Number of Neoblasts in the Intact Body of *Euplanaria torva* and *Dendrocoelum lacteum*

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INTRODUCTION

The regeneration blastema in planarians is constituted by the totipotent neoblasts which migrate to the wound (Dubois, 1949). The rate of regeneration, measured by appearance of eyes in the blastema, shows a characteristic variation throughout the planarian body, thus constituting a static, time-graded regeneration field (Brøndsted, 1946). We do not know the mechanism underlying this species-specific feature. Neither do we know why some species, e.g. *Dendrocoelum lacteum*, are able to regenerate a head only from the part of the body lying anteriorly to the pharynx, whereas other species, e.g. *Euplanaria torva*, can regenerate a head from almost every part of the body. A possible explanation might be the number of available neoblasts. This idea was formulated by Curtis & Schulze (1934). They claim that in *Procotyla fluviatilis*, a species closely related to *D. lacteum*, the inability to regenerate a head from parts behind the pharynx is due to scarcity of neoblasts, as compared with species with greater regeneration ability. This claim, however, is disputed by some authors, although supported in varying degree by others (for references, see Brøndsted, 1955).

As far as we know, the actual number of neoblasts in the planarian body has never been reported. We therefore thought it necessary to get quantitative information about the problem, particularly as *Dendrocoelum* and other species (e.g. *Bdellocephala punctata*) with restricted head-regeneration ability are often able to regenerate tail and other parts quite easily from all parts of the body. We also think that a clarification of the problem has some significance for regeneration problems in general.

MATERIAL AND METHODS

*D. lacteum* and *E. torva* from Lake Furesø near Copenhagen were selected on account of their very differing power of head regeneration, as mentioned in the introduction. The animals were fixed in various fixatives; the best proved to be Zenker. Sections were stained with pyronin-methyl green by the method of Kurnick (1952, 1955), embedded in paraffin, and cut in transverse sections of 10 μ in order to transect as few neoblasts as possible without too great impairment.

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of transparency. The neoblasts are rich in RNA (Pedersen, 1959), and in the sections they stand out clearly with an intense dark purple colour against other cell types, which may also be stained by pyronin. During microscopical observation the colour may be intensified by a dark yellow filter. The counting was done at a magnification of 100. To obtain as objective readings as possible, one of us counted the neoblasts in every section throughout the whole body of the animal, the other noted the dictated numbers without comment on any trend in the numbers. Several sections were examined twice at varying time intervals, and the counts of neoblasts never differed by more than 5 per cent. of the total.

We are, of course, quite aware of the error introduced by the risk of counting a transversely cut neoblast twice in two consecutive sections. The error was minimized by omitting a count if a neoblast seemed to be without a nucleus or had only a small portion of one; we therefore deemed it unnecessary to correct our findings by using the formulae given by various authors for proportionality of cell size and thickness of section. We have found no noteworthy difference in nuclear size of neoblasts in the two species, nor in other planarian species we have had occasion so far to investigate; the curves for the two species used here are therefore quite comparable as to the distribution of neoblasts measured with our method.

The volume of the fixed and embedded animals was measured by drawing the contour of every 5th section on squared paper and computing the areas of the sections; the figures found were plotted along the longitudinal axis of the animal (Figs. 1, 2).

RESULTS

*D. lacteum.* In a specimen, 15 mm. in the living and outstretched state, 37,826 neoblasts were found; they often lie in clusters, especially ventrally in the neighbourhood of the large lateral nerve-cords, but a statement of their more precise location is beyond the scope of this paper. Here we present only their distribution along the antero-posterior axis of the animal. Text-fig. 1 shows three curves: one representing the cross-sectional area of the animal along the main axis, in the fixed and embedded state; this curve is somewhat irregular on account of the unavoidable contractions taking place during fixation. The second curve represents the time-graded capability of head-regeneration, and the third curve gives the number of neoblasts. As will be seen, no neoblasts are found in the foremost part of the head region; from here the numbers rise suddenly, reaching a peak somewhat in front of the pharynx; the numbers fall in the pharyngeal region, rising again to a new peak around the genital apparatus, then falling abruptly to the most posterior part of the body.

Two points stand out: the distribution of the neoblasts coincides neither with the volume of the body nor with the capability of head regeneration.

*E. torva.* In a specimen, 15 mm. in the living and outstretched state, 31,320 neoblasts were found. Text-fig. 2 shows two distribution peaks placed in about
the same region as in Dendrocoelum. A slight difference may, however, be noted: the first peak is situated somewhat more anteriorly, and the caudal peak is slightly higher than the cranial. No correlation with cross-sectional area or time-graded field can be observed in this species either.

Fig. 1. D. lacteum. --- cross-sectional area of the animal. --- numbers of neoblasts. — head-regeneration ability along the antero-posterior axis. The scale on the left indicates numbers of neoblasts.
DISCUSSION

The hypothesis of Curtis & Schulze (1934), that the number of neoblasts might be decisive for the capacity to regenerate in species differing in this respect, has to be abandoned. It has been shown in this paper that the number and distribution of neoblasts do not furnish an adequate explanation why some planarian species are able to regenerate heads from every part of the body, although at different rates, whereas other species are able to do this only from the forepart of the body. A. & H. V. Brøndsted (1954) have shown that the rate of head regeneration is the same from small and large segments of the body, but that the size of the blastema formed depends on the neoblasts available (Brøndsted, 1956).

The species-specific character of the time-graded head-regeneration field must therefore be due to other factors, which are still completely obscure. Lender, in a series of papers (1950–6), has shown that the regenerating cephalic ganglia are responsible for eye regeneration. This fact is, of course, of great interest, but does not furnish a clue to understanding the underlying cause of the existence of the time-graded regeneration field. We are still ignorant on the question why the cephalic ganglia during regeneration originate at different rates at the various levels of the planarian body. We think that the fundamental problem of competence is involved, which has still to be unravelled.
Another question, probably quite separate from the one mentioned, is why the neoblasts are distributed in such a characteristic manner. This must involve delicately balanced biochemical processes, and is equally obscure. Both questions are being pursued in our laboratory.

It is interesting to compare our results in planarians with those of Tardent (1952–4) in *Hydra* and *Tubularia*. In these animals the RNA-packed neoblasts, designated I-cells by Tardent, are situated almost exclusively in the main body, not in the peristome and the tentacles; they are arranged in an antero-posterior gradient so that their numbers are greatest just behind the peristome. In Tardent’s opinion, and he gives rather convincing reasons, this arrangement means that the neoblasts originate continuously at the high-point just behind the peristomal zone, and that they migrate continuously from here to places where physiological loss of cells has to be replaced by neoblasts by differentiation. Tardent has, however, not given any account of possible mitoses in the neoblasts in the zone of origin. No mitoses were seen in planarians.

The neoblasts in Hydroids are also responsible for true regeneration, and Tardent has shown that the regeneration ability follows the same gradient as the distribution of the neoblasts. When we compare these findings with those in the planarians, we see that other mechanisms must exist in the more highly organized planarians than those detected by Tardent in the much more lowly organized coelenterates.

It may be of some interest to note that the total volume of neoblasts in *E. torva* is about 0.0063 cu. mm., the average diameter of the neoblasts (in the fixed state) being about 6 μ (the cytoplasm being rather scanty). The total volume of the inspected specimen (in the fixed state) is 6.2 cu. mm., thus exceeding the volume of the neoblasts by a factor of 1,000.

*Note added in proof:* While this paper was in the press, two papers on the number of neoblasts in *Dugesia lugubris* appeared (Lender & Gabriel, 1960 a, b). They also reported two maxima, one just behind the eyes, the other just in front of the pharynx.

**SUMMARY**

1. The total numbers of neoblasts were counted in a specimen of *D. lacteum* and in a specimen of *E. torva*. The numbers were respectively 37,826 and 31,320.
2. The distribution of the neoblasts throughout the planarian body does not coincide either with head-regeneration ability or with cross-sectional area along the cephalo-caudal axis of these animals.
3. Although the distribution curve of the neoblasts shows fair agreement in the two species, the time-graded regeneration field is quite different, in *Dendrocoelum* stopping short just in front of the pharynx, in *Euplanaria* distributed throughout the animal (A. & H. V. Brondsted, 1952).
4. The planarian time-graded regeneration field cannot therefore be ascribed
to the available numbers of neoblasts at the level of the cut, as is the case in
hydroids.

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