Mitotic Pattern in the Chick Pronephric Duct

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WITH ONE PLATE

INTRODUCTION

By means of a variety of ingenious experiments it has been shown that the pronephric duct originates anteriorly and grows caudally to join the cloaca (for review, see Burns, 1955). It has been concluded on the basis of histological study (Lillie, 1919), and from operative experiments in which the pronephric duct path was blocked by grafts (Waddington, 1938), that in the chick the pronephric duct extends posteriorly by proliferation of its own cells. In the present study, the proliferative pattern in the outgrowing duct of the chick has been examined in normal and in colchicine-treated embryos. Results indicate that proliferation is particularly marked towards the posterior duct tip. It was also noted that tensions of the blastoderm (see Spratt, 1956) which are disrupted by colchicine treatment appear to play some part in maintenance of normal diameter and posterior elongation of the duct.

MATERIALS AND METHODS

In this work attention was largely confined to chick embryos at stage 14 (Hamilton & Hamburger, 1951), though in a few cases stages 13 to 15 were included. The material consisted of both normal and colchicine-treated embryos. The normal group included embryos fixed at 21 to 24 somites. In the second group embryos of 20 to 22 somites were treated with colchicine by dropping 1.5 c.c. of colchicine 1:10^4 made up in Tyrode on top of the blastoderm through a window in the shell, resealing the egg, and incubating it 4 hours. With this treatment three-quarters or more of the embryos survived 4 hours as indicated by heart-beat. All were preserved. This group was at a developmental stage comparable to that of the first group despite the longer incubation period, since some cooling occurred during the treatment.

Counts were made of resting and dividing cells in both groups of colchicine-treated embryos. Embryos were sectioned at 10 μ and stained in Harris haematoxylin. Counts were made with an oil-immersion objective. They included

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stages from late prophase to telophase in normal embryos and late prophase to metaphase in colchicine-treated embryos. In the pronephric duct counts were made from the posterior tip to the first branching tubule. The number of mitoses in anterior regions where new tubules join the duct appears to be high, possibly due to inductive activity in this region (see Bellairs, 1955). This anterior level was excluded from consideration. Mitoses were counted in every section of the duct, those occurring in more than one section being recorded only once. The number of resting nuclei in each section was also counted. The mitotic frequency was expressed as mitoses per 1,000 resting nuclei and was calculated for successive units of ten sections, or 100 \( \mu \) lengths of the duct. Since ducts were of different lengths in different embryos, or even on right and left sides of the same embryo, the posterior tip of the duct was always taken as the point of reference in averaging counts from different cases.

Mitotic counts were made in somites and neural tube for comparative purposes at the most posterior level, which included both pronephric duct cords. The counts were made in six alternate sections, in two of which resting nuclei were also counted. The number of resting nuclei was then tripled in estimating mitotic frequency. The total numbers of resting cells in somite or spinal cord which appear in Table 1 are thus three times the number actually counted. In these counts the number of mitoses was usually recorded accurately but the number of resting nuclei occasionally differed by as much as 3 to 5 per cent. in successive counts of the same section. Because of differences in the counting method of mitotic figures in the duct and other regions, duct counts were adjusted for comparative purposes to take into account the section thickness, and it was assumed that the mitotic figure measured 4 \( \mu \) (see Abercrombie, 1946). These adjusted counts were used in calculating the indices which appear in Table 1. This method can, of course, give only a rough measure of mitotic frequency. However, it seems adequate for comparing differences between various embryonic regions, and the mitotic indices obtained here are, in general, consistent with those reported by other workers (Schultz, 1922; Derrick, 1937; Bellairs, 1955).

RESULTS

Spatial mitotic pattern

Counts made throughout the duct and mitotic indices calculated for 100-\( \mu \) duct lengths suggest that to a large extent the duct elongates through terminal proliferation. As indicated in Text-fig. 1 (open circles), average values based on counts in 24 ducts of normal embryos support this opinion. This trend was evident in many but not all individual cases. Such individual variation could be explained by the fact that the cell count for a given antero-posterior location might be as low as 50. When counts were repeated in embryos which had been exposed to colchicine for 4 hours, a marked and consistent posterior increase occurred. Averages for mitotic indices in 16 cases are plotted in Text-fig. 1 (solid
circles). Since there is an over-all increase in the mitotic index in colchicine-treated embryos, the differences between anterior and posterior regions is accentuated. A rough correspondence between the two curves may be noted, since anteriorly the mitotic index increases from about 15 to 60, or four times, and at the tip the increase is from 30 to 135, again approximately four times. A very close correspondence would not be expected since, as will be described below, colchicine treatment produces some morphological distortion of the embryo.

The data indicate that a definite spatial pattern occurs in mitotic frequency and, presumably, in proliferative activity. Thus proliferation would be highest towards the elongating tip.

This general antero-posterior pattern of mitotic activity is not confined to the duct alone, but is evident on inspection in colchicine-treated embryos throughout the axial mesoderm (see Derrick, 1937) and in the neural tube.

Text-fig. 1. Spatial distribution of mitotic activity in the elongating pronephric duct. Open circles, normal embryos; solid circles, colchicine-treated embryos. For explanation, see text.
Relative mitotic frequency

Although mitotic activity is high at the duct tip compared to more cephalic levels, it is not higher than that in other embryonic regions at the same anterior-posterior level. The situation is summarized in Table 1, which compares nuclear and mitotic counts in the most posterior 100-μ length of the duct with those in somite and spinal cord. In both somite and spinal cord the mitotic index in colchicine-treated embryos is 3.7 times the normal value. Although the increase produced by colchicine in the pronephric duct is greater than this, if counts in two unusually high cases, both in the same animal, are deleted, then the value for the pronephric duct is 3.8 times (Table 1, figures in parentheses), suggesting that there is no difference in this regard between pronephric duct, somite, and spinal cord. A comparison of mitotic indices in the three regions indicates that mitotic frequency in the pronephric duct is the same or possibly somewhat lower than that in adjacent mesoderm, while values for both mesodermal regions are lower than that for the spinal cord.

Counts in individual cases showed some over-all variation, most readily explained as due to differences in cooling. Since counts in various regions are compared here between whole groups, this variation is not apparent.

Colchicine distortions

In the colchicine-treated embryo both resting and dividing cells tend to become rounded. Such changes in cell shape are associated with a marked shrinkage of the area vasculosa radially and an antero-posterior contraction of the embryo proper, particularly in the trunk region, combined with abolition of normal elasticity of the blastoderm. A detailed study of these colchicine effects is described elsewhere (Overton, 1958). After colchicine treatment the pronephric duct is shortened and considerably thickened (Plate, fig. A). Effects of the antero-posterior contraction are particularly evident in resulting convolutions of the neural tube (Plate, fig. B). The consequent increase in cell count may be noted.

### Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of cases</th>
<th>Resting nuclei (total)</th>
<th>Mitoses (total)</th>
<th>Mitotic index</th>
<th>Indices colchicine/normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pronephric duct (normal)</td>
<td>24</td>
<td>1,043</td>
<td>32 (123)</td>
<td>41 (155-6)</td>
<td>3.7 (3.8)</td>
</tr>
<tr>
<td>Pronephric duct (colchicine)</td>
<td>16</td>
<td>1,257</td>
<td>173 (172)</td>
<td>192 (344-2)</td>
<td>4.7</td>
</tr>
<tr>
<td>(14)*</td>
<td></td>
<td>(1,105)</td>
<td>(123)</td>
<td>(172)</td>
<td></td>
</tr>
<tr>
<td>Somite (normal)</td>
<td>12</td>
<td>6,261</td>
<td>328</td>
<td>52-4</td>
<td>3.7</td>
</tr>
<tr>
<td>Somite (colchicine)</td>
<td>8</td>
<td>4,932</td>
<td>948</td>
<td>192-2</td>
<td>3.7</td>
</tr>
<tr>
<td>Spinal cord (normal)</td>
<td>12</td>
<td>9,906</td>
<td>929</td>
<td>93-8</td>
<td>3.7</td>
</tr>
<tr>
<td>Spinal cord (colchicine)</td>
<td>8</td>
<td>6,852</td>
<td>2,359</td>
<td>344-2</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* See text.
from data in Table 1. The greatest increase occurs in the pronephric duct where the cell count is approximately doubled. The increase is more moderate in the somite and least conspicuous in the spinal cord.

The shortening of the duct appears to a large extent to be a reflection of the rounding up of cells which are normally elongated in the direction of the antero-posterior axis. It may be contrasted, for example, with effects in the spinal cord where cells tend to be elongate at right angles to the antero-posterior axis. The duct tip, then, is composed of cells which are normally markedly elongate in the direction in which the duct is extending, a condition suggestive of migration. Attempts to study migratory activity of these cells directly by culturing sections of the trunk in plasma clots were unsuccessful. The increase in diameter of the pronephric duct with colchicine treatment must also be in part the result of distortions in other parts of the embryo, since shortening of the trunk may be sufficiently extensive to bend the notochord, for example.

DISCUSSION

Backward growth of the pronephric duct in the chick has been ascribed to proliferation of duct cells on the basis of histological study (Lillie, 1919) and surgical interruption (Waddington, 1938). The 'growth energy' of cells in terminal portions of the duct was noted by Gruenwald (1942) in cases where the duct was damaged a short distance anterior to its growing end and the part caudal to the injury grew normally and persisted. The mitotic pattern in the duct described here is consistent with the results of these operative experiments in suggesting that normally elongation of the duct is due largely to activity of cells at the posterior duct tip. Since a marked antero-posterior gradient in mitotic frequency occurs throughout the axial region, the proliferative pattern of the duct corresponds, at least roughly, to that throughout the trunk, and the mitotic index in the duct tip is similar to that in neighbouring mesoderm.

Although there is an early report of terminal mitoses in the outgrowing pronephric duct of Triton alpestris (Mollier, 1890), this spatial pattern is not characteristic of a number of Ambystoma species (Overton, 1959) in which mitoses appear to be rather uniformly distributed along the length of the duct. The amphibian duct seems to extend posteriorly as a result of the activity of cells throughout its length, while in the chick the actively growing region is more localized.

In colchicine-treated chick embryos there is a conspicuous shortening of the duct and an increase in its diameter. This distortion is most marked in the duct tip, where it results to a large extent from changes in shape of duct cells which become rounded. Duct cells thus tend to be elongate, particularly towards the tip, suggesting that cells here are involved in migration as well as in multiplication. Changes in cell shape in other regions associated with loss of elasticity of the blastoderm and with antero-posterior shortening of the embryo also affect
the arrangement of duct cells indirectly. These distortions emphasize the role of normal tensions of the blastoderm in maintaining the typical diameter and extent of the pronephric duct.

SUMMARY

1. Studies carried out on the stage 14 chick embryo indicate that in normal and in colchicine-treated embryos mitotic frequency is greatest towards the posterior tip of the elongating pronephric duct.

2. Although the mitotic frequency in the duct tip is high compared to that in the duct at more cephalic levels, it is similar to that in other mesodermal regions at the same level.

3. Distortions of the embryo which occur with colchicine treatment suggest that migratory activity is prominent towards the duct tip, and emphasize the dependence of normal morphology of the duct on tensions in the blastoderm.

ACKNOWLEDGEMENTS

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REFERENCES


EXPLANATION OF PLATE

Fig. A. Cochineal-treated chick embryo; cross-section. ca. x 475.

Fig. B. Cochineal-treated chick embryo; fixed preparation. ca. x 37.

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