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Retinoids and pattern formation in a hydroid

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SUMMARY

The retinoids (retinol, retinal, retinoic acid) cause alterations in the pattern of limb elements in vertebrates (Summerbell & Harvey, 1983). As shown here, retinoids also influence pattern specification in hydroid polyps (*Hydractinia echinata*) in a way suggesting interference with the generation and transmission of signals responsible for the dimension and spacing of structures. A pulse-type application of low doses (e.g. retinoic acid 10^{-6} to 10^{-10} M, 4 h) causes metamorphosing primary polyps to develop more tentacles but fewer stolons per unit circumference, to shorten the length of the hydranth while the stolon elongates, and to bud secondary hydranths at high frequency 2–3 days after treatment (Fig. 3). Dose-response curves display optimum peaks. It is argued that the increase in budding rate is due to a reduction of the range of spacing signals emitted by the primary hydranth. In regenerating hydranths, low doses (10^{-10} to 10^{-9} M) improve the rate of head formation, whilst medium doses (10^{-8} to 10^{-6} M) result in more tentacles being regenerated. However, prolonged treatment with high doses (10^{-6} to 10^{-5} M) causes the animals to reduce all head structures and to transform eventually into stolons, in contravention of the rule of distal transformation that they normally obey (Fig. 8). The effects of the retinoids are counteracted by a putative morphogen, the endogenous inhibitor isolated from *Hydra* by Berking (1977). The *Hydra*-derived 'head-activator' displayed no stimulating effect on the number of tentacles and buds formed.

INTRODUCTION

In vertebrates, two pattern-forming systems have been shown to be dramatically altered by vitamin A (retinal) and its derivatives (Summerbell & Harvey, 1983). (1) In regenerating limbs of larval axolotls, the proximodistal sequence of pattern elements is affected. The positional value of blastema cells is reset to lower values. A new sequence is added starting from a more proximal level, in contravention of the rule of distal transformation (Maden, 1982). (2) In the chick limb bud, retinoic acid mimics the action of the polarizing region (Tickle, Alberts, Wolpert & Lee, 1982). In *Rana*, both the proximodistal axis and the anteroposterior pattern of digits are affected (Maden, 1983). The rule of distal transformation is valid not only in outgrowing limb rudiments. Some hydroid polyps obey this rule as well: at a given level along the body column, only more distal structures (in the last instance a hypostome or 'head'), can be formed, and not proximal structures, such as stolons. This is best exemplified in *Tubularia* and *Hydractinia* (Müller, 1982). Can retinoids lower the positional value, and provoke the formation of stolons? This question was suggested to me by Dr D. Summerbell.

The signalling action of the polarizing zone bears a formal analogy to the polarizing action of the hypostome of polyps. When brought into contact with tissue of proximal origin the head can evoke distal transformation of the neighbouring recipient, even against its normal polarity (Müller, 1982). The question, therefore, was: would we see distal transformation, proximal transformation, or no effect at all?

For this initial study, I chose the marine hydroid *Hydractinia echinata*, since pattern formation in this species can be studied during both regeneration and metamorphosis. During metamorphosis of a planula larva into a primary polyp, both stolons and head structures are formed, whereas regenerating hydranths form only heads, following the rule of distal transformation.

METHODS

Animals and their normal development

Colonies of *Hydractinia echinata* in reproductive condition were procured from the Biological Station at Helgoland. The colonies were reared at 19 °C and subjected to a light–dark cycle of 16 h L: 8 h D. Planulae were obtained in fairly large numbers (some hundreds per female colony per day) from spawnings. Embryos were raised in glass bowls at 19 °C; 3-day-old planulae were reared in plastic Petri dishes at 4 °C until induction of metamorphosis was attempted.

Metamorphosis of the planulae into primary polyps was induced by synchronously treating the larvae for 3 h with sea water containing caesium ions (Müller, Wieker & Eiben, 1976). The Cs^+ sea water solution consisted of one part CsCl stock solution (986 mg CsCl in 10 ml of distilled water) and nine parts of sea water. After Cs^+ treatment, the larvae were washed several times.

Retransfer of the larvae into sea water defines time zero. About 8 h later, stolons and tentacles begin to appear. The larvae have developed into primary polyps, the founders of a colony (Figs 1, 2). As a rule, their branching stolons do not bud secondary hydranths until the primary hydranth has been fed. For this study, the polyps were not fed unless otherwise stated. In unfed animals, the numbers of tentacles and stolons reach constant levels on the third day of development, and only 1–10 % of the primary polyps bud secondary hydranths. Incubation with retinoids started 3 h after initiation of metamorphosis, i.e. 5 h before tentacles or stolons emerge.

For regeneration studies, nutritive hydranths, which look like a bud-less hydra, were collected from colonies by a transverse cut just above their stolonal substratum. A second cut just beneath the tentacle whorl removed the head. The isolated gastric columns were synchronously transferred to test dishes 0·5 to 2 h after cutting and incubated at densities of about five pieces/ml for various times.

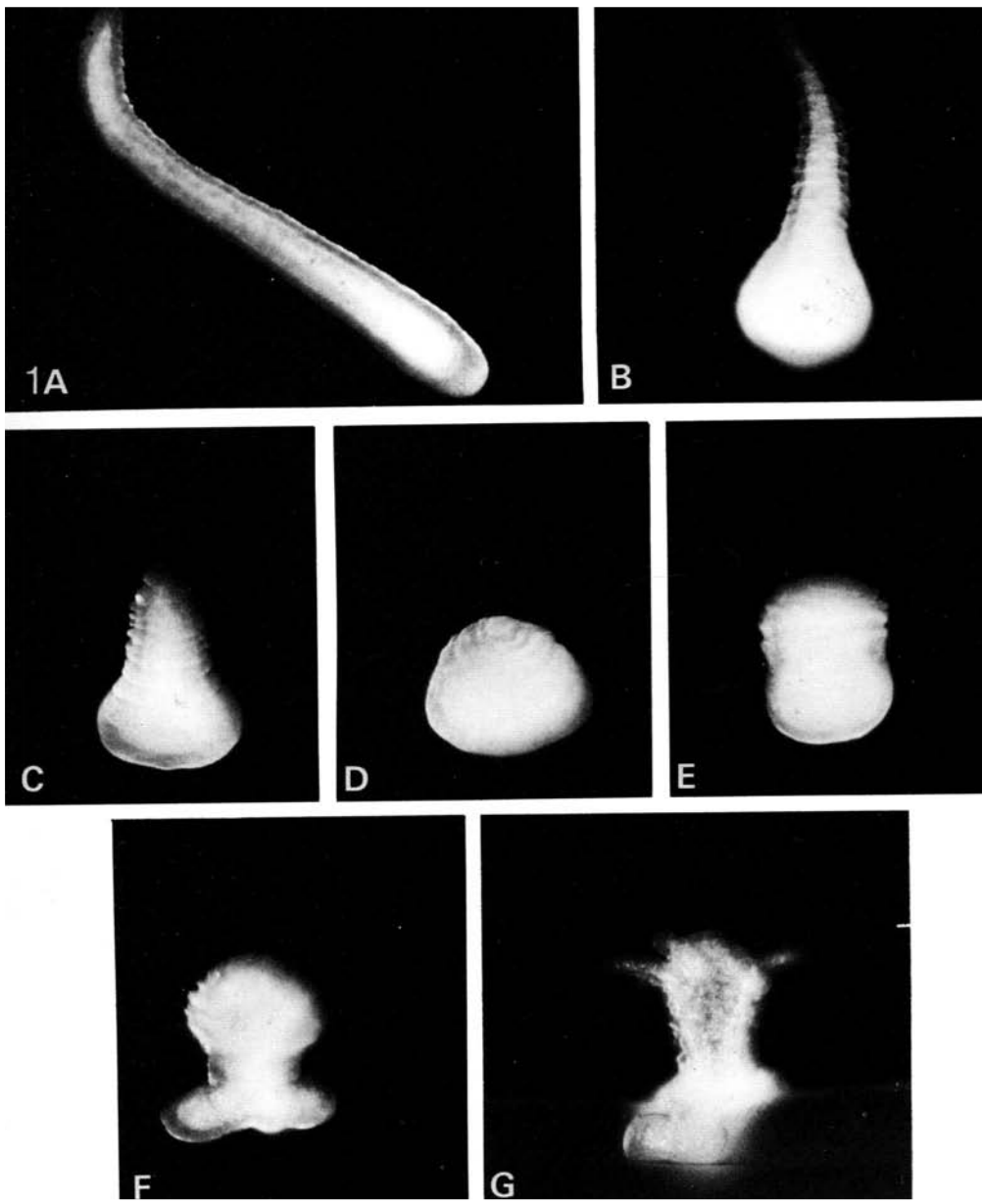


Fig. 1. *Hydractinia echinata*. Metamorphosis of the planula larva into a primary polyp. The length of the larva is about 1 mm. Upper series (A, B, C) initial period which depends on the presence of an external inducing stimulus. In the laboratory, Cs^+ ions substitute for a natural inducer released by environmental bacteria. Lower series (D, E, F, G) autonomous morphogenesis after removal of the inducing Cs^+ ions. Retinoids were administered during phase D–E. Photographs by Berking.

Retinoids and morphogens

Retinoids were bought in their all-trans form from Sigma (Munich) or Serva (Heidelberg). Fresh solutions were prepared immediately before use. The rather hydrophobic substances were predissolved in methanol, and dilution series were established in methanol. From each dilution step, 5 μl were put into Petri dishes containing about 120 metamorphosing animals, or 50 regenerating hydranths in 10 ml of sea water. 5 μl of pure methanol was added to the controls. In such low concentrations, methanol does not have any perceptible effects.

The 'endogenous inhibitor' from hydra was a gift from S. Berking, and the 'head activator from hydra' was either a natural product highly purified by the group of Ch. Schaller (Schaller & Bodenmüller, 1981) or a synthetic product (Birr, Zachmann, Bodenmüller & Schaller, 1981) procured from Bachem, Switzerland. The batches used were tested for quality in the laboratory of Ch. Schaller.

Evaluation of data

Tentacle number was monitored 2 and 3 days after initiation of metamorphosis or amputation, respectively. Budding of secondary hydranths by the stolons of primary polyps was scored at 3 and 4 days. Some experiments were followed for 2–4 weeks. In critical cases, budding rates were evaluated using the χ^2 analysis and tentacle number compared using the F-test (Flechtner, Lesh-Laurie & Abott, 1981).

RESULTS

I. *Metamorphosis*

Pattern of tentacle and stolon generation

During metamorphosis, the first visible sign of an altered pattern specification is a narrow spacing of the tentacles. Tentacles normally appear in a characteristic spatiotemporal order. Initially, three to five tentacles are formed simultaneously. Only when these have reached a certain length are new tentacles inserted by intercalation (Figs 2, 3). Two days after initiation of metamorphosis, the mean number is $7{\cdot}7 \pm 0{\cdot}6$ per hydranth. After treatment with retinoids, tentacles appear more synchronously. The animals frequently start with eight or more primordia simultaneously. The final number can reach an average value close to ten. The tentacles, however, grow more slowly: apparently, too much mitotic activity is demanded to sustain normal elongation of all tentacles simultaneously. With higher doses, even more tentacle primordia may appear without any interspace, but most are unstable and the final number of tentacles eventually is lower than in controls. The dose-response curve, therefore, displays an optimum (Figs 4, 5). The position of the optimum and its height are dependent on the retinoid chosen and the duration of the incubation period. With retinoid acid and an

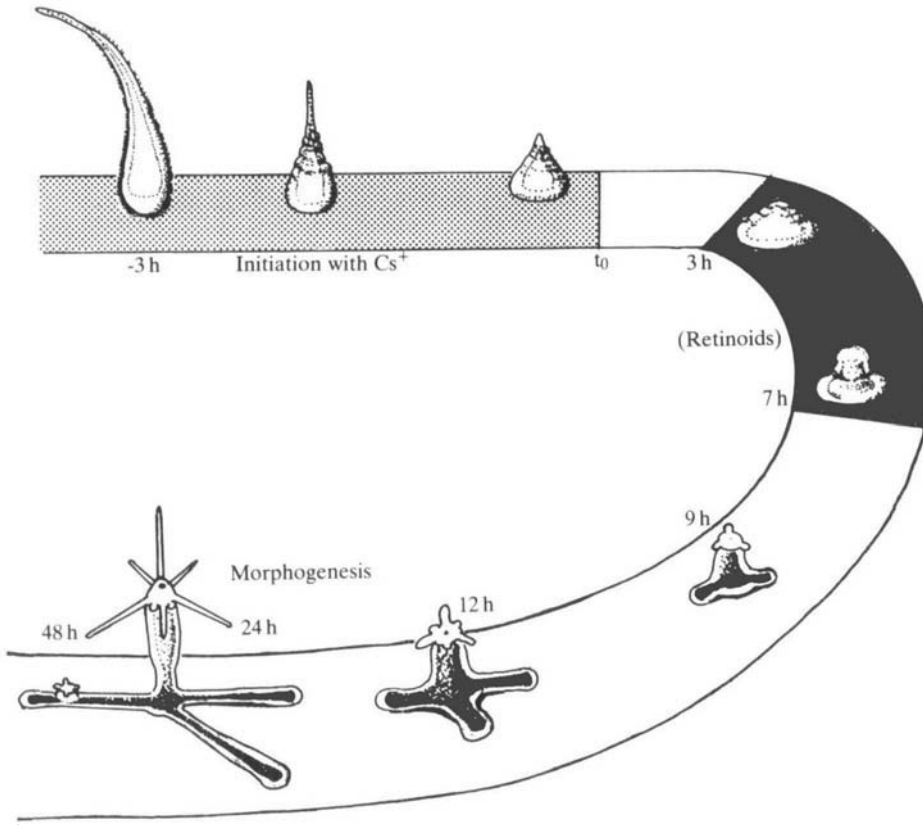


Fig. 2. Metamorphosis. Time scale and period of treatment with vitamin A. Note also the temporal pattern of tentacle emergence.

incubation period of 4 h, most tentacles were formed at 10^{-7} or 10^{-6} M (Figs 4, 5A). Occasionally, even 10^{-10} M gave significantly increased values.

Metamorphosing larvae develop a second radial and periodic pattern, the stellate arrangement of stolon tips around their base (Figs 2, 3). Larvae treated with retinoids form fewer stolon tips. The resulting primary polyps, therefore, display the symptoms of 'oralization' (Müller, Mitze, Wickhorst & Meier-Menge, 1977), combining an increase in tentacle number with a decrease in stolon number (Figs 4, 5).

For theoretical considerations, it is of importance to know whether there exists a limited phase of sensitivity in early metamorphosis. If oralization were brought about only in early metamorphosis before tentacles and stolons emerge, it could be explained in terms of an altered longitudinal prepattern: more larval material would have been invested in head formation, less material in the formation of the first stolon tips at the former anterior pole of the larva.

However, the data do not support such an interpretation: at least mean tentacle number can still be increased on subsequent days as long as the unfed hydranths generate new tentacles. The yield, of course, decreases with time.

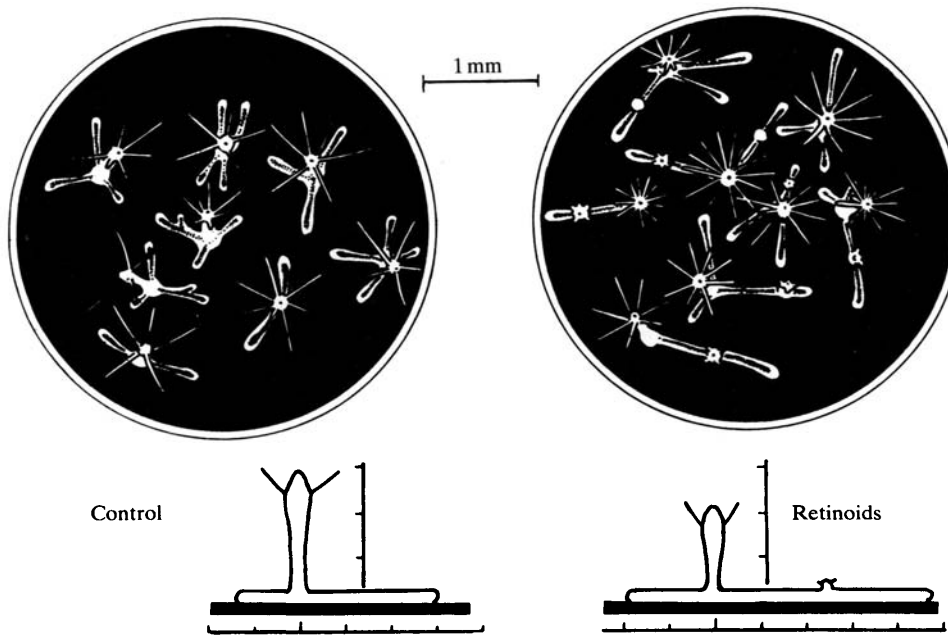


Fig. 3. Primary polyps, three days after initiation of metamorphosis. Note the following differences between control and experimental animals. (1) Number and length of tentacles, (2) number of stolons originating from the basal disc of the primary polyp, (3) length of the stolons, and (4) buds of secondary hydranths on the stolons. Appearance of buds is restricted to retinal treated animals. In reality, comparatively larger dishes were used containing about 20 times more polyps but at a lower density.

II. *Postmetamorphosis*

Budding frequency and stolon elongation

An unexpected finding is of particular interest: at the second and third day after the onset of metamorphosis, stolons which did not exist at the time of treatment bud secondary hydranths in high frequency (Fig. 3). Up to 74 % of the primary polyps started to establish a colony by generating at least one bud compared to control levels of 0–10 % (Figs 4, 6, 7). The occurrence of hydranth buds is preceded by another striking phenomenon: stolons elongate rapidly, doing so at the expense of the hydranth. Hydranths export cells into the elongating stolon. Their proximal body column is, therefore, shortened. On the third day after treatment with 10^{-5} M retinoic acid, the hydranth length was 194 ± 4 μm, as compared to 273 ± 5 μm in untreated controls. Incorporation of emigrated cells enabled 62 % of the stolons (controls 26 %) to grow longer than 1000 μm, and such elongated stolons generate new hydranths (Fig. 3). However, the budding rate was also significantly increased on short stolons (500–100 μm).

The distance between the primary hydranth and its first bud was reduced from 725 ± 144 to 553 ± 144 μm, and sometimes two or three buds arose in series.

An increase in the budding rate can still be induced, though with a lower yield,

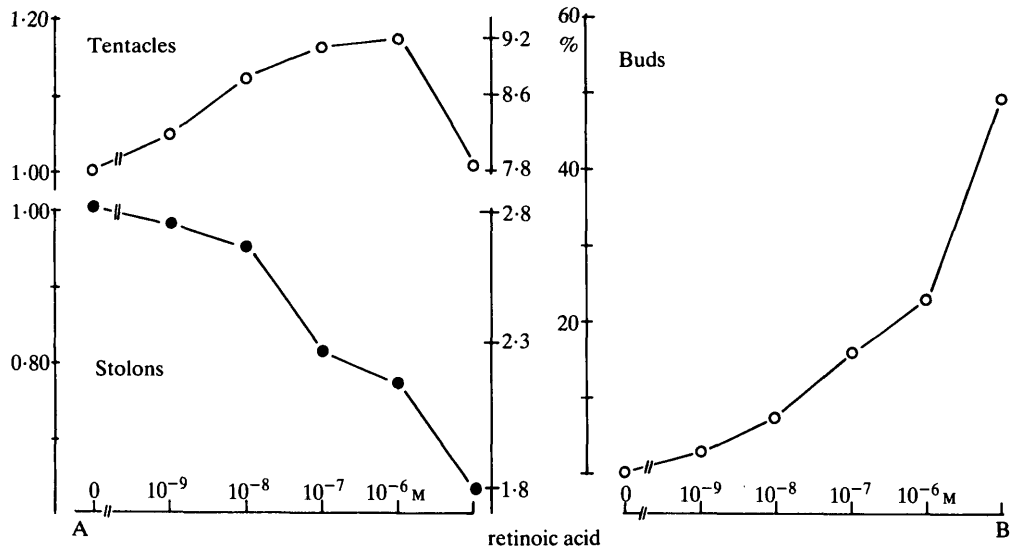


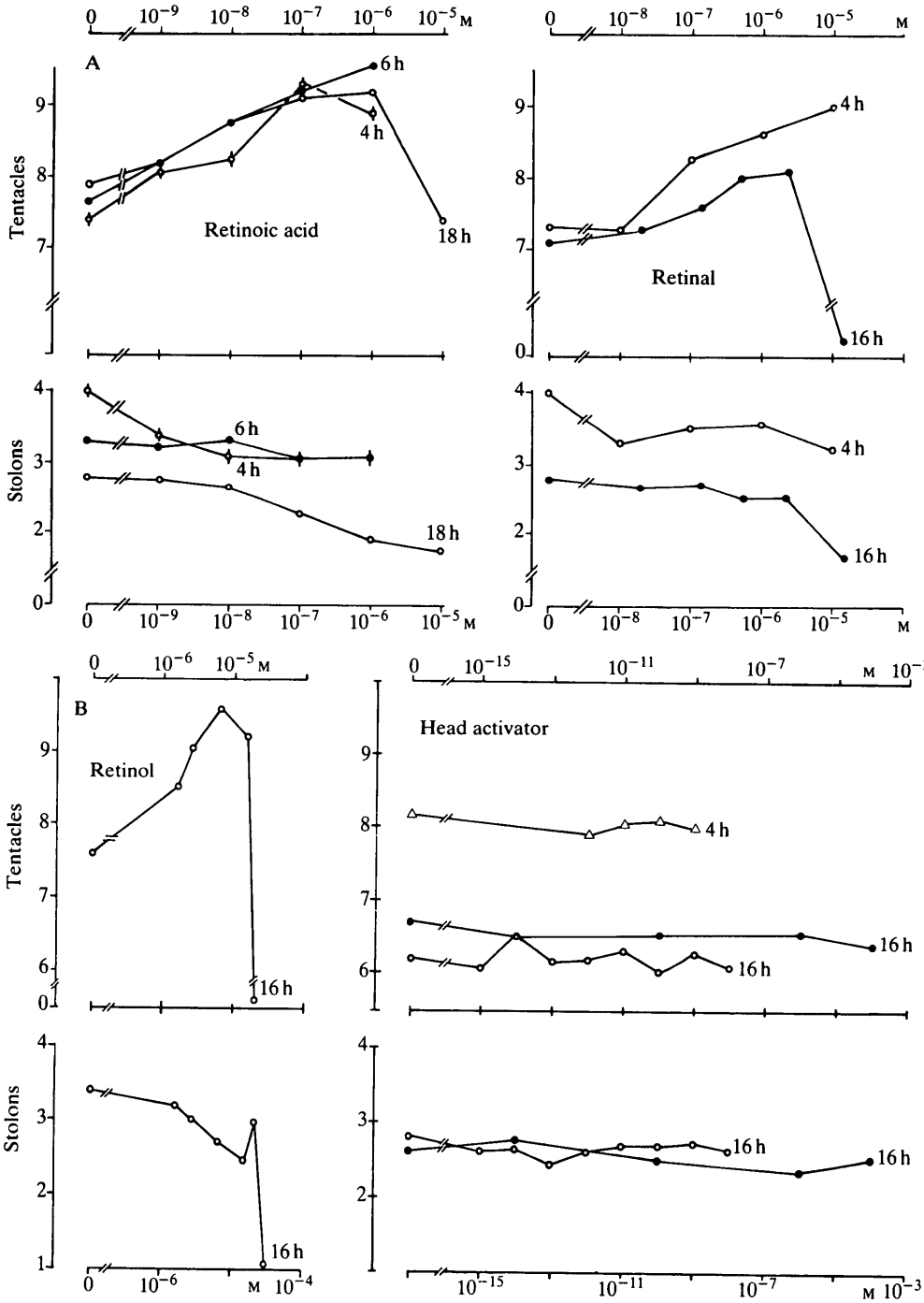
Fig. 4. Metamorphosis and postmetamorphosis. Numbers of tentacles, stolons, and hydranth buds per primary polyp on the third day following initiation of metamorphosis. Retinoic acid was applied for 4 h during early metamorphosis (3–7 h). Left ordinate: relative values as compared to the control level. Right ordinate: actual values per primary polyp. Standard deviations are between ±0·8 and ±1·2 for tentacles as well as for stolons. Results are from a single experiment.

one or two days after initiation of metamorphosis, when stolon elongation has begun to slow down. Quite high doses (up to 10^{-5} M at an incubation period of 4 h) can be used, but significant values have also been scored using 10^{-9} M for 4 h or 10^{-10} M for 20 h.

It should be pointed out that the influence of a retinoic pulse on budding frequency is a long-term effect, and not restricted to metamorphosis. Young colonies, 1–2 weeks old and fed once or twice, responded to a pulse-type treatment equally well. A series of experiments using such young colonies revealed that a single 4 h pulse of 10^{-6} M retinoic acid induces increased budding for days. A second pulse then finds the colonies, which now consist of several hydranths, in a 'refractory' state, i.e. there is no additional increase in budding.

III. *Regeneration*

Experimental developmental biology in coelenterates mainly means regeneration studies. For the present study, nutritive hydranths, which look like a hydra, were chosen. Gastric columns were isolated by two transverse cuts and synchronously transferred to test dishes 0·5 and 2 h after cutting, then incubated with retinoids for various periods of time. Again, the number of tentacles constituting the crown is increased (Figs 8, 9). Moreover, in batches with regeneration rates below 100 %, a higher portion of the gastric pieces treated with low doses formed a head (Fig. 9). This improvement was statistically insignificant in


A
Retinoic acid
Retinal
Tentacles
Stolons
6 h
4 h
18 h
16 h
B
Retinol
Head activator
M

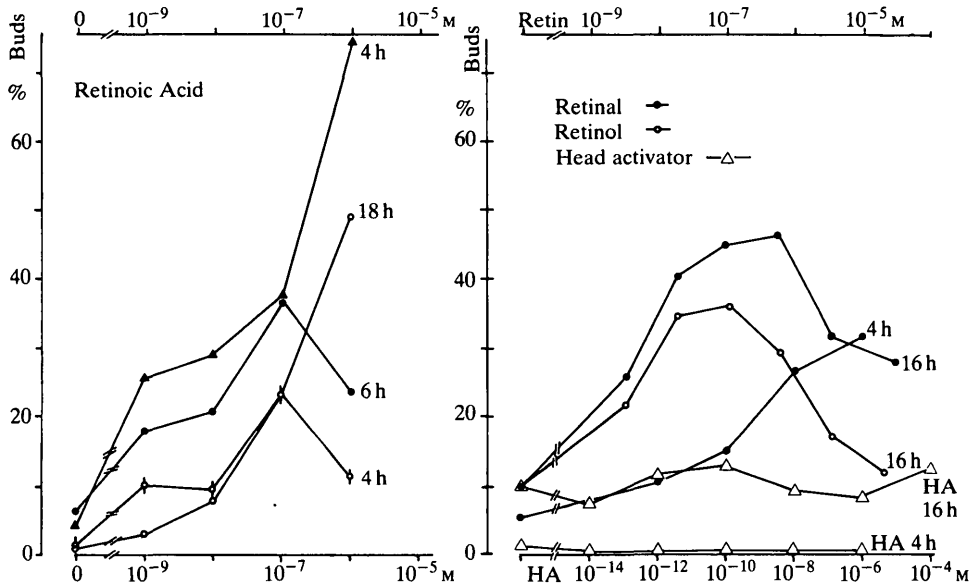


Fig. 6. Postmetamorphosis – budding rate. Hydranth buds emerging from the stolons of primary polyps were monitored over a 4–5 day period following induction of metamorphosis. Shown are the summarized values. They indicate the fraction of primary polyps with at least one bud. Primary polyps: n = 76–1000 per point. Times at right end of curves indicate the length of treatment.

each single experiment but the values eventually summed up to a final result which no longer allowed acceptance of the null hypothesis.

The most conspicuous forms are animals developing heads at both ends simultaneously. In contrast to most species of *Hydra*, *Hydractinia* hydranths occasionally form heads also at their proximal end (Fig. 10) driven by those unknown self-enhancing processes which are subsumed under the term 'distal transformation'. Double head formation terminates in a mirror image duplication of the distal body pattern (Müller, 1982). Spontaneous double head formation demonstrates a low expression of polarity. In the course of the present experiments, only one clone was found showing a labile polarity. The scored maximum increase in double head formation, from 6 to 16 %, (caused by 10^{-9} M retinal) is statistically not significant.

Prolonged (48 h) or repeated treatment with high doses (retinoic acid, retinal

Fig. 5. Metamorphosis. Final tentacle and stolon numbers per primary polyp. The points on the left end of the curves represent the control values. Their different heights reflect the biological variability among different batches of larvae. Times at the right end of the curves indicate the time after which the test solution was removed and replaced by normal sea water. The effective incubation period may have been shorter, due to depletion of the agent by uptake and decay. Standard deviations are omitted for clarity. They were ±0·8–1·2 with n = 60–120 per point.

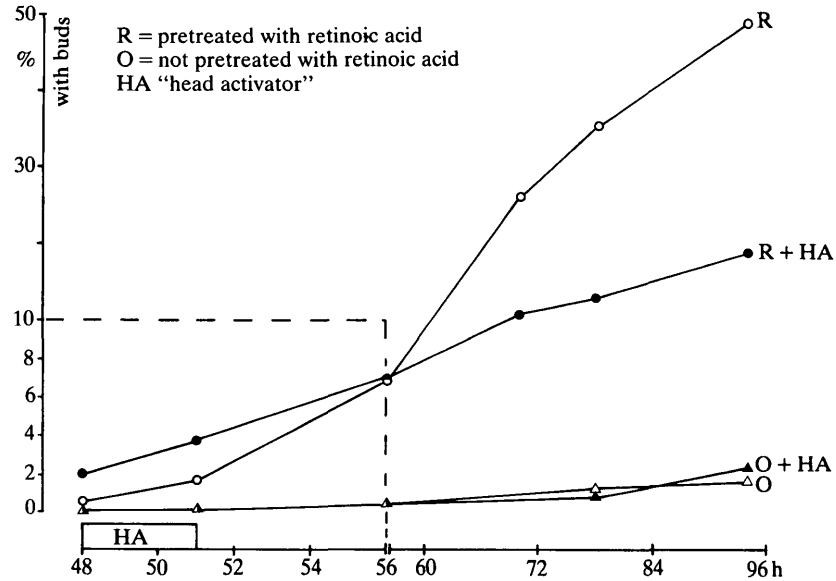


Fig. 7. Postmetamorphosis. R: these animals were pretreated with 10^{-6} M retinoic acid during early metamorphosis (3–7 h). Budding started at about 48 h. At this time, one half of the pretreated animals were incubated with 10^{-9} M 'head activator' (HA) for 3 h (R+HA). Primary polyps derived from untreated planulae (0 = control) developed only few hydranth buds, and did not respond to incubation with 'head activator' (0+HA). The only significant effect of HA was a depression of the budding rate in animals which had been induced to bud at a higher frequency by previous application of retinoic acid (compare R+HA with R). Within the dotted rectangle the scales are expanded.

$>10^{-8}$ M) causes adverse effects in all cases: even complete hypostomes reduce their tentacles and are eventually totally resorbed. Such hydranths may temporarily form a head at their proximal end but after a few days, all head-specific characteristics are lost, and the hydranths transform into elongated tubes. Their contractility identifies them as gastric columns. Finally, they may grow stolons, in contravention to the rule of distal transformation (Fig. 8).

A comparison of the dose-response curves for all the morphological changes scored yields characteristic sequences in the position of the optimum peak. Values refer to retinal, since most data were collected using this compound.

10^{-9} M×4 h: improvement of the regeneration rate,
10^{-7} M×4 h: increase in the tentacle number,
10^{-6} M×16 h: total transformation into stolonal tissue

Retinoids cause long-term alterations in hydranth behaviour not only at high doses and not only in regenerating animals. A final experiment demonstrates this:

Beheaded hydranths as well as intact polyps were treated without (control), or with 5×10^{-10} M and 10^{-6} M retinoic acid, respectively, for 18 h. Tentacle

number was monitored over a 12-day period following treatment. Amputation stimulated regenerative tentacle formation in all samples. Yet, the values were not stable. By the 12th day, only a fraction of the hydranths still bore tentacles:

Control samples:	50% of the regenerated, 65 % of the intact hydranths,
5×10^{-10}M:	62 % of the regenerated, 94 % of the intact hydranths,
10^{-6} M:	16 % of the regenerated, 5·5 % of the intact hydranths,

n was 49–135.

Low doses stabilize the state of differentiation.

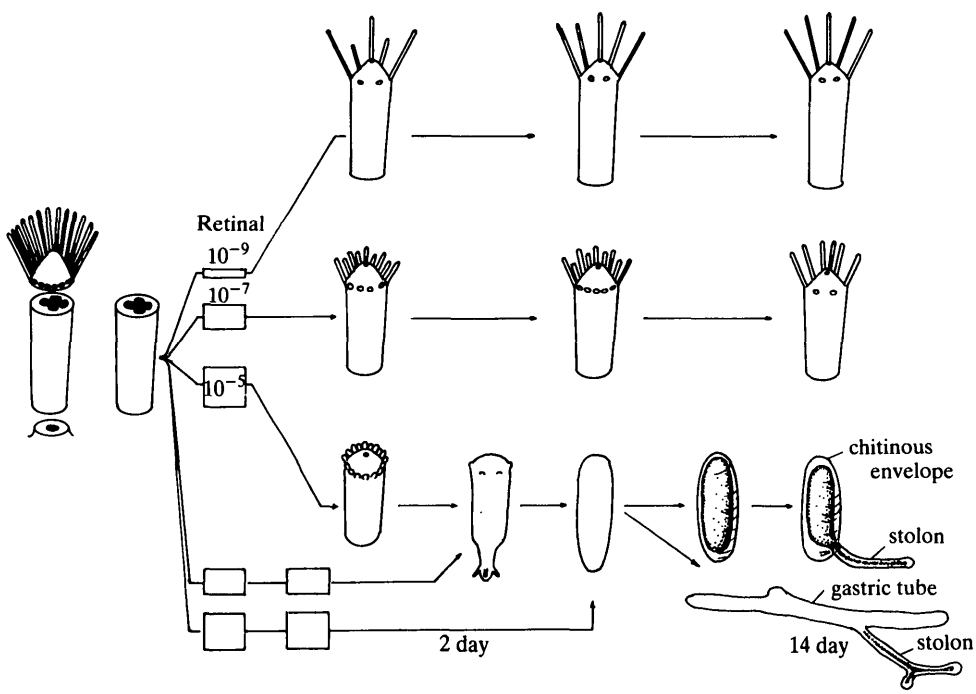


Fig. 8. Retinal and regeneration. Gastric pieces were pulse treated for 4 h with retinal immediately after isolation. Some samples were treated with a first pulse on day 0 and a second pulse on day 1. Upper series: low doses do not alter the tentacular pattern as compared to controls. However, regeneration is accelerated and the newly formed head structures are exceptionally stable. Middle series: increase in tentacle number. Some tentacles may later be reduced. Lower series: high doses or repeated treatment produce various effects. After a burst in the formation of tentacle rudiments and/or a transitory polarity reversal the regenerates reduce their head structures. Eventually, the elliptic or tube-like vestigial masses stolonize.

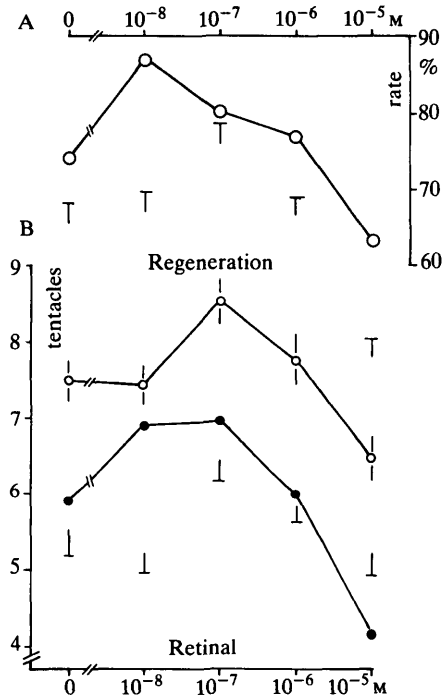


Fig. 9. Regeneration. Influence of retinal on the rate of regeneration and the final tentacle pattern. (A) Regeneration rate: fraction of gastric columns having regenerated at least two observable tentacles 48 h post amputation. (B) Tentacle formation upper curve: mean values displayed by the fraction of regenerating hydranths only. This curve reflects the actual pattern. Tentacles lower curve: mean values related to all input animals. Because in regeneration biological variability is very high, even when all hydranths are taken from the same colony, standard deviations enclose a range three times as large as in metamorphosis.

IV. *Retinoids and 'morphogens'*

The effects of retinoids are counteracted by a putative morphogen, the 'endogenous inhibitor' isolated from *Hydra* and other cnidarian sources by Berking (1977, 1983).

In terms of their effects on regenerative pattern formation, the retinoids and the inhibitor are antagonists. This statement implies an unexpected finding: when applied simultaneously with retinal, the inhibitor not only reduces the effectiveness of suboptimal retinal doses, but also compensates, within certain limits, for the deleterious effect of supraoptimal concentrations, thus improving their specific stimulating effectiveness. Most tentacles have been counted after simultaneous application of a high, by itself toxic, retinal dose with a high, by itself strongly retarding, inhibitor dose (Fig. 11). Details will be given in a separate report.

Morphological changes similar to those exerted by retinoids, in particular an

increase in the number of tentacles (and in the frequency of double head formation), could also be induced by applying extracts from various cnidarian sources. Dose-response curves also displayed optima (Müller *et al.* 1977; Müller & Meier-Menge, 1980). Hitherto, we attributed the biological activity to a putative peptide known under the name 'head activator' (Schaller, 1973; Schaller, Schmidt & Grimmelikhuijzen, 1979). This 'head activator' has been proposed to govern polarity and pattern formation in *Hydra* in cooperation with an antagonist designated 'head inhibitor'. Arguments in favour of this hypothesis were derived from assays based on the acceleration of regeneration, tentacle number and budding rate (Schaller, 1973; Schaller & Bodenmüller, 1981).

In the course of this study, several batches of the synthetic peptide, as well as a sample of the highly purified natural product, were made available. These samples had no effect on the pattern of head structures in *Hydractinia* in the concentration range from 10^{-3} M to 10^{-14} M (Figs 5B, 6). In contrast, instead of stimulating budding, the 'activator' reduced the budding rate. This reduction was particularly significant in polyps that, three days earlier, had been induced to bud in high frequency by a 4 h pulse of 10^{-6} M retinoic acid during metamorphosis. 10^{-9} M head activator, applied for 3 h after the first buds arose, reduced the subsequent budding rate from 48 to 19 % (Fig. 7). The behaviour of the primary polyps, therefore, is in apparent contrast to that of *Hydra*.

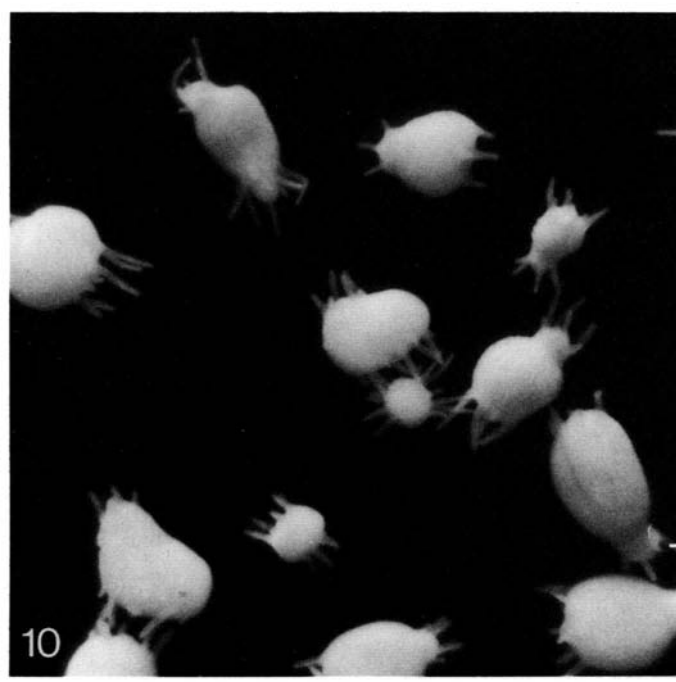


Fig. 10. Double head formation in regenerating isolated gastric columns derived from nutritive polyps (gastrozooids).

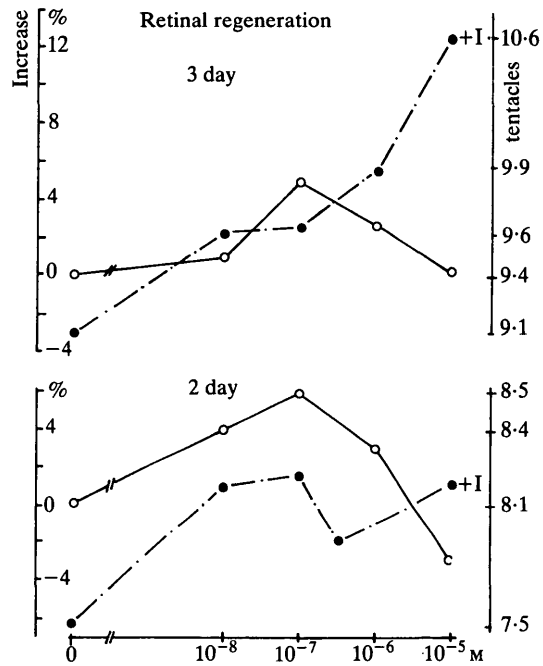


Fig. 11. Regeneration. Tentacle number in retinal-stimulated regenerating hydranths, with or without simultaneous application of a constant amount of endogenous inhibitor isolated from *Hydra*. 2 day: values scored 48 h post amputation, 3 day: final values counted at 72 h. % means relative increase or decrease as compared to the control level.

DISCUSSION

In pure phenomenological terms, the effects of the retinoids can be classified into three categories:

(1) *Radial pattern*. Tentacles and primary stolons are radially arranged structures. Their spacing must be controlled by circular pattern-forming systems. Retinoids modify the pattern-forming mechanisms at the distal and proximal body in a reciprocal way. The number of tentacles formed along the circumference is increased, whilst the number of stolon tips is decreased.

(2) *Longitudinal pattern*. The boundary between hydranth and stolon cells is shifted at the expense of the hydranth. This pattern is analogous to the prestalk-prespore pattern in *Dictyostelium* (e.g. Sternfeld & David, 1981). Total transformation of the hydranth into a giant stolon, as caused by high retinoid concentrations, means a shift of the boundary to the left end of the scale (compare Fig. 12).

(3) *Colonial pattern*. The distances between the primary and the secondary hydranths are reduced. This reduction expresses itself as increased budding rate.

The initiation of morphogenetic processes, such as tentacle formation and hydranth budding, can be attributed either to the stimulating action of an activating, or removal of an inhibitory, principle. The contemporary developmental biology of *Hydra* derives pattern specification from the crosscatalytic interaction of both activating and inhibitory morphogens. These putative morphogens are delivered by undefined sources (Meinhardt, 1982; Mac Williams, 1983). It has been proposed that the dominant sources are nerve cells, producing, for example,

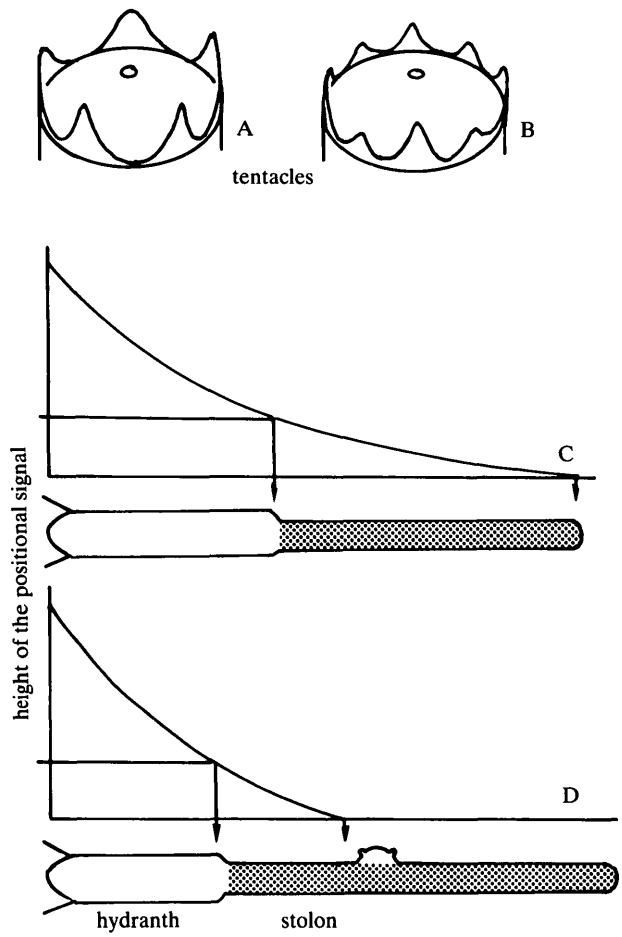


Fig. 12. Reduced ranges of positional or spacing signals, respectively, may account for several results. (A, B) If the diffusion constant of a hypothetic tentacle inhibitor were reduced, a Gierer-Meinhardt system would specify more tentacles in a circular series of cells (according to computer simulations). (C, D) The slope and extension of a morphogen gradient determines the hydranth/stolon boundary and the distance to the next hydranth bud. Reducing the range causes elongation of the stolon at the expense of the hydranth and a shift of the bud's position towards the founder hydranth. Buds arise, therefore, even on short stolons. (In a Gierer-Meinhardt system, the hydranth/stolon junction might reflect the range of an activator, the position of the bud the range of an inhibitor.)

a neuropeptide named 'head activator' (Schaller & Gierer, 1973; Schaller *et al.* 1979; Schaller & Bodenmüller, 1981). In addition, inhibitory activities, which might be interpreted as morphogens, have been extracted (Schaller *et al.* 1979; Berking, 1977, 1983).

Are retinoids activators?

At first glance, most of the morphological changes caused by retinol, retinal, or retinoic acid are similar to effects ascribed to the putative 'head activator' in *Hydra*. These include improvement of the rate of regeneration, increase in the number of tentacles and increased budding rate. Moreover, methanolic extracts from *Hydra* and fractions thereof, prepared according to Schaller *et al.* 1979, in order to separate the 'activator' from inhibitory components, stimulated tentacle formation in metamorphosing planulae of *Hydractinia* (Müller *et al.* 1977; Müller & Meier-Menge, 1980). Therefore, it first appeared that retinoids might act through stimulation of the release of neurogenic substances, in particular of the head activator.

However, the purified peptide used in the present study had neither a stimulating effect on tentacle formation nor on the budding rate. Other components might have accounted for the biological activity of the extracts, such as glutamate (Flechtner *et al.* 1981) and retinoids themselves, since retinoids should have been present in methanolic extracts.

In this context, one should bear in mind that, until 1981, an 'activator' existed merely as a putative common denominator of various biological activities present in chromatographic fractions. Furthermore, the assay used to monitor the last purification steps and assess the activity of synthetic peptides did not measure the frequency of bud initiation, but the velocity of bud outgrowth (Schaller & Bodenmüller, 1981; Birr *et al.* 1981). The interval between administration of the peptides and bud appearance was 3 h, whilst the interval between bud specification and bud appearance is 12 h (Berking, 1977).

In *Hydractinia*, I could not see an acceleration of bud emergence, perhaps because the number of stimulated rudiments was too low. The budding period induced by a previous retinoid pulse extends over 2 to 3 days and only few bud anlagen may have been in the appropriate state during the 3 h period the head activator was present. However that may be, in the long term the activator depressed the subsequent budding activity.

Retinoids increase the budding frequency. If added in low doses, they promote regenerative head development. They are countered by an *Hydra*-derived inhibitor known to reversibly block head regeneration and budding (Berking, 1979, 1983). It might be tempting, therefore, to interpret retinoids, or still unknown derivatives, as morphogens that activate head formation by themselves. Yet, such a straightforward interpretation would be difficult: the secondary hydranths arise on stolons which did not exist at the time of treatment. In addition, whilst the buds emerge to form a head, the primary hydranth is

simultaneously reduced while the stolon elongates. My conclusion is, therefore, that retinoids are not morphogens fulfilling the function of a 'head activator'.

Interpretation of the results

The spacing of hydranth buds is controlled by inhibitory signals emitted by existing hydranths (Plickert in *Eirene viridula*, in prep.). Not until the signal strength along the stolon has dropped below a critical value can new hydranth buds be specified. Accordingly, buds appear in high frequency when the primary hydranths are removed (Müller, unpublished supplementary observations in *Hydractinia*). Treatment with high doses of retinoids is equivalent to removal of the founder hydranth. This similarity of events provides a clue to understanding most of the retinoid effects, in particular the effects on the longitudinal and the colonial pattern.

My interpretation assumes the following: retinoids interfere with signalling systems responsible for the dimension of structures and spacing of periodic pattern elements. The range of positional signals emitted by the hypostome (Wolpert, Hornbruch & Clarke, 1974; Meinhardt, 1983; Mac Williams, 1983) is reduced. These signals control the length of the body column. When the signal strength falls below a critical threshold, cells of the proximal body emigrate to the stolons. The stolons elongate at the expense of the hydranth.

At least formally, the spacing signal which controls the distance between hydranths can be considered an extension of the positional signal in hydranths. A first threshold value may determine the boundary of the hydranth–stolon junction, and a second the position of the next hydranth (Fig. 12). In terms of the more elaborated model of Gierer & Meinhardt (Meinhardt, 1982; Mac Williams, 1983) the dimension of the hydranth may reflect the range of an activating morphogen, the position of the next bud the range of an inhibitory morphogen. Again, the effect of retinoids could be defined as reduction of the signalling ranges. Presently, no prediction can be made as to whether the reduction in the signalling range is due to a reduced signal generation, emission or transmission, or to an increased signal loss.

Prolonged treatment with high doses finally reduces the signalling range to zero, possibly by destroying the whole transmitting set. The positional value decreases everywhere, and as a consequence, head structures are reduced while stolons grow out. This destructive effect, however, is not specific to retinoids. It can also be evoked by a long-term administration of agents such as dimercaptopropanol (Müller & Spindler, 1972).

Tentatively, the promoting effect of low doses of retinoids on the rate of regeneration and the stability of differentiated head structures might be interpreted as a consequence of a transitory stimulation of morphogen release as well as a subsequent regenerative source renewal. Aged sources would become replaced by newly produced ones.

No uniform explanation can be given for the increase in the tentacle number

and simultaneous decrease in stolon number. The spacing mechanisms at the distal and proximal body ends appear to be inversely affected.

Pattern formation in metamorphosis is a two-step process. (1) The longitudinal body pattern is essentially specified early on embryogenesis. The future use of the cellular inventory of the larva is already preprogrammed: the anterior region is committed to become stolons, the posterior region to develop head structures (Müller *et al.* 1977). This prepattern is, however, not invariant. (2) Based upon this longitudinal pattern secondary subsystems create the periodic, radial pattern of tentacles and stolon tips along the circumference.

A change in this radial pattern may be the result of altered properties of the radial subsystems or of a previous shift of the primary longitudinal pattern. Such a shift may place more or less material at the disposal of either head or stolon formation. Oralization and aboralization as caused by caesium ions or serotonin, respectively, has been explained in terms of altered longitudinal proportions (Müller *et al.* 1977).

However, in contrast to agents such as serotonin, the retinoids are effective not only in a temporally limited period of the early metamorphosis. The pattern of tentacles can still be modified when the position of the tentacle whorl is already determined.

If we take the number of tentacles as a result of a circular pattern-forming system working along an unaltered circumference, and if we rely on only one pattern-forming model system, the basic Gierer-Meinhardt system (Meinhardt, 1982, p. 14), then an explanation is easily possible: in a circular series of cells the number of concentration peaks of a hypothetic 'tentacle activator' can best be increased by reducing the diffusion range of the corresponding inhibitor (Kemmner & Müller, in prep.) However, can one assume that uptake of retinoids lowers the diffusion constant of a 'tentacle inhibitor' but increases the diffusion constant of a 'stolon tip inhibitor'?

Even more problems would arise if one aimed at interpreting the effects of retinoids on pattern formation in vertebrate limbs and hydroid polyps by a common general principle. Such a principle has still to be found.

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