Cellular Source in Hydroid Regeneration

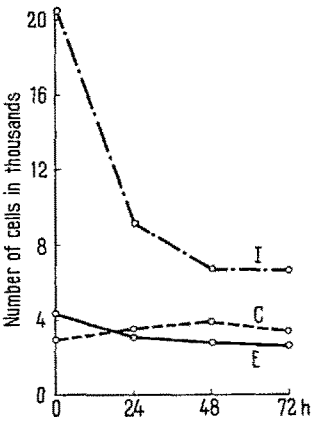
The question of the source of regenerating tissue is an unsolved issue. Whether the differentiated cells come from the division of the existing differentiated cells, or from continuous differentiation of the interstitial cells, is an old and controversial problem of hydroid histogenesis. McConnel[1] has shown that the differentiated cells of hydra can divide mitotically; his figures are not conclusive to decide the issue, and the opinions of Strelin[2] and many others that none but interstitial cells are able to divide are not yet invalidated. The second view that interstitial cells provide other cells is still current, as Tardent[3] has recently shown that in regenerating hydra the relative number of differentiated cells in the anterior region increases and that of interstitial cells decreases. In this context, a survey of changes in the total population of three cell-types viz. epitheliomuscular, interstitial and cnidoblast, in the ectoderm of hydra during regeneration has been undertaken.

Hydra (*Hydra vulgaris orientalis*) were amputated just below the hypostome and were allowed to regenerate. The cut surface is first healed up and rounded; tentacle rudiments appear after 48 h and after 72 h fully formed hypostome and tentacles are seen. Numbers of epitheliomuscular, interstitial and cnidoblast cells in the ectoderm of hydra after amputation, and after 24, 48, and 72 h regeneration have been estimated by direct counting of alternate serial transverse sections of 8 μ thickness after staining them in toluidine blue. Total number of each cell-type in a regenerate was obtained by doubling the figures. Three individuals were examined in each case.

A close proximity in the number of these cells is observed in the three cases presented (Table). In the ectoderm of an amputated hydra, the aggregate varies from 25 to 29 thousands, in which the interstitial cells constitute the overwhelming majority ranging from 19 to 21 thousands. The epitheliomuscular and cnidoblast cells are much lower in number and range from 3 to 5 thousands and from 2 to 3 thousands respectively. A huge fall in the aggregate of three cell-types is recorded after 24 h regeneration. In a total loss of cells, from 27 thousands after amputation to 15 thousands after 24 h, the interstitial cells are most affected and are reduced to less than half. The number varies from 7 to 10 thousands. But the number of epitheliomuscular cells is only slightly lowered from 4 to 3 thousands on the average. In comparison, cnidoblast cells are slightly increased in number. Similar trends of cell changes are observed in 48 h regenerates, but to a much lesser extent. Further, very little change is found in the cell population of 72 h regenerates. A noticeable difference is the decrease in number of cnidoblasts which was so far on the increase. Epitheliomuscular cells are very slightly lowered, the interstitial cells remaining more or less constant. Morphological change of the regenerates during this period is restricted to the elongation of the tentacles. These latter being longer than the body column they were not enumerated throughout their length, but instead counts were made starting from the base of the tentacles. Thus it is seen the cell population of the regenerates does not change appreciably after 48 h, i.e., after the tentacle rudiments have appeared; the slight loss of cells encountered is due to the omission of the excess length of the tentacles. This is further indicated by the constancy of interstitial cells during this period, because these cells are not present in tentacles.

Changes in the total population of epitheliomuscular, interstitial and cnidoblast cells during regeneration of hydra.

Hours of regeneration	Serial No.	Epithelio-muscular	Interstitial	Cnidoblast
After amputation	1	3754	19692	2490
	2	5328	20678	3910
	3	3818	21264	2416
	Mean	4300	20544	2938
24 h	1	3224	9628	3390
	2	3038	10032	3656
	3	3094	7648	3566
	Mean	3118	9102	3537
48 h	1	3052	7792	4136
	2	2314	5888	3144
	3	3098	6374	4636
	Mean	2821	6684	3971
72 h	1	2706	6406	3108
	2	2204	4204	2754
	3	3194	9322	4450
	Mean	2701	6644	3437



Showing the total number of cells in regenerating hydra at different intervals. I, Interstitial cells; E, Epitheliomuscular cells; C, Cnidoblast cells.

The above data first show that overall cell population in the ectoderm of hydra does not increase during regeneration; on the contrary, there occurs a huge loss of cells, most conspicuous in case of interstitial cells and also to some extent in epitheliomuscular cells. Cnidoblast cells with their nematocysts gradually increase in number (Figure). It is reasonably clear that during regeneration interstitial cells in hydra do not differentiate to form epitheliomuscular cells, and hence possibly to other differentiated cells, except to cnidoblasts. However, possible transformation of interstitial cells to other types in the general mechanism of growth and histogenesis of hydra[4]

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cannot be ruled out. It may be concluded that factors of hydranth regeneration largely involve reorientation of the cellular configuration in the available material. A detailed report on the manner of cell disposition and its relation to morphogenesis in hydra will be published elsewhere[5].

Zusammenfassung. An regenerierenden Süsswasserpolypen (*Hydra vulgaris orientalis*) wurde die Gesamtmenge der Epithelmuskelzellen, der interstitiellen Zellen und der Cnidoblasten bestimmt. Dabei wurden sehr grosse Zellverluste, speziell bei den interstitiellen Zellen, in geringerem Masse bei den Epithelmuskelzellen festgestellt. Die Zahl der Cnidoblasten nahm dagegen etwas zu.

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A Possible Correlation Between Reverse Mutation and Complementation

Results obtained in *Neurospora crassa* (see FINCHAM[1] for references) indicate that mutants exhibiting intra-locus complementation in a heterokaryon form a protein (C.R.M.) related serologically to the functional enzyme of the wild-type strain. Non-complementing mutants form apparently no C.R.M. Recently CRICK et al.[2] have suggested that mutations induced by proflavine and the majority of spontaneous mutations (FREESE[3]) are due to the addition or subtraction of base pairs in the DNA, and result in the formation of no specific protein or to one greatly different from the wild-type.

These results may perhaps indicate that mutants arising by addition-subtraction type mutation would produce no C.R.M., and hence be non-complementing when occurring at loci at which intra-locus complementation is known to occur.

Mutants of the complementing type might be expected to be due to transition type mutations, affecting only one base pair of one triplet (FREESE[3]), and having only one amino acid difference between C.R.M. and the wild-type enzyme (WITTMANN[4]). If this hypothesis is true, complementing mutants would revert on treatment with mutagens causing transitions, such as base-analogues and nitrous acid.

True non-complementing mutants, besides producing no C.R.M., might be expected not to revert with mutagens causing transitions. Further, if no, or only a grossly altered protein is produced they would not be expected to be leaky or temperature-sensitive mutants. Results obtained by LEUPOLD ([5] and personal communication) for the ad-1 and ad-6 loci of *Schizosaccharomyces pombe* show at least 38 of the 40 incompletely blocked mutants tested to be of the complementing type.

Preliminary results obtained with ten mutants at the ad-1 locus of *Schiz. pombe* indicate that at least five of six complementing mutants will revert after treatment with nitrous acid. Three of the four non-complementing mutants will not do so. The fourth non-complementing mutant responds to nitrous acid treatment. It should be pointed out, however, that classification of a mutant as non-complementing is to some extent uncertain, since a given mutant may complement with very few other mutants at the same locus (CATCHESIDE[6]).

This hypothesis, that non-complementing point mutations producing no C.R.M. will not give true back-mutation on treatment with nitrous acid, and other transition-type mutagens, is open to test at a number of loci in Neurospora (CATCHESIDE[7]) Schizosaccharomyces and Salmonella (HARTMAN, HARTMAN and SERMAN[8]).

Résumé. Les mutants présentant la complémentation intra-allélique sont probablement susceptibles aussi de mutation reversé vraie sous l'effet de l'acide nitreux.

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Institute of Animal Genetics, Edinburgh (Scotland U.K.), June 21, 1962.

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Presynaptic Inhibition in the Lumbar Cord Evoked from the Brain Stem

It has recently been shown that the presynaptic terminals of primary afferents may be depolarized through spinal reflex actions and that synaptic actions to motoneurones by this mechanism may be inhibited[1]. Ia afferents are depolarized from group I afferents predominantly of flexor muscles, the flexor reflex afferents (FRA), on the other hand, from group I afferents and the FRA. Primary afferents may also be depolarized from higher centres, volleys in the pyramidal tract depolarize Ib, cutaneous and high threshold muscle afferents but not Ia afferents[2].

The present experiments (decerebrate, unanaesthetized cats) have revealed the existence of a brain stem centre from which depolarization can be evoked not only in the above mentioned categories of afferents, which are influenced from the pyramidal tract, *but also in Ia afferents.*

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