factors[3,7]. This hypothesis could explain the results obtained in the kidney[7] and liver grafts since these shared a common blood supply with the host organ and would hence have been affected by any change in the rate of blood flow following unilateral pneumonectomy.

Increase of blood supply to the liver in situ was not found to stimulate liver growth[10], an observation which may appear inconsistent with our hypothesis. The liver has a system of anastomoses between the larger branches and tributaries of the hepatic artery, portal vein and hepatic vein[11] and diversion of blood via these anastomoses reduces flow through the small vessels of the sinusoids[11,12]. We would suggest that it is the rate of flow of blood through the small vessels, with their intimate contact with the hepatic tissue, that regulates the potential for increased cell division. Because of the system of anastomoses, an increased blood supply to the liver[10] would not necessarily produce in increased flow through the small vessels of the sinusoids.

The rate of cell division in the liver grafts was lower than in the liver in situ, a result consistent with previous results on mitosis and DNA synthesis[13] and growth[14] in ectopic liver grafts. In view of the proximity to the well-vascularized capillary bed of the surrounding lung tissue it seems unlikely that this effect was due to ischemia and the explanation may be found in the absence of normal bile drainage or nerve connections. Stimulation of hepatic cell division was observed in the absence of any injury to the liver graft or to the liver in situ. We consider this inconsistent with the hypotheses[15,16] which propose an organ-specific humoral control system.

Mitotic indices (proportions of mitoses per 10^4 cells) and SD in organs of Xenopus laevis 2 days after removal of the right lung (unilateral pneumonectomy)

Organ	Mitotic index Unilateral pneumonectomy	Control
Left lung	6.2 ± 3.7 ($p < 0.05$)	2.7 ± 1.2
Right lung	2.2 ± 1.2 (NS)	2.2 ± 0.3
Liver graft	6.6 ± 1.1 ($p < 0.001$)	3.7 ± 1.0
Liver	16.1 ± 3.5 (NS)	14.7 ± 2.9
Kidney	9.5 ± 2.9 ($p < 0.05$)	13.8 ± 3.0

The number of animals in the unilaterally pneumonectomized group was 7 and in the control group 6. The value for each animal was the mean of 5 sample mitotic counts. Significance of differences (p-values in parentheses) was calculated by Student's t-test with the number of degrees of freedom (11) taken as the number of animals minus 2. For the difference between liver and liver grafts $p < 0.001$.

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Effect of botulinum toxin on the choline acetyltransferase activity in salivary glands of cats[1]

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Summary. The choline acetyltransferase activity of submandibular glands that had previously received a retrograde injection of botulinum toxin via their ducts was found to be markedly lower than in the untreated contralateral glands. In the parotid glands exposed to the same treatment the activity of this enzyme was less affected.

Botulinum toxin causes paralysis of peripheral cholinergic mechanisms, and this is considered to be the consequence of impaired release of acetylcholine from the nerve endings[2]. In the cat, Emmelin[3] showed that injection of the toxin through salivary ducts causes the submandibular and parotid glands to develop within a few weeks a supersensitivity which lasts for several months, similar to that found after parasympathetic denervation. The acetylcholine-synthesizing enzyme, choline acetyltransferase, is confined to the cholinergic nerves, and it was wondered whether the botulinum toxin would have any affect on the activity of this enzyme. Therefore intraductal injections of botulinum toxin have been made into submandibular and parotid glands of cats, and their choline acetyltransferase activity was determined after different time periods. At the end of each experimental period, the sensitivity to chemical stimuli was tested in order to assess the efficiency of the original injection of botulinum toxin.

Material and methods. 14 cats of either sex, weighing 2.0–4.4 kg, were used. Under nembutal anaesthesia (36 mg/kg i.p.), either the submandibular duct or the