Cell Degeneration in the Larval Ventral Horn of *Xenopus laevis* (Daudin)

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*With one plate*

Cell death accompanies a variety of developmental processes in many tissues and systems of organs. A comprehensive review by Glücksmann (1951) has revealed how widespread is the occurrence of degenerating cells in vertebrate embryos. Within the developing nervous system, the earliest descriptions of cellular degeneration are due to Barbieri (1905) in Amphibia, and to Collin (1906) in the chick embryo. Since that time, destruction of cells has been observed in the morphogenesis of the neural tube, most recently by Boyd (1955) and by Källén (1955) in early mammalian embryos; in the establishment of regional differences between limb and non-limb-­levels in spinal ganglia of the chick (Hamburger & Levi-Montalcini, 1949), and also in the histogenesis of Anuran ventral horn cells (*Xenopus*, Hughes & Tschumi, 1958; *Eleutherodactylus*, Hughes, 1959).

The present study is also concerned with ventral horn cells in *Xenopus*. Here the aim has been to draw up a cell balance-sheet during development based both on counts of numbers of ventral horn neuroblasts throughout their differentiation, and also of the number of degenerating cells among them at each stage. Attention has been confined to the more posterior pair of ventral horns concerned with the innervation of the hind limbs. It is conveniently referred to as the lumbar ventral horn from its correspondence with that of higher vertebrates. The Anuran ventral horn is well suited for quantitative studies, for from its first appearance it is composed of a discrete group of cells distinct both from the cells of the mantle layer from which the ventral horn neuroblasts are derived, and also from the primary motor-cells which innervate the axial musculature of the larva, and which are present from a much earlier period of development (Hughes & Tschumi, 1958). Again, the total number of ventral horn cells in Anura is far below that found in the spinal cord of higher vertebrates. In the adult *Xenopus* there are only about 1,200 lumbar ventral horn neurones on each side, though even so, during the course of the present work and in the

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experimental studies to be described in subsequent papers, it has been necessary to count over a quarter of a million cells in the lumbar ventral horns of upwards of seventy larvae. Sharrard (1954) has estimated the number of motor-cells in each human lumbo-sacral ventral horn as 24,600.

**MATERIAL AND METHODS**

Eggs and larvae of *Xenopus* have been obtained from the same stock of animals and by the same methods as have been used in previous studies (e.g. Tschumi, 1957). The larvae are staged according to the descriptions given in Nieuwkoop & Faber (1956), although our larvae kept at a lower temperature develop more slowly and are of a smaller size at each stage than those described by the Dutch authors. The length of the hind limb was used as a more exact means of comparison between individual larvae. During the course of this work it became clear that the number of cell degenerations present in the ventral horn at certain stages, particularly around N. & F. 55, depends on the rate at which the larva is developing, which in turn depends not only on the temperature of the environment, but also varies from one larva to another within a batch kept under identical conditions. Accordingly, it became necessary to adopt standards such as are expressed by the parallel scales of stages, length of hind limb, and time of development in Text-fig. 1. These refer to an average rate of growth for larvae kept at 20° C., and reared as far as possible under uniform conditions.

Tadpoles were fixed either in Bouin’s fluid at half strength, or in an aqueous formol-acetic fixative. To ensure adequate penetration it was found necessary at the time of fixation to cut transversely through the body-axis some distance to either side of the lumbar ventral horn, and to expose the spinal cord from below by opening the sheath of the notochord. An intact notochord may contract during preparation to such an extent that the cord becomes laterally compressed. Sections were prepared from this material and stained either in Ehrlich’s haematoxylin and eosin, or by the Feulgen technique. Cells were counted under a 4 mm. apochromatic objective with a squared grid within an eyepiece of the microscope. A schematic reconstruction of the ventral horn in each section series was prepared by counting the number of ventral horn cells on either side in each section, and representing these figures graphically as points on a series of parallel co-ordinates, at right angles to which the axis of the spinal cord was projected in terms of the number of sections in the series (Text-fig. 2). Through the points thus plotted, an outline was drawn which shows the average number of cells present at all levels throughout the ventral horn. The area between the curve and the longitudinal axis of the graph represents, in appropriate units, the total number of viable cells present. These areas were estimated by counting squares. Along the axis of each graph the distribution of degenerating cells in each ventral horn was also indicated.
TEXT-FIG. 1. Numbers of viable cells and of degenerations in lumbar ventral horn of *Xenopus*, both larval and adult. The scales of stages, of hind-limb length, and of time refer to the standard course of development for which this data applies. The scale of cell death-rate is based on a degeneration time of 3·2 hours.
**OBSERVATIONS**

The ventral horn cells

The earliest appearance of the lumbar ventral horn in *Xenopus* at stages N. & F. 50–52 has been described by Hughes & Tschumi (1958). The ventral horn neuroblasts are clearly distinguishable from the cells of the mantle layer, both in position and appearance, for their nuclei are larger and within them the stainable material is dispersed through a greater volume of nuclear sap, so that they appear lighter in colour than the nuclei of the mantle cells. These criteria make it possible to distinguish the two types of cell even at the cranial and caudal poles of the ventral horn, where the number of ventral horn cells gradually decrease until they no longer protrude laterally into the white matter.

The nucleus of the ventral horn cell steadily increases in size during development (Plate, figs. 1–4). In early stages (N. & F. 51–52) it is markedly ovoid, with a long axis of about 10 μ; the nucleolus is then between 1 and 2 μ in diameter. Subsequently, the nucleus of the ventral horn cell becomes more rounded as it enlarges, and the nucleolus increases markedly in size. In the largest ventral horn cells of the adult animal the nucleus is over 20 μ in diameter, with a nucleolus
of nearly 4 μ. A chromosomal network is always visible within the nuclear sap in material stained with haematoxylin, but in Feulgen preparations the increase in the volume of the nuclear contents has diluted the DNA of the nucleus by stage 56 to such an extent that there is only a faint purple apparent, while elsewhere in the cord smaller nuclei are deeply stained.

After the earliest phases of differentiation of the ventral horn, the constituent cells do not develop further at a uniform pace, but exhibit a wide range of stages among them. New cells which continuously join the ventral horn from the mantle layer maintain a proportion of the smallest neuroblasts.

The cytoplasm of the most advanced ventral horn cells first becomes conspicuous at about stage N. & F. 53. The cells are then obviously bipolar with a dense cap of cytoplasm over each pole of the nucleus. By stage 56 the majority of the ventral horn cells show a positive Koelle reaction for cholinesterase, and in ultra-violet photomicrographs it is then evident that the cytoplasmic nucleotides are no longer compact but have a flocculent distribution. They are then assuming the form of the Nissl substance (Hughes & Lewis, 1960).

Cell degeneration

The course of degeneration of a nucleus in the neural tube of a human embryo was described by Glücksmann (1930), and it was compared with observations on the same process in other material in his subsequent review (Glücksmann, 1951). Whereas in the human neuroblast nuclear sap is expelled into the cytoplasm in the earliest recognizable stage of degeneration ('feinen Pyknosierung'), the first phase in the Anuran ventral horn nucleus occurs within an uncontracted nuclear membrane (Plate, figs. 5, 6, 11). The earliest stage of all (Plate, fig. 5) precedes any alteration in cytoplasmic texture, and resembles the first signs of a normal prophase. Were it not for the fact that ventral horn cells are all post-mitotic, it would not be possible to assign this early phase to nuclear degeneration rather than to nuclear division. The aggregation of Feulgen-positive material within such a nucleus, however, soon becomes too coarse for a nucleus in prophase (Plate, figs. 6, 11). The earliest stage of all (Plate, fig. 5) precedes any alteration in cytoplasmic texture, and resembles the first signs of a normal prophase. Were it not for the fact that ventral horn cells are all post-mitotic, it would not be possible to assign this early phase to nuclear degeneration rather than to nuclear division. The aggregation of Feulgen-positive material within such a nucleus, however, soon becomes too coarse for a nucleus in prophase (Plate, figs. 6, 11). This material may either remain for some time in separate blocks (Plate, figs. 6, 11) or may clump around the nucleolus into a single mass, distinguishable from a normal nucleolus both by its larger size (Plate, figs. 7, 8) and by its dense reaction to the Feulgen reagent. Next, the cytoplasm begins to shrink in volume and the nuclear membrane contracts. At this stage the basophilic material of the cytoplasm becomes concentrated in a perinuclear ring of densely staining material, which superficially resembles that seen in cells during periods of active synthesis. The nuclear membrane is then dissolved and the nuclear contents are set free as a dense Feulgen-positive granule (Plate, fig. 10). The cytoplasm also breaks up into rounded droplets, which stain more lightly in haematoxylin preparations than does the nuclear granule. By this time microglial phagocytes have appeared in the neighbourhood (Plate, fig. 12) and then proceed to engulf the cellular
remains. The site of a recently degenerated neurone is often indicated by a gap among the ventral horn cells. Among the smaller nuclei of the mantle layer, the same course of degeneration can be recognized, though the early stages are less conspicuous than in the ventral horn. In late larvae scattered throughout the mantle layer pycnotic nuclei in their final granular form are a striking feature of Feulgen preparations.

Quantitative observations

To assist in judging the significance of the data which are presented below, some points in the procedure observed in counting cells in this material must first be discussed. To enumerate a class of objects in the field of a microscope it is necessary not only to count them but in the first place to distinguish them from other structures. A uniform definition of each category must then be maintained. Within the ventral horn of Xenopus, the larger neurones are clearly distinct from the cells of the mantle layer. At the other extreme, however, are some neuroblasts which approximate in size and appearance to the mantle cells; these have presumably only recently migrated into the ventral horn. Only those cells are reckoned as ventral horn cells in which the nucleus is considered to be larger than those of the adjacent mantle neuroblasts. Cells are counted solely by their nucleoli, for in this way errors arising by the spread of a cell through adjacent sections are minimized (Hughes & Tschumi, 1958).

The earlier phases of cell degeneration are also clearly distinguishable, but in the final stages, which follow the collapse of the nuclear membrane, the dense knot of chromatic material which condenses round the nucleolus can be confused with the nucleus of a microglial phagocyte. Microglia are first found among the ventral horn cells after stage N. & F. 54, at a time when cell degenerations first become conspicuous. In enumerating degenerating cells, the smaller dense residues of chromatin have not by themselves been counted unless accompanied by some paler droplets of cytoplasmic material or found within a space vacated by the recent degeneration of a cell.

A further difficulty is that the debris which results from the death of a cell tends to spread through adjacent sections. Even if one attempts to allow for this, it is still possible that cells may degenerate in contiguous groups. For these reasons, counts of degenerations tend to be more variable than those of viable cells.

To assess the importance of these factors, a section series through a normal cord at stage N. & F. 56 was chosen, and in one row of sixteen consecutive sections the number of ventral horn cells on each side was counted five times. The mean value of these five counts and its standard error was calculated, and the results are shown in Table 1. Again, through the whole ventral horn the number of degenerations were also counted five times over, and the totals were treated in the same manner. It will be seen from Table 1 that the standard errors expressed as percentages of the mean are about four times larger for counts of
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Degenerations as for those of viable neurones. In neither case does it seem that more than a small proportion of the variability of the numerical results shown in Text-fig. 1 is due to inconsistency in counting.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Side</th>
<th>Mean</th>
<th>Standard error; and the same as percentage of mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable ventral horn cells (sixteen consecutive sections)</td>
<td>Left</td>
<td>270</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>261</td>
<td>6.2</td>
</tr>
<tr>
<td>Degenerations throughout ventral horn (165 consecutive sections)</td>
<td>Left</td>
<td>48</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>63</td>
<td>4.9</td>
</tr>
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</table>

**Viable ventral horn cells.** In the early phase of development of the lumbar ventral horn, with hind limbs up to 3 mm in length, the number of viable cells on each side varies between 3,000 and 4,500 in different individuals (Text-fig. 1). In tadpoles of relatively slow growth the hind limbs are smaller than in other larvae of the same age, though the growth in length of the larvae is less retarded. In such animals are found ventral horns with the largest number of cells.

After stage N. & F. 55, the number of cells in the lumbar horn is closely correlated with stage of development. Their number decreases sharply until about stage 58, when some 1,500 cells are present on each side. From thence until the approach of metamorphosis there is a further slow decline to about 1,200 cells, a number which is maintained into adult life. It is of interest that in the tiny Anuran *Eleutherodactylus* there are about half this number of cells in each ventral horn (Hughes, 1959).

The outline of the ventral horn varies in shape during development (Text-fig. 2). The early horn is relatively short, but then contains more cells at each level than at any later stage (Text-fig. 2A). The number of neuroblasts in each section is at a maximum at a point about two-thirds along the axis. During later development the ventral horn becomes elongated and narrowed, partly by the further growth in length of the whole animal, and partly through the reduction in number of cells in each section (Text-fig. 2 B, C, D). The effects of limb amputation on the distribution of ventral horn cells will be described in a succeeding section.

**Degenerating ventral horn cells.** At stages N. & F. 50–51, when the ventral horn is first discernible, it is free from degenerations. Some neuroblasts undergo cell death at about stage 52, and thereafter the number of degenerating cells in the ventral horn increases sharply to a maximum at about stage 56 (Text-fig. 1). At this time, nearly half the sections through the ventral horn will contain at least one pycnotic nucleus (Plate, fig. 2). Although the total number of degenerations
within the ventral horn then varies widely, there is always a clear maximum at
this point though it is variable in its intensity. After stage 56 the number of
degenerations declines, at first with a rapidity equal to that of the previous rise.
Pycnotic nuclei are relatively rare by stage 58 and disappear entirely after stage
62. The peak in number of degenerating cells thus coincides with the period of
most rapid decline in viable cell number. Moreover, the inflexion of this curve
after stage 58 to a slower rate of loss corresponds with the onset of the later
period of less frequent degenerations.

**Estimation of the time of cell degeneration.** To attempt a further analysis of
these observations it is necessary to know the duration of the observed stages in
cell degeneration. This period may be estimated by modifying the normal rate of
degeneration by some sudden experimental treatment, and observing its sub-
sequent effects along a measured and relatively short time-scale. If it is assumed
that the rate of recruitment from the mantle layer is not significantly altered by
the treatment during this short time, an increased rate of population decline
induced by the treatment directly reflects an increased number of degenerations,
and from this the average duration of degeneration can be calculated. For this
purpose, amputation of a hind limb has been employed at stages when the leg
is from 3-5 to 6 mm. in length.

The effect of removal of the limb area at earlier stages has already been de-
scribed by Hughes & Tschumi (1958), who showed that the differentiation of the
ventral horn in the first place is dependent on some stimulus which emanates
from the limb-bud and is transmitted to the cord along dorsal sensory pathways.
However, after extirpation of the limb area at an early stage, the subsequent
regeneration of even a small limb-bud is sufficient to evoke the formation of
a ventral horn of nearly the normal size, even though the size of the dorsal root
ganglia at the level of the limb plexus is reduced.

The effect of amputation at the thigh of a hind limb 3-5-6 mm. long on the
already partly differentiated ventral horn is to cause a temporary increase in the
number of cell degenerations among the neuroblasts of the amputated side
(Text-fig. 2e). These extra degenerations appear between 1 and 2½ days after
operation (Text-fig. 3). In consequence, the number of cells in the ventral horn
on the operated side falls below that on the other. By the third day, however,
the degeneration count once more becomes even in both ventral horns, and the
deficit in viable cell number may then gradually be made good during the
succeeding days. In order to see whether these effects of amputation on the
ventral horn are statistically valid, it is necessary to see whether the differences
between the ventral horns on the two sides in operated animals are at any time
significantly greater than the random differences in the numbers of viable and
degenerating cells on the two sides of control animals. Accordingly, paired
*t*-tests for significance (Snedecor, 1938) were applied first to the data from a set
of thirteen control larvae, and then to the seven tadpoles of Text-fig. 3 which had
been amputated for periods between 30 and 60 hours. The results obtained are
set out in Table 2. Furthermore, an unpaired $t$-test was used to determine whether amputation on one side exerts any effect on the contralateral ventral horn. Here it is necessary to choose a group of control and amputated larvae all of the same stage, because of the changes in number of both viable and degenerating cells during normal development. Four control and three amputated larvae between 44 and 60 hours, respectively, all at stage 56, were chosen. The data

![Text-fig. 3. Effect of amputation of one hind limb on numbers of viable and degenerating cells in two groups of *Xenopus* larvae from 1 to 8 days after operation. Each pair of linked points represent the differences in these two counts between the two sides. The slope of the line $AB$ drawn through the differences in viable cell number from 1 to 2 days corresponds with a mean rate of loss of cells of 15.4 per hour.](image)

from both sides of the controls were included, as it has already been shown (Table 2A) that there are no significant differences in the ventral horns between right and left sides in normal animals. These results are also included in Table 2.

Of the three larvae described in Text-fig. 3 and fixed 24 hours after the operation, in two no extra degenerations have yet appeared, while in the third the process must only recently have begun, for the ventral horn on the amputated side exhibits an abnormally large proportion of the earliest phases of degeneration. In the eight subsequent examples up to $2\frac{1}{2}$ days after the operation there is an average number of 49.0 extra degenerations over this period. If the deficit in the number of ventral horn cells on the operated side begins at 24 hours, it may be considered to progress at a mean rate that can be derived from the data...
for animals between 30 and 60 hours after amputation (Text-fig. 3 A, B). This rate is 15·4 cells lost per hour as the result of the operation.

**Table 2**

*Statistical tests of data from ventral horn counts*

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<tr>
<th></th>
<th>Viable cells</th>
<th>Degenerations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degrees of freedom</td>
<td>Mean difference</td>
</tr>
<tr>
<td>A. Paired t-test of left and right horns in thirteen control larvae</td>
<td>12</td>
<td>45</td>
</tr>
<tr>
<td>B. Paired t-test of left and right horns in eight amputated larvae</td>
<td>7</td>
<td>318</td>
</tr>
<tr>
<td>C. Unpaired t-test between total numbers in four controls and contra-lateral sides of three amputated larvae, all at stage 56</td>
<td>9</td>
<td>330</td>
</tr>
</tbody>
</table>

From these figures a time of degeneration may be inferred as follows. If cell death occupied 1 hour, and every hour only one cell entered degeneration, then the number of degenerations visible would always be unity. Longer periods of degeneration would increase the number proportionately, as would the death of larger numbers of cells. Hence if 49·0 degenerations are seen and correspond with a rate of cell loss of 15·4 per hour, the time of degeneration is 3·2 hours. This estimate is comparable with that of 1·3 hours quoted by Glücksmann (1951) for neuroblasts in the larval frog retina, and of 7 hours for mammalian tumour cells. In the larval ventral horn of *Xenopus* the proportion of the degenerating cells at early stages with an intact nuclear membrane varies around 4 per cent., a figure which suggests a duration for this phase of the process of 8–9 minutes, though this estimate leans heavily on the assumption that cells enter degeneration at a steady and even rate.

Among other errors to which these estimates are subject, we neglect the regenerative movement of cells from the mantle layer into the ventral horn by means of which an even number of cells is restored on the two sides in subsequent days. We have no evidence when this movement begins, though from the data for the third day after operation and onwards, it seems rapid only for the more advanced tadpoles. This factor could reduce the cell deficit during the
second day, and so lead to an overestimate of the time of degeneration. It must also be remembered, however, that in the stages of degeneration which are counted the end phases are excluded for reasons of consistency (p. 274). The rate of the final removal of cell debris, as Glücksmann (1951) suggests, may vary with the extent of degeneration.

The cellular mechanism which is responsible for the extra degenerations in the ventral horn following the operation is at present obscure. The course of events shown in Text-fig. 3 holds only for tadpoles during the period of high normal degeneration in the ventral horn, from stages N. & F. 55–57, with hind limbs from 3.5 to 6.0 mm. long. A larva at stage 59 showed no effect of amputation on the ventral horn after 48 hours, and others at stage 54 reacted to the operation more slowly than did the main group of larvae. Dr. P. R. Lewis (unpublished) has made some relevant observations on the spread of neuromuscular junctions through the developing hind limbs, as revealed by the esterase reaction on the whole limb (Lewis, 1958). Cholinesterase is first evident in the thigh region at stage 53, and does not spread distally throughout the leg before stage 56. One factor concerned in the extent to which degeneration is provoked by amputation may thus be the proportion of ventral horn cells whose axones have already entered the limb and which are severed by the operation. The temporary loss of cells after amputation at the thigh affects the ventral horn evenly throughout its length (Text-fig. 2e) and there is no indication at these stages of development of any regional grouping of the ventral horn cells.

The equilibrium in number of ventral horn cells in amputated larvae, which is restored in some by the end of the first week after the operation, persists for a varying time which may extend for a further 2 weeks, during which there may be an actual excess of cells on the operated side. Ultimately, however, there is a second and final loss of cells from the ventral horn, which in animals at metamorphosis is thereby reduced to about a third of its normal size (Text-fig. 1f). The loss of cells is then most severe towards the caudal pole of the differentiated ventral horn, a region which is thus shown to be mainly concerned with the innervation of the distal segments of the limb.

Most larvae with hind limbs 3 mm. and more in length which are amputated at the thigh show little or no attempt at regeneration of the distal segments, either before metamorphosis or in subsequent adult life.

Cell turnover in the ventral horn

Given the estimate of the time of degeneration of ventral horn cells as 3.2 hours, once can place in Text-fig. 1 a second scale alongside that of the number of degenerations in terms of loss of actual numbers of cells, if every 3.2 degenerations observed is equated with a rate of loss of 1 cell per hour. The total number of cells which degenerate within the ventral horn during the whole of its development can then be estimated by measuring the area beneath the curve of degeneration in Text-fig. 1. This was assessed by counting squares in appropriate units.
A figure of 10,150 cells results, which implies that for every neurone which finally differentiates in the ventral horn, between eight and nine other cells have died during development. Little emphasis need be placed on this actual figure. If our estimate of the time of degeneration is too great, then the ratio of cells which degenerate to those which survive is still higher. The main point which emerges is that many more cells degenerate in the ventral horn than can be accounted for by the observed decline in cell numbers during development. This conclusion can only be set aside by assuming an improbably long duration for the process of degeneration.

A continuous migration of cells from the mantle layer into the ventral horn must be an important feature in the development of the spinal cord; some assessment of the scale of this movement can now be made. During the period of most rapid decrease in total cell number in the ventral horn from stages 55 to 58, the slope of this part of the curve drawn by eye in Text-fig. 1 corresponds to a rate of cell loss per day of 160. During the same period an average value for the cell death-rate is 15 per hour, or 360 each day. During that time, therefore, some 200 cells per day must enter the ventral horn from the mantle layer. After this rapid period of cell turnover, from stage 59 onwards to metamorphosis the average rate of actual cell loss from the ventral horn is only 8 per day, but the death-rate is even then nearly ten times this figure. So the migration of neuroblasts continues, even though many mantle cells are then differentiating into neurones.

Although no estimate of the rate of cell loss from the ventral horn in the early period up to stage 55 will be attempted owing to the wide variation in total cell number in the early ventral horn, it is clear from Text-fig. 1 that this rate, and consequently that of the migration of mantle cells into the ventral horn, must then be smaller than during the middle period of its development. It is of interest, therefore, that the restoration of the number of ventral horn cells from the third day of amputation indicated by Text-fig. 3 is more rapid for larvae at the stages when the normal movement of neuroblasts into the ventral horn is most abundant.

DISCUSSION

In his review, Glücksmann (1951) distinguishes three categories of cell degeneration, each of which is associated with a particular aspect of embryogenesis. There can be little doubt that those with which we have here been concerned belong to the group of 'histiogenetic degenerations', and are associated with the differentiation of the ventral horn cell. They are most abundant just at the time when the majority of these cells are synthesizing cholinesterase, and are organizing the cytoplasmic pattern of the Nissl substance. The same is equally true of the mantle layer, though within it degenerations are most frequent and neurones differentiate at a time subsequent to the same events in the ventral horns.
The association of histogenesis in the cord with the occurrence of cell death is illustrated by some experimental evidence. If by heterotopic grafting, a segment of the spinal cord of the *Xenopus* larva is deprived of the influence both of the limbs and of the remainder of the neural axis, no ventral horns are formed, no neurones differentiate within the mantle layer, and nor are any degenerating cells seen within the grafted cords (Hughes & Tschumi, 1960). The mantle layer undergoes a hypertrophy of about twofold.

In discussing these results we tentatively suggested that if cell degeneration had continuously been suppressed within these grafts, this factor alone might be sufficient to account for the abnormally large number of cells in the mantle layer. The present results suggest that the normal migration of cells out of the mantle layer to degenerate within the ventral horns is on a sufficient scale to account for this difference between the grafted and normal cords.

The factors which in general control the number of cells within each tissue and organ during development are at present almost wholly obscure. In the ventral horns we see that a temporary depletion in cell number may be restored after only a few days. The factors responsible for the maintenance of the normal population of ventral horn cells must at this stage be intrinsic to the cord, for the appropriate number is restored in the absence of the leg, even though at an earlier phase of development the limb is responsible for the first appearance of the ventral horn (Hughes & Tschumi, 1958). Once the latter has been induced, and is evident as a discrete group of cells, we can speak of a 'ventral horn field' with its own regenerative capacity.

We are at present however entirely ignorant of the cellular mechanisms at work throughout its development. In the ventral horn there are two separate processes, one the degeneration of cells within it, and the second the entry of fresh neuroblasts, which are governed by some equilibrium which holds the cell population at a certain level at each stage of development. There is evidence that these two processes are to some extent interdependent, for a preliminary study of the effects of radiation on the developing ventral horn (Hughes & Fozzard, 1961) has shown that it is possible to arrest degeneration for some time with little or no effect on the normal changes in cell population.

We do not know whether a neuroblast degenerates wholly as a result of intrinsic instability, or whether some factor external to the cell is in part responsible which the viable cell is able to resist. The tendency for ventral horn cells to degenerate in groups favours the second alternative.

If the concept of cell-turnover, which the present results suggest, be accepted, we then need to know the average duration of survival of the individual cell during development and whether any of the neurones which finally differentiate have been present from the early stage of the ventral horn. We thus need some means of labelling cells as such, a much more difficult matter than labelling the atoms and molecules within them. There are broadly two possibilities. Either the cells which early take up their position and differentiate first thereby become
more stable than later arrivals, or the developing neuroblast at all stages is
equally mortal, or nearly so. It is certainly true that degenerations do occur
among the largest cells of the ventral horn.

This question has an important bearing on the course of maturation of the
individual nerve-cell. The series of changes through which a future motor
neuroblast is thought to pass—outgrowth of the axon, formation of multiple
dendrites, and the later withdrawal of most of the latter—may not so much
represent stages in the differentiation of a single cell as the forms of successive
tenants at foci within the developing nervous system. Close study of the distribu-
tion of individual differences within cell populations at different stages would
be required for an attack on these problems.

SUMMARY

1. The development of the lumbar ventral horn of the spinal cord in the larva
of *Xenopus* is described. The population of viable cells at each stage is counted,
as is also the number of degenerating cells present. Stages in cell death as seen
in the ventral horn are illustrated.

2. A total of between 3,000 and 4,000 cells is reduced to 1,200 by metamor-
phosis. The most rapid period of decline in cell numbers is accompanied by
a peak in number of degenerations.

3. The duration of degeneration was estimated from the early events within
the ventral horn which follow the amputation of a limb. Between 1 and 2½
days after the operation an average excess of 49 degenerations was seen within the
ventral horn on the operated side. These resulted in a continuous loss of
ventral horn cells at a mean rate of 15·4 per hour. These figures suggest a time
of degeneration of 3·2 hours.

4. Given this figure, the total number of cell deaths in the course of develop-
ment of the ventral horn may be estimated. It seems that for every neurone
which finally differentiates, some eight or nine neuroblasts undergo degeneration.
The rate of migration of mantle cells into the ventral horn needed to maintain
its cell population has also been estimated.

5. Possible implications of these observations for the development of the
nervous system are discussed.

RÉSUMÉ

*Dégénérescence cellulaire dans la corne ventrale de la moelle épinière du têtard de
Xénope*

1. On décrit le développement de la corne ventrale lombaire de la moelle
épinière de la larve de *Xenopus*. On dénombre la population de cellules viables
à chaque stade, ainsi que les cellules en dégénérescence présentes au même
moment. Les phases successives de la mort des cellules font l’objet d’illustrations.

2. Un total de 3 à 4.000 cellules se trouve réduit à 1.200 à la métamorphose.
La période la plus active de diminution du nombre des cellules s'accompagne d'un maximum de dégénérescences cellulaires.

3. La durée de la dégénérescence a été estimée à partir des premiers phénomènes consécutifs, dans la corne ventrale, à l'amputation d'un membre. Entre 1 et 2 jours 1/2 après l'opération, on a observé un excès moyen de 49 dégénérescences du côté opéré. Celles-ci aboutissent à une perte continue de cellules de la corne ventrale au rythme moyen de 15,4 par heure. Ces données suggèrent une durée de dégénérescence de 3,2 heures.

4. Ceci posé, on peut estimer le nombre total de morts cellulaires au cours du développement de la corne ventrale. Il semble que, pour chaque neurone qui se différencie finalement, huit ou neuf neuroblastes dégénèrent. On a également estimé le rythme de migration dans la corne ventrale des cellules périphériques indispensables au maintien de sa population.

5. On discute les implications possibles de ces observations sur l'étude du développement du système nerveux.

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**REFERENCES**


EXPLANATION OF PLATE

Figs. 1–4 are of stages in the development of the lumbar ventral horn of *Xenopus*. Right hand of picture lateral to left. Haematoxylin and eosin; all figs. ×220.

Fig. 1. Stage 54.

Fig. 2. Stage 56. Degenerations are visible among the ventral horn cells.

Fig. 3. Stage 59. The peak of degeneration is now over.

Fig. 4. Adult animal.

Figs. 5–12 are of stages in the degeneration of ventral horn cells selected from various larvae near the peak period of degeneration. Haematoxylin and eosin; all figs. ×1,692.

Fig. 5. Prophase-like stage with cytoplasm yet unaffected.

Figs. 6, 11. Nuclear membrane still uncontracted, but with cytoplasmic reduction.

Figs. 7 left, 8. Precipitation of chromatin round nucleolus, with dense ring about nuclear membrane.

Fig. 7 right. Normal neurone.

Fig. 9. The contraction of the nuclear membrane has begun.

Fig. 10. Nuclear membrane has now disappeared.

Fig. 12. Shows end-stage of degeneration, with both cytoplasmic droplets and chromatinic granules, together with two microglia and two normal neurones.

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