STUDIES ON THE ORGANIZER PROBLEM IN *PELMATOHYDRA OLIGACTIS*

I. THE INDUCTION POTENCY OF THE IMPLANTS AND THE NATURE OF THE INDUCED HYDRANTH

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(With Three Text-figures)

The existence of a physiological gradient in *Hydra* has been repeatedly demonstrated by a number of investigators, and the hypostome, which is supposed to be the region of highest metabolic activity, has generally been regarded as the organization centre of the animal. Earlier transplantation experiments (Browne, 1909; Rand, Bovard and Minnich, 1926; Mutz, 1930) indicated that when a piece of hypostome is grafted, it can act as an organizer and induce a new hydranth. Although these are all well-known facts, the underlying mechanism in the induction process remains obscure. The present work is the first step in an attempted physiological analysis of the problem, with special reference to the induction potency of implants derived from different body levels, and to the nature of the induced hydranth.

MATERIAL AND METHODS

The common brown *Hydra*, *Pelmatohydra oligactis*, was used in this investigation. The specimens were kept in the laboratory in glass aquaria containing well water and some *Hydrilla* plant, and fed regularly on *Daphnia*. The technique was that of homoplastic transplantation, using only uniform non-budding animals, which had not been fed for 2 or 3 days, as hosts and donors respectively. The grafts were pieces taken from different body levels of a *Hydra* sectioned in the manner shown in Fig. 1, and stained supravitally with nile blue sulphate before implantation. Without exception grafts were made in the middle region of the body. After the take of the implants, the operated *Hydras* were cultured individually in porcelain dishes containing sterilized tap water changed at regular intervals, and kept in the dark at room temperature. Observations were made on alternate days following implantation, and the fate of the implants was recorded.

EXPERIMENTAL RESULTS

(1) *Transplantation experiments with a hypostome fragment as graft*

Pioneer work of Browne (1909) had demonstrated that a new hydranth is produced when a small piece of hypostome is grafted into another individual, and that the new individual determined by the graft is derived to a considerable extent from the host body. Later, Rand *et al.* (1926), and especially Mutz (1930), performed similar experiments. They did not, however, consider the constitution of the induced

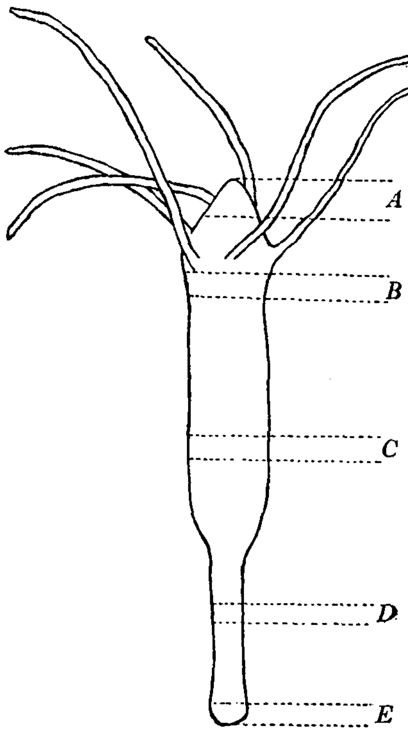

Fig. 1. A semidiagrammatic sketch showing the planes of section for different kinds of grafts used in the transplantation experiments. *A*, hypostome; *B*, subtentacular region; *C*, middle body region; *D*, stalk region; *E*, pedal disk.

hydranth. In the present cases, the demarcation between the stained graft and the unstained host tissue was so sharply defined that the determination of the constitution of the induced hydranth was a matter of ease. It was found that, in the majority of cases, the hypostome of an induced bud was supplied by the implant itself, and the rest, including the tentacles and the body proper, was derived from the host, as shown in Fig. 2. Sometimes, however, the induction process was accompanied by a certain degree of self-reconstitution of the implant, giving an induced bud, with tentacles formed at the expense of both host and implant materials. Table 1 summarizes some of the results thus far obtained.

Table 1. *The fate of implanted one-quarter hypostome fragments of* Pelmatohydra oligactis *grafted into the middle region of the host body of an individual of the same species*

Exp. series	No. of cases	Fate of implant (in number of cases)				% of induction
		Assimilated	Transformed into a single tentacle	Induction of new hydranth		
				Typical induction	Induction accompanied by self-reconstitution	
1	16	2	—	12	2	87
2	13	2	1	7	3	77
3	16	—	2	13	1	87

Table 2. *The fate of implanted fragments from the subtentacular region of* Pelmatohydra oligactis *grafted as indicated in Table 1*

Exp. series	No. of cases	Size of implant	Fate of implant		
			Assimilated	Self-reconstitution	Induction of new hydranth
1	42	$\frac{1}{6}$ ring	42	—	—
2	10	$\frac{1}{8}$ ring	10	—	—

Table 3. *The fate of implanted fragments from the middle body region of* Pelmatohydra oligactis *grafted as indicated in Table 1*

Exp. series	No. of cases	Size of implant	Assimilated	Fate of implant		
				Self-reconstitution	Differentiated into a pedal disk	Induction of new hydranth
1	20	$\frac{1}{4}$ ring	18	—	2	—
2	25	$\frac{1}{6}$ ring	24	1	—	—
3	16	$\frac{1}{6}$–$\frac{1}{8}$ ring	15	1	—	—

From the table, it can be seen that a piece of hypostome one-fourth of the whole in size will be able to give, on an average, 84% inductions, most of which are typical. In spite of the relatively small number of cases, therefore, it seems safe to say that under experimental conditions the implant is a highly potent system, in most instances able to induce the formation of a new hydranth.

(2) *Transplantation experiments with a fragment from the subtentacular region as graft*

The results of transplantation experiments with this kind of graft are given in Table 2. The grafts were found to be assimilated in all cases. Neither induction nor self-reconstitution has yet [redacted]n recorded.

(3) *Transplantation experiments with a fragment from the middle of the body as graft*

The fate of implants obtained from this level of section fell into two groups. They were either assimilated by the host, becoming a part of its body wall, or reorganized themselves into new hydranths through a process similar to the reconstitution of an isolated fragment. Such a reconstituted hydranth, therefore, consists of implant materials only. In a few exceptional cases (2 out of 61), the graft reorganized itself into a distinct pedal disk. Some of the data are presented in Table 3.

(4) *Transplantation experiments with stalk as graft*

Animals were first sectioned along plane *D* in Fig. 1 to obtain rings of tissues, which were then bisected and used as grafts. The grafts taken were invariably found to be assimilated at an early or later period. In accordance with their inability to regenerate (Goetsch, 1920; Weimer, 1928), no selfre[redacted]titution of such grafts has yet been observed. Sometimes the implant remained as a well-defined outgrowth showing pedal characters. Furthermore, owing to the readiness with which they disintegrate after bisection, it was very difficult to get a sufficient number of satisfactory grafts. Out of a total of twenty-four experiments so far performed, the implants were assimilated in twenty-two cases, and differentiated themselves into pedal disks in the remaining two.

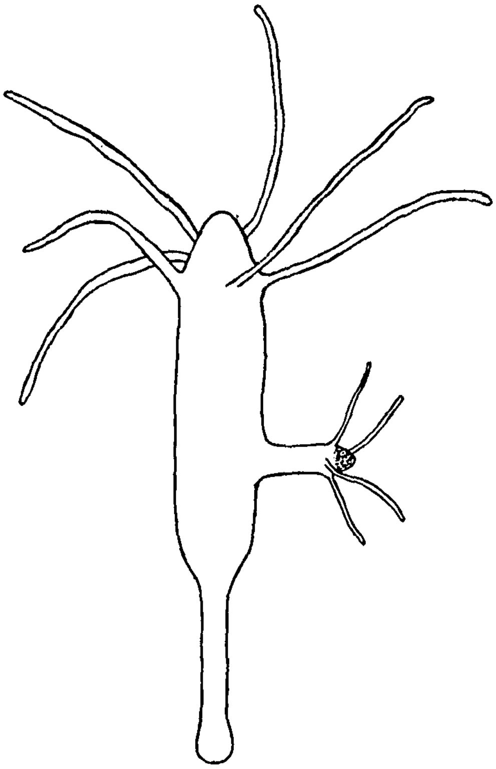

Fig. 2. A sketch showing typical induction. Note that the dotted implant material is confined to the hypostome region of an induced bud, while the tentacles and the body proper of the latter are all supplied by the host tissues.

(5) *Transplantation experiments with pedal disk as graft*

The pedal disk of *Hydra* has the characteristic properties of mucus secretion and gas production. When pieces of it (one-half to one-fourth of the original size) were grafted in, and taken by, the host, they were generally not assimilated; on the contrary, their characteristics remained unchanged for 3–5 weeks or even longer. Thus, in only three out of thirty-seven experimental individuals were the implants eventually absorbed; the rest were each provided with two pedal disks, indistinguishable in all respects except that of size (Fig. 3).

DISCUSSION

The presence of an 'axial gradient' in fresh-water *Hydra* is suggested by gradated properties of various kinds, such as the rate of reconstitution of isolated fragments (Weimer, 1928), the rate of disintegration in cyanide (Child & Hyman, 1919) and the rate of methylene-blue reduction (Child, 1934). Recently, a gradient in tentacle development was reported (Rulon & Child, 1937). In view of these well-established facts, it was of considerable interest to test whether or not there is a correlation between

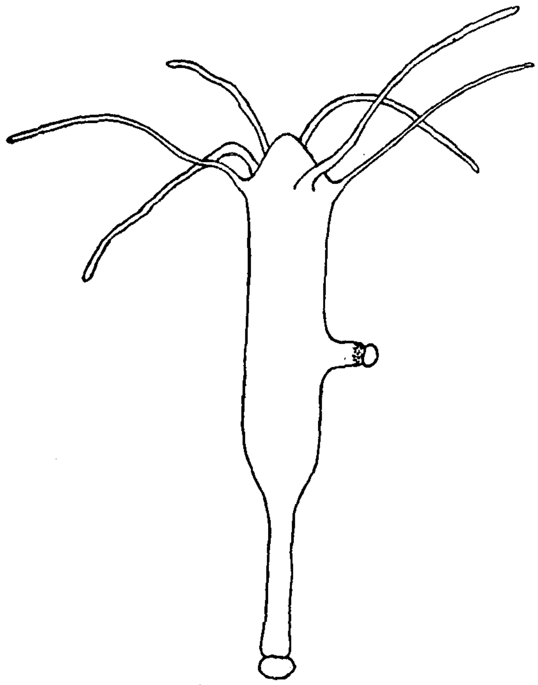

Fig. 3. A sketch showing that the characteristic properties of a pedal disk are retained after transplantation. The implant secretes a gas bubble just as does the pedal disk of the host.

the phenomenon of induction and the physiological gradient. If the induction potency were also a gradient character, then it might be revealed by transplantation experiments in terms of relative frequency of induction. From the data given here, however, it may be concluded that such a gradient of induction potency does not exist: induction ability is confined to the cells of the hypostome. A typical induced bud is composed of hypostome derived from the implant itself while the rest originates from the host tissues. Not infrequently, however, the implant materials also give rise to tentacles. This is understandable since the ability of an isolated hypostome fragment to give complete regeneration under favourable conditions has been proved.

Assimilation is found to occur most frequently when a piece of stalk is used as implant. In a few cases the differentiation of the graft into a pedal disk may be related to the suggestion of Burt (1925) that the pedal region of a *Hydra* has its own unipotent system. A similar fate of assimilation may befall grafts derived from the middle as well as from the subtentacular region. In the experiments undertaken thus far, no induction could be obtained with these grafts, and self-reconstitution occurred only rarely. These results lead to the conclusion that assimilation takes place more easily if the graft is composed of tissues the potency of which is the same as, or at least very similar to, that of the host tissues in the region of implantation. Moreover, the assimilation process can, perhaps, best be explained by the regulative action of the host.

The fact that implanted pedal disks appear to retain their own characteristics clearly indicates that the disk is composed of a group of specifically differentiated cells. Furthermore, its low frequency of assimilation seems to show that the differentiation process is irreversible. The incapacity of a piece of pedal disk to perform any induction agrees wi[redacted]e fact that in isolated culture such pieces are unable to regenerate new *Hydras* and may disintegrate within 10 days.

SUMMARY

1. Homoplastic transplantation in *Pelmatohydra oligactis* has been studied with implants obtained from different body levels.

2. As a result of the regulation of the host, assimilation of the graft has been recorded in all cases. The frequency of assimilation is higher when graft and host are composed of cells having the same (or nearly the same) developmental potentiality.

3. Pedal disk is usually not assimilated, but maintains its own properties following implantation.

4. No gradient of induction potency can be demonstrated in this animal. Perhaps owing to unknown intrinsic factors, the induction power is limited to the cells of the hypostome.

5. A typical induced hydranth is always composed of hypostome supplied by the graft itself, and of tentacles and body derived from the host.

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