

Changes in mitotic rate during compensatory renal growth in *Xenopus laevis* tadpoles after unilateral pronephrectomy

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In many organs partial extirpation is followed by compensatory hypertrophy in the residual tissue which is generally brought about by a temporary increase in the rate of cell division. After unilateral nephrectomy in the rat, cell proliferation in the contralateral kidney starts after a latent period of 24 h and reaches its maximum rate about 24 h later (Goss & Rankin, 1960). Similar results were obtained in immature metamorphosed *Xenopus laevis* after partial extirpation of liver and kidney. In this case the increased cell proliferation starts at 3 days and reaches a maximum 6 days after the operation (Chopra, unpublished results). Although the timing of the response may thus vary according to the experimental conditions the pattern of a latent period followed by a rapid increase in the mitotic rate seems to be a general phenomenon.

Two main hypotheses have been put forward to explain the mechanisms by which compensatory growth is regulated. One favours the idea of tissue-specific growth regulators, compensatory hypertrophy being accounted for by a decrease in the concentration of a growth inhibitor caused by the extirpation of the organ (Bullough, 1965). According to the other hypothesis compensatory response may be due to the increased functional load on the residual tissue of the partially extirpated organ (Goss, 1964). Recent work involving kidney grafting (Fox, 1960) and parabiosis of normal and partially nephrectomized animals (Johnson & Vera Roman, 1968) has suggested that renal hypertrophy is mainly due to functional overload and that in some cases the role of postulated specific mitotic inhibitors may be discounted.

In the present work attempts have been made to re-examine this problem through experiments with the regenerating *Xenopus laevis* larval kidney. There are two main features which make this a suitable material for this type of experiment: at NF stage 48 (Nieuwkoop & Faber, 1967) the larvae contain two types of kidney, the pronephros and the mesonephros which have different embryological origins and which may possibly contain different growth regulators; and

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at this stage the pronephros is functional while the mesonephros does not appear to start functioning until later.

MATERIAL AND METHODS

Adult *Xenopus laevis* males and females were made to breed by injection of gonadotrophins and fertilized eggs were collected and reared up to NF stage 48 (early feeding tadpole). Both stock and experimental larvae were fed on nettle powder and cleaned twice a week.

At this stage it is possible to see the pronephros under the dissecting microscope ($\times 12.5$), lying on either side of the body at the junction between the gill pouches and gut region. Experimental larvae were anaesthetized with 0.1 % MS 222 and the pronephros of one side was completely removed by cauterization with fine tungsten needles attached to a crystal-controlled diathermy apparatus. Subsequently the larvae were allowed to recover and heal in Niu and Twitty solution for 4–6 h. The procedure is fairly simple and a survival of 80–85 % can be obtained by practice. After healing of the wounds the larvae were divided into groups of ten and each group transferred to a Pyrex vessel containing 400 ml of standing tap water at a temperature of 16–18 °C. Controls were treated in a similar manner but no operations were performed.

When tadpoles are placed in a Colcemid solution (0.5 mg/20 ml water) mitosis is successfully blocked at metaphase in all tissues (Chopra & Simnett, unpublished results) and this technique is the basis for our present study of the rate of mitosis.

Mitotic incidence (MI) was measured in groups of nephrectomized and control larvae at 1, 3, 6 and 10 days after the operation and in the nephrectomized animals additional measurements were made at 2 and 4 days. Each group contained five larvae (nephrectomized or control), with the exception of the 10-day nephrectomized group which only contained three. On the appropriate day each group was treated separately for 4 h in 20 ml of a solution containing 25 μg of Colcemid per ml. The larvae were then fixed for 20 h in Worcester's fluid (10 % acetic acid and 10 % formaldehyde in saturated mercuric chloride) after which their heads and tails were removed to leave the trunk region containing pronephros and mesonephros. The material was dehydrated in cellosolve, embedded in paraffin wax, sectioned serially at 7 μ and stained in Weigert's haematoxylin.

In each tadpole the number of arrested metaphases was counted in two samples of 1000–1500 nuclei each, one for pronephros and one for mesonephros. The proportion of arrested metaphases to total nuclei, i.e. the mitotic incidence (MI), was calculated in percentage for the pronephros and mesonephros of each tadpole and mean and standard deviations for each group were estimated from these values. When calculating mitotic rate from the MI obtained after Colcemid treatment the proportion of metaphases in untreated tissue should generally be

taken into account. However, since according to our findings this value was very small it has been disregarded and MI is taken as representing the proportion of cells entering mitosis in a 4 h period. From stage 53 the pronephros begins to degenerate (Nieuwkoop & Faber, 1967) but there appears to be no cell loss at the stage used in the present experiments. The doubling time was therefore calculated from the mitotic rate on the assumption that even if cell loss did occur in the pronephros and mesonephros the rate was too low to constitute a significant source of error.

RESULTS

The data for MI of pronephros and mesonephros in control and pronephrectomized larvae are given in Table 1 and these results are plotted graphically in Figs. 1 and 2.

Table 1. *Mitotic incidence in pro- and mesonephros after unilateral pronephrectomy compared with control values in non-pronephrectomized animals*

Days after pronephrectomy	Pronephros		Mesonephros	
	Control	Pronephrectomy	Control	Pronephrectomy
1	0.55 ± 0.24	1.60 ± 0.76	3.57 ± 2.40	5.60 ± 1.58
2	—	2.61 ± 1.17	—	10.31 ± 3.18
3	0.57 ± 0.27	0.48 ± 0.15	3.20 ± 1.24	2.44 ± 1.31
4	—	0.46 ± 0.25	—	5.20 ± 0.99
6	0.47 ± 0.17	0.42 ± 0.14	2.25 ± 0.86	4.64 ± 0.59
10	0.13 ± 0.07	0.23 ± 0.15	1.34 ± 0.36	1.10 ± 0.03

Mitotic incidence (MI) in control larvae. Both in respect of pronephros and mesonephros no statistically significant difference was found between the MI for the 1st and 3rd days. For these 2 days the mean MI in mesonephros (3.38 %) was 6 times that in pronephros (0.56 %), which corresponds to doubling times of 118 h and 714 h respectively. The doubling time in pronephros is considerably longer than the time required for the whole larva to double its size, which agrees with the fact that at this point in development the pronephros, though functional, has virtually attained its maximum size and will soon start to regress (Fox, 1963; Nieuwkoop & Faber, 1967).

During the 10-day experimental period there was a gradual but significant decrease ($P < 0.02$) in the MI of both organs. However, even at the end of this period the MI in mesonephros (1.34 %) corresponded to a doubling time of 300 h, indicating that this organ was still growing. By this time the MI in the pronephros (0.13 %) corresponded to a doubling time of 3100 h, showing that the growth of the pronephros had almost ceased.

Mitotic incidence in experimental larvae. As expected, unilateral removal of

the pronephros was followed by a highly significant ($P < 0.001$) increase in the MI of the contralateral pronephros and a similar response was also found in the unoperated mesonephros. In both organs the increased MI was apparent 1 day after the operation, reaching a maximum on the 2nd day. On the 1st and 2nd days the MI in pronephros was 1.6% and 2.61% and in mesonephros 5.6% and 10.31% respectively. This rapid response in the larval organs contrasts with the situation in newly metamorphosed *Xenopus laevis*, where in the liver and mesonephric kidney the peak response was not obtained until 6 days after partial ablation (Chopra & Simnett, unpublished results). By the 3rd day the MI in operated larvae had returned to the level found in the controls and subsequently no significant difference between control and experimental values was found.

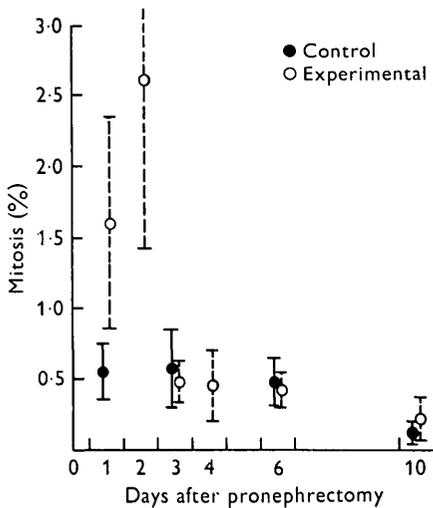


Fig. 1

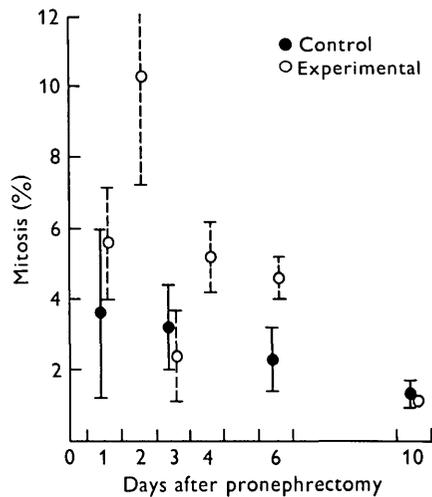


Fig. 2

Fig. 1. Pronephros. Mean mitotic incidence and standard deviation. Data from Table 1.

Fig. 2. Mesonephros. Mean mitotic incidence and standard deviation. Data from Table 1.

Relative degrees of compensation in pronephros and mesonephros. In order to establish if the extent of compensation for pronephrectomy differed in these two organs the MI in experimental larvae was compared at different time intervals with the corresponding values in control animals. The first step in this procedure was to construct from Table 1 a graphical line of best fit (Moroney, 1956) for the control values in pronephros and mesonephros. The experimental values at different intervals after operation were then divided by the corresponding control values derived from the graph to give the factor by which the experimental value exceeded the control value. These statistics are presented in Table 2.

The mitotic response occurred mainly on the 1st and 2nd post-operative days

and during this period the extent of the compensation was significantly higher in the pronephros ($P < 0.02$). At first it appeared that this could be due to a delayed response in the mesonephros, but on comparing all values up to the 10th post-operative day this hypothesis had to be rejected since the ratio was higher in pronephros for the entire period of the experiment (Table 2). It would therefore seem reasonable to conclude that the pronephros gives a persistently higher response than the mesonephros.

Table 2. *Relative degrees of compensation in pro- and mesonephros after unilateral pronephrectomy: the factor by which values for mitotic incidence in pronephrectomized larvae exceed those in non-pronephrectomized controls*

Days after operation	Pronephros		Mesonephros
1	2.49		1.65
2	4.58		3.12
Mean of days 1 and 2	3.54	$P < 0.02$	2.34
3	0.96		0.84
4	1.04		2.03
6	1.12		2.10
10	0.97		0.69
Mean of days 1-10	1.86		1.72

The proportion of cells reduplicating during the period of increased mitotic incidence. When the data for MI in pro- and mesonephros at 1, 2 and 3 days after the operation were plotted graphically, asymmetric curves were obtained which were integrated to give the total proportions of cells dividing during the period of increased mitotic incidence. From these values were subtracted the proportions of the original populations (derived from control values) which would have divided during the normal growth process. This gave the proportions by which the two cell populations increased as the result of unilateral pronephrectomy: 21% for the pronephros and 74% for the mesonephros. Complete restoration of tissue after unilateral pronephrectomy would involve the production of a mass of cells equal in number to 100% of the cells in one pronephros. To compare this value with the actual figures obtained for compensation in pro- and mesonephros it was necessary to measure the relative volumes of the two organs and to compare the number of cells per unit volume. The volumes of pronephros (one side only) and mesonephros (entire organ), measured from serial sections, were 3.27×10^7 and $3.08 \times 10^7 \mu^3$ respectively. Allowing for errors in measurement, these two values can therefore be taken as approximately equal, which means that a correction for size differences between pro- and mesonephros is not required. In contrast, the number of cells per unit volume of solid tissue in mesonephros was 5.2 times higher than in pronephros. Thus, a 74% increase in the cell population of the mesonephros will produce a

mass of cells equivalent to 5.2×74 or 385 % of the cells present in the one remaining pronephros. We consider that this almost four-fold increase is well outside the range of possible experimental error and it thus appears that the mesonephros overcompensates for unilateral pronephrectomy.

As shown previously, the mitotic response is higher in the pronephros in terms of the difference between experimental and control values. However, the mesonephros has higher control values and contains a much larger number of cells than the pronephros. Consequently, even though the increase in MI after pronephrectomy is proportionately smaller in mesonephros the number of cells produced is much higher than in the pronephros.

Although no direct measurements of over-all size were made for the compensating pro- and mesonephros it is highly probable that an increase in cell number produces an equivalent increase in organ mass. We would therefore predict a 21 % increase in the mass of the remaining pronephros and a 74 % increase in the mesonephros. Since the whole mesonephros is, as we have shown, approximately the same size as one pronephros the total increase in mass will be equal to 95 % of the extirpated pronephros—virtually complete restoration. It appears that the increased rate of mitosis serves to restore not the original number of cells but the original mass of tissue.

DISCUSSION

An attempt will now be made to evaluate our data obtained for compensatory growth in *Xenopus* larval kidney after unilateral pronephrectomy in terms of the two main hypotheses for growth regulation: that growth is due to a temporary deficiency of a mitotic inhibitor or chalone (Bullough, 1965) or to the increased physiological load placed on the organ (Goss, 1964).

Unilateral pronephrectomy resulted in an increased MI both in pronephros and mesonephros, and if we suggest that this compensatory growth is due to a temporary depletion of organ-specific mitotic inhibitors (Bullough, 1965) we must also suggest that at least some of these substances must be common to the two types of larval kidney. Some evidence in support of this theory is provided by the observation that the MI of explanted *Xenopus laevis* embryonic pronephros is depressed by the addition of a cell-free extract of adult *Xenopus* mesonephric kidney to the culture medium (Chopra, unpublished).

Since the mesonephros is believed to be non-functional at the larval stage used in the present experiments it might be argued that the increased MI in the mesonephros could not be a response to functional overload, a point in favour of the organ-specific regulator hypothesis. A similar inference has been made in the case of the regenerating pre-functional metanephros of the 12- to 13-day chick embryo (Goss, 1964). In our opinion this reasoning has several weak points. Once an organ has differentiated to the state where it contains the structural elements appropriate to its function—and this is true for the mesonephros

of NF stage 48 larvae—conclusive evidence would be required to prove that the organ was completely without function. All that can be said in such circumstances is that no functional activity can be detected. Moreover, even if the organ could be shown to be non-functional there seems to be no *a priori* reason why the appropriate physiological stimulation should not evoke a response in it or even cause it to function prematurely. Accordingly, the mitotic response of the mesonephros obtained in our experiments cannot necessarily be interpreted as an argument against the physiological overload hypothesis.

The observation that compensation is greater in the pronephros can apparently be accounted for by either hypothesis. If physiological load were the determining factor we would expect compensation to be greater in the functional pronephros than in the prefunctional mesonephros. According to the alternative hypothesis we might expect some differences between the two organs in respect of their specific regulatory products, in which case the pronephros would respond more readily than the mesonephros.

Other authors claim to have obtained more definite evidence in favour of the role of functional overload (Fox, 1957, 1960). After unilateral blocking of the pronephric duct in larval urodeles the contralateral organ increased in size. Since no pronephric tissue was removed it was argued that there could have been no depletion of the postulated growth regulators and that hypertrophy must have been due to functional overload. In a separate series of experiments Fox (1960) grafted pronephric rudiments into unilaterally pronephrectomized urodele larvae. He suggested that if the grafts contained growth regulators these would be liberated into the circulation, thus preventing hypertrophy of the host kidney. In fact, the host kidney did increase in size and Fox concluded that growth regulators were unimportant, the hypertrophy in this case being due to the graft not functioning and the host kidney hence being subjected to a functional overload.

Recently the evidence for humoral control of compensatory renal hypertrophy was re-examined by parabiosis experiments between normal and unilaterally nephrectomized rats (Johnson & Vera Roman, 1968). The compensatory response measured by the autoradiographic labelling index for DNA synthesis in the nephrectomized partner was similar to that in non-parabiosed nephrectomized animals, while in the intact parabiotic partner there was no significant increase in labelling index as compared with intact non-parabiosed rats. The authors concluded that if the kidney produced a humoral factor regulating mitosis this would have circulated between the two partners and that the labelling index should have been the same in both animals. They therefore postulated that kidney hypertrophy must be controlled not by humoral mitotic regulators but by functional overload.

In these experiments it has been tacitly assumed that the experimental technique has no significant effect on the rate of exchange or diffusion of the postulated mitotic regulator. It is possible that this assumption may be unjustified, as may be seen if we try to interpret the difference in growth patterns

between some other organs in terms of Bullough's (1965) mitotic inhibitor or chalone hypothesis. In some, e.g. the epidermis (Bullough & Laurence, 1960), the lens epithelium (Reddan & Rothstein, 1966) and the lacrymal gland (Goss, 1964), there is a purely local response, whereas in others such as the kidney and liver (Goss, 1964) and lung (Romanova, 1960) there is usually a response in homologous tissues situated at some distance from the site of the tissue extirpation. This difference could be explained in terms of the rate of breakdown of the regulator. Where this is high no appreciable systemic pool would be formed and the response would consequently be purely local. Conversely, where the rate of breakdown is low a systemic pool would be formed and in this case tissue extirpation would lead to a depletion of this pool and consequently to a response in distant homologous tissues. One must therefore take the stability of the regulator into consideration. An impairment of blood supply to and drainage from an organ might cause a significant decrease in the amount of regulator released into the blood stream. Blocking of the pronephric duct might conceivably result in blood being diverted to the contralateral pronephros, while grafting or parabiosis might produce circulating concentrations of regulators which were less efficient than those released by the kidney in its normal situation. Johnson & Vera Roman (1968) found that in rats joined by a carotid-jugular anastomosis an almost complete equilibrium between the two circulations was achieved 30 min after the injection of a radioactive tracer. However, the half-life of many hormonal growth regulators may be less than 5 min (Diem, 1962), and if the postulated tissue-specific growth regulators were equally unstable a balance between the two parabiostic partners could not be achieved. The reduced rate of vascular exchange via the anastomosis was recognized by Kurnick & Lindsay (1968) as a possible factor to be considered in the interpretation of results from parabiosis experiments. The assumption of unimpeded circulation of postulated regulators therefore seems open to challenge, and for this reason it is difficult to accept the arguments presented by Fox (1957, 1960) and Johnson & Vera Roman (1968) as conclusive evidence in favour of the functional overload hypothesis.

Controversy still exists as to whether compensatory renal growth is due principally to an increase in cell size or in cell number. Following unilateral pronephrectomy in *Ambystoma* larvae Fox (1960) observed an increase in the tubular cell size without any significant increase in the size of the cell population. In the present work on *Xenopus laevis* larvae the mitotic response actually attained a peak on the 2nd day and declined to the control level by the 3rd day. Consequently Fox's failure to demonstrate an increased cell production may be due to the fact that his measurements were not made until 10 days after the operation. Goss & Rankin (1960) have recorded a mitotic peak 48 h after unilateral pronephrectomy in mice but from measurements made on the rat Johnson & Vera Roman (1966) have demonstrated that only 25% of the increase in organ mass was due to cell division, the remainder being due to an increase in cell size. It seems probable that increase both of cell size and cell number occurs

in the compensating kidney, but that the relative importance of these two factors may vary according to the experimental material. In *Xenopus* larvae the increased mitotic rate by itself was sufficient to achieve almost complete restoration of the extirpated tissue.

In conclusion our results show that compensatory hypertrophy following unilateral pronephrectomy in *Xenopus* larvae is mainly due to an increase in cell number and that the data can be explained equally well by the mitotic regulator or the functional load hypothesis.

SUMMARY

1. Early feeding tadpoles of *Xenopus laevis* (NF stage 48) were subjected to unilateral pronephrectomy and the mitotic incidence (MI) in the mesonephros and remaining pronephros was measured using the Colcemid technique.

2. In both organs an increase in MI was apparent 1 day after the operation and maximum values were obtained after 2 days. By the 3rd post-operative day the MI had returned to the level found in the controls.

3. In terms of the difference between nephrectomized and control values the pronephros exhibited a greater response than the mesonephros, but since the mesonephros had higher control values and a larger number of cells per unit volume of tissue the absolute number of cells produced in the mesonephros in response to the operation was much higher than in the pronephros.

4. The total number of cells produced during the period of increased MI was approximately 4 times the number contained in the extirpated pronephros, but since these were mainly small, densely packed mesonephric cells it was estimated that the mass of tissue produced would be approximately equal to that of the extirpated pronephros.

5. The data can be interpreted equally well on the basis of either of the main hypotheses for the control of growth and regeneration: regulation by tissue-specific growth inhibitors or by the degree of physiological activity required of the tissue.

RÉSUMÉ

Modifications de la vitesse mitotique pendant la croissance rénale compensatrice à la suite d'une pronéphrectomie unilatérale chez le têtard de Xenopus laevis

1. De jeunes têtards de *Xenopus laevis* (NF stade 48) ont été soumis à une pronephrectomie unilatérale et l'incidence mitotique (MI) dans le mésonéphros et dans le pronéphros restant a été mesurée par la technique de Colcemid.

2. Dans les deux organes, une augmentation du MI est apparue le lendemain de l'opération et les valeurs maximum ont été atteintes après 2 jours. Le 3e jour postopératoire, le MI est retombé au niveau des témoins.

3. En termes de différence entre les valeurs du néphrectomisé et du témoin, le pronéphros montre une réponse plus grande que le mésonéphros. Mais étant donné que le mésonéphros aurait des valeurs témoins plus élevées et un plus

grand nombre de cellules par unité de volume de tissu, le nombre absolu de cellules produites dans le mésonéphros en réponse à l'opération est beaucoup plus élevé que dans le pronéphros.

4. Le nombre total de cellules produites pendant la période d'augmentation du MI est d'environ 4 fois le nombre de cellules contenues dans le pronéphros enlevé, mais étant donné que ce sont principalement de petites cellules serrées les unes contre les autres, il a été estimé que la masse du tissu régénéré est environ égale à celle du pronéphros enlevé.

5. Ces résultats peuvent être interprétés sur l'une ou l'autre des principales hypothèses concernant le contrôle de la croissance et de la régénération: une régulation par des inhibiteurs de croissance spécifique d'un tissu ou par le degré d'activité physiologique nécessaire au tissu.

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