The Science Reports of the Kanazawa University, Vol. V, No. 2, pp. 77—84, May, 1959

Location of the New Pharynx in Regenerating Piece of Planaria

By

Tetsuji KIDO *)

(Received February 3, 1959)

In the process of regeneration, regenerating part takes at first a much disharmonious form to a remainder of an organism, but in most cases it gradually restores a well proportioned form. This morphological regulation is an important event in regeneration.

Planaria has a great regenerating power and is able even to undergo simply the regulation by morphallaxis. For example, the first site of a new pharynx in a regenerating piece is disproportional to the whole at the onset of its development, but it takes easily a final position that is proportional to the organism as a whole. Morgan ('00) pointed out that the first position of the new pharynx in a regenerating piece of Planaria has a definite relation to the original region in the worm, from which the piece was taken out. Child ('06, '12, '13) stated, moreover, that the more posterior the original region of the piece, the more anterior was the position of the new pharynx in the regenerating piece. He ascribed this to the regional difference due to the gradient of the physiological activities. Buchanan ('27) carried out a more precise experiment by means of estimating an acculate position of the new pharynx in the piece removed from the various levels of *Phagocata gracilis*. According to him the factor which determines the site of the new pharynx is essentially physiological and quantitative in character, like the claim of Child. Afterwards this gradient conception is stressed by many investigators (Abeloos, '30; Sivickis, '33; Buchanan, '33; Watanabe, '35), e. g., head frequency decreases from anterior rearwards in the original worm. However, there remains a question as to whether the site of the new pharynx estimated by the previous authors indicates accurately the very same position in which the new pharynx actually begins to develop, because a final position of the pharynx becomes different from the first one due to accomplishment of regulation. Therefore, it is required to measure the site of the new pharynx immediately after it is first settled. The present experiment was undertaken with caution for this point.

The writer wishes here to express his hearty thanks to prof. Dr. M. Ichikawa of the University of Kyoto, for his encouragement and reading the manuscript.

Material and Methods

The material used in the present experiment was *Dugesia gonocephala* collected in

* Biological Institute, Faculty of Science, Kanazawa University

the vicinity of Kanazawa City. They were exclusively asexual form 16–18 mm long.

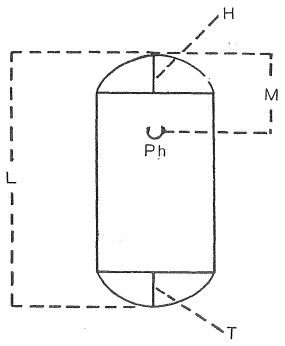

Fig. 1. Scheme showing the parts to be measured in the regenerating piece. H; length of the new tissue regenerated from the anterior end of the piece. T; length of the new tissue regenerated from the posterior end of the piece. L; total length of the regenerating piece. M; distance from the tip of the anterior new tissue to the middle of a new pharynx. Ph; new pharynx.

After starvation over a week, animals of approximately same size were selected and cut each into a certain number of pieces. The pieces excepting a head were cultured respectively in a Petri dish filled with tap water. Caution was paied for detecting the position of the new pharynx as soon as possible and for measuring the lengths of the following portions of a piece: (1) total length of the piece (designated as *L*), (2) distance from the tip of a new head to the middle of a new pharyx (*M*), and (3) length of a new tissue regenerated from the anterior cut surface (*H*) or from the posterior cut one (*T*), as sown in Fig. 1. In order to measure as accurately as possible, the specimen was brought under a binocular microscope after fixing it in one per cent solution of hydrochloric acid. The fixation did not at all affect the structure of the regenerating worm. Occasionally, the heteromorphic individuals appeared, but they were excluded from the measurement, for they were unsuitable for the present purpose of the experiment.

Experimental Results

Experiment I. *Position of the new pharynx in the regenerating piece on the third or fourth day after cutting.*

Measurements of 50 specimens coming from each level, except for the pharyngeal one (D), were made immediately after the new pharynx became externally visible, or on the third or the fourth day after cutting.

The results are summarized in Table 1, but for the sake of convenience, the explanation of the results will be given separately according to the length of the cut pieces.

Table 1. Measurements of the regenerating pieces on the third or the fourth day after cutting.

Levels of regenerating pieces	Length of parts (in mm.)*				Ratio
	H	T	L	M	M/L
A	0.24	0.19	1.74	1.56	0.89
B	0.24	0.18	1.74	1.50	0.86
C	0.18	0.18	1.68	1.44	0.86
E	0.30	0.24	2.46	0.90	0.36
F	0.30	0.18	2.58	0.90	0.34
G	0.24	.....	3.18	0.84	0.26
AB	0.24	0.24	2.82	2.58	0.92
EF	0.30	0.18	3.18	1.08	0.34

* Numerals in each column represent the average value of the measurements of 50 specimens.

(a) *Position of the new pharynx in the short piece.* The headless animal was cut transversely into seven pieces along the body axis, but the size of each piece was somewhat different from level to level (cf. Fig. 2 and the upper 6 rows in Table 1). On the third or fourth day after cutting, the pieces from the prepharyngeal region (A/7, B/7 and C/7)* indicated each a small but distinct accumulation of dark pigment granules at the middle point of a posterrior border line between the old and new tissues, regardless of the level from which the test piece was taken. The pharynx was always found as a semitransparent spot just behind this pigment accumulation, and it began to grow gradually into the old tissue. Such mode of appearance of the new pharynx has previously been reported by Okada and Sugino ('37), Okada and Kido ('43), Kido ('52), whereas another mode of appearance was stated by Child ('06) and Teshirogi ('55) with different species in which the new pharynx developed from the first in the old tissue. The site of the new pharynx with respect to the total length of the piece at the time of its first appearance was approximately the same with one other (0.89 in A/7, 0.86 in B/7 and C/7), as can be seen in Table 1 as the ratio M/L.

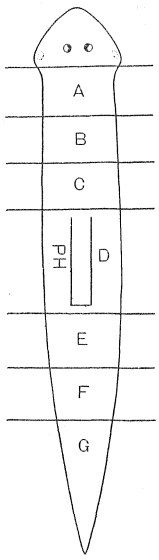

Fig. 2. Scheme showing the levels of seven pieces (A–G) in the worm. PH; original pharynx.

At that time, both regenerates from the anterior and posterior cut surfaces were yet very poor in growth, and their length differed very slightly, the anterior being a little longer than the posterior. The eye-spots were hardly discernible from outside.

In the pieces from the pharyngeal region (D/7), the old pharynx occupied the main part of the piece. In the case in which the old pharynx was left uninjured, it was cut apart from its own base (autotomy), and thrown out of the body. The new pharynx developed from the base of the atrium. In the case in which the old pharynx was left without followed by the autotomy, the old one was absorbed gradually to disappear, and the new one arose from the middle of the atrial wall on either side. In short, the mode of development of the new pharynx was not uniform due to presence or absence of the original pharynx. In addition, pharyngeal appearance was more delayed in the later than in the former case. The data measured with respect to the piece D/7 were omitted from the table for the reason of this time difference of the pharyngeal appearance and consequently the difference of the ratio M/L between the former and the later case.

In all pieces from the postpharyngeal region (E/7, F/7 and G/7, cf. Fig. 2), the new pharynx occurred always in the old tissue near the anterior cut surface, i. e., the ratio M/L of these piece decreased to 0.36, 0.34 and 0.24 in respective piece coming from the anterior to the posterior level of the worm, and the pharyngeal appearance was slightly delayed in comparison with that in the prepharyngeal region.

*Alphabet of numerator, A, B or C represents the level in the worm of the test piece taken from anterior rearwards, and denominator indicates the number of the cut pieces from the same individual.

(b) *Position of the new pharynx in the long piece.* In the above experimental series, the pieces cut out from the prepharyngeal region were always shorter than those from the postpharyngeal one. Therefore, there will arise a question concerning the above mentioned locality of the new pharynx. In other words, whether the different values of the ratio M/L between the pre- and postpharyngeal pieces really reflect the difference of the region of the worm or merely result from the difference of the length of the piece. In order to settle this question, the further experiment was carried out. For this purpose the long pieces were cut out from the pre- and postpharyngeal regions. The length of the piece AB from the prepharyngeal region was equal to the length of A/7 plus B/7 of the short pieces of the previous experiment and likewise the length of the piece EF from the postpharyngeal region was equal to the length of E/7 plus F/7 (cf. Fig. 2). Even in the long piece AB, a new pharynx was also formed at a posterior border line between the new and old tissues as was found in the short pieces. The ratio M/L in this case was 0.92 and in the case of the short piece it was 0.89 in A/7 and 0.86 in B/7. The long piece EF showed the onset of the new pharyngeal development in the old tissue near the anterior border, just as the respective short piece E and F did. Namely, the ratio M/L was 0.34 in EF, 0.36 in E/7 and 0.34 in F/7 (cf. the lower two rows in Table 1). From these facts it is evident that even if the pieces were different in length, so far as they were taken from the same region, first position of the new pharynx and its mode of appearance are both nearly the same

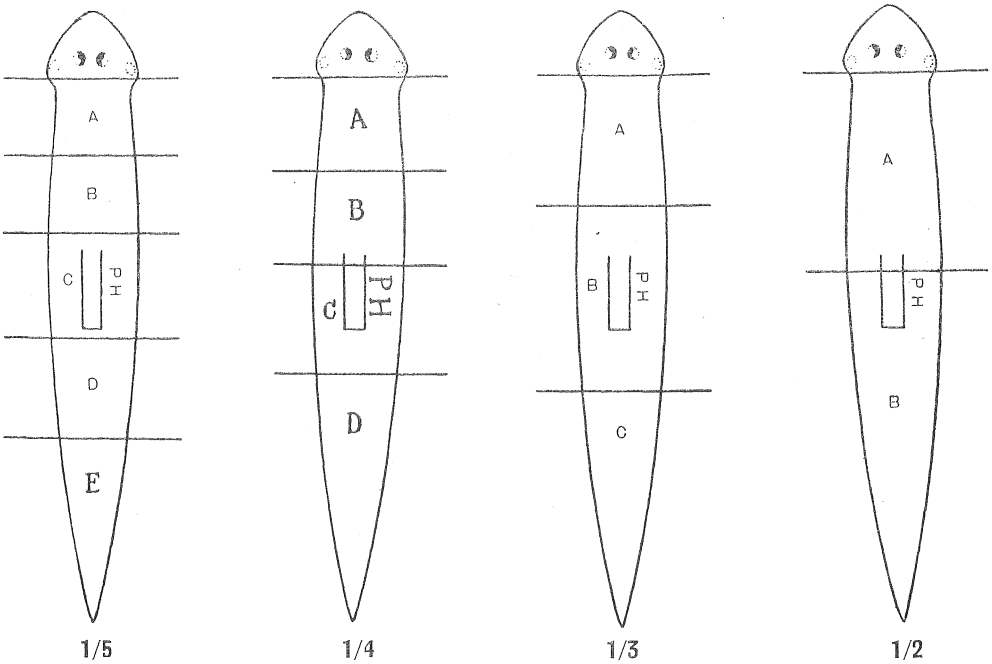

Fig. 3. Scheme showing the origins of the pieces of half, one-third, one-fourth and one-fifth of the worm. Each piece is termed A/2, B/3, D/5 and so on, and e. g., D/5 means D region when the worm body was divided into five pieces.

as in the short piece.

Furthermore, the speed of regeneration at both anterior and posterior cut surfaces was not at all influenced, at least for a first few days, by the length of the regenerating piece.

Experiment II. *Position of the new pharynx at fourteen days after cutting.*

In experiment I, it is exhibited that the new pharynx begins to develop at a definite position of the cut piece, so far as the source of the test pieces is the same level in the worm. However, as regeneration goes further, the new pharynx gradually changes its position towards the middle of the piece. To know more precisely this regulation the test pieces were cut in different size from the different regions of the animals, as is shown in Fig. 3. In the pieces derived from the prepharyngeal region such as A/3, A/4, A/5, B/5, A/7, B/7 and C/7, the new pharynx always began to develop on the posterior line of demarcation between the old and new tissues, regardless of the length of the piece.

In the pieces including the whole old pharynx such as B/3, C/5 and D/7, the new pharynx developed according to a rule which was described in the previous experiment. When the cutting was made a little behind the base of the pharynx, the divided pharynx in the respective piece was thrown out of the body, but the atrium remained in situ. In the cases in which a basal fragment of atrium remained in the piece such as A/2 and B/4, the new pharynx developed from this fragment. But, in the cases in which most of the old atrium except for its base was included as in B/2 or in C/4, the new pharynx arose from a point where the cut ends of both sides of the atrial wall fused with each other.

In the pieces coming from the postpharyngeal region such as C/3, D/4, D/5, E/5, E/7 and G/7, the new pharynx made its appearance in the old tissue behind the regenerating new head. In short, the position and the mode of appearance of the new pharynx were quite similar to those in the case where a worm was divided into seven pieces in the previous experiment.

At fourteen days after cutting, the specimens were sacrificed and the measurements of the ratio M/L were made with fifty specimens in each group. But the length of the new head (H) and the new tail (T) could not be measured, because the boundary between the old and new tissues regenerated from the cut surface became obscure by that time. The shape of the new head and tail approached nearly to the normal, the new pharynx elongated considerably and the pharyngeal opening was recognized on the ventral side of the regenerating worm. The average values of the measurements are arranged in Table 2.

The table indicates that it is a rule that the ratio M/L gradually approaches to the normal value of 0.50 from the anterior piece to the posterior one in every group regardless of the length of the test piece. This finding is in line with Buchanan's observation on another Planaria, *Phagocata gracilis*.

Table 2. Measurements of the regenerating pieces at fourteen days after cutting.

Levels of regenerating pieces		Average values of measurement (in mm)*		Ratio
		L	M	M/L
1/2	A/2	5.46	3.30	0.64
	B/2	5.58	2.34	0.40
1/3	A/3	4.32	2.64	0.61
	B/3	5.16	2.46	0.48
	C/3	4.32	1.86	0.43
1/4	A/4	3.60	2.28	0.63
	B/4	4.20	2.22	0.53
	C/4	4.14	1.98	0.48
	D/4	5.08	1.86	0.46
1/5	A/5	4.26	2.58	0.61
	B/5	4.44	2.28	0.53
	C/5	4.38	2.16	0.49
	D/5	4.62	2.28	0.48
	E/5	4.74	1.98	0.41
1/7	A/7	2.04	1.50	0.73
	B/7	2.28	1.44	0.65
	C/7	2.58	1.64	0.63
	D/7	4.31	2.16	0.50
	E/7	2.58	1.38	0.54
	F/7	2.28	1.32	0.58
	G/7	2.28	1.14	0.50
Control (Intact animal).....		14.32	7.08	0.50

* Numerals in each column represent the average value of the measurements of 50 specimens.

Discassion

As to the initial site of the new pharynx occurring in the planarian regenerating piece, Buchanan ('27) found with *Phagocata gracilis* that it is more posteriorly located in the piece taken from the more anterior level of the worm, and it is nothing to do with the length of the piece. Child and his school elucidated this fact as a phenomenon which arose according to the physiological gradient existing along the antero-posterior body axis. However, the measurements in the present experiment at the time of the first appearance of the new pharynx revealed that Child's elucidation based on the Buchanan's report is not applicable to our material *Dugesia gonocephala*. In our species, the new pharyngeal formation occurs on the posterior line of demarcation between the old and new tissues of the test piece, regardless of the level of the piece located in the original worm, so far as it lies in the prepharyngeal region. In the postpharyngeal pieces, this pharyngeal formation always appears in the old tissue at a point definitely distant from the tip of the new head and not at a point that would be expected from

Buchanan's experiment.

As the regeneration proceeds on, the new pharynx is found nearer and nearer to the middle of the regenerating piece (cf. Table 2). Therefore, the pharynx seems to shift its position cephalad in the prepharyngeal piece, but caudad in the postpharyngeal piece. Such seeming shift of the pharyngeal position is always recognized in every piece regardless of its original length. As to the cause responsible for this seeming shift we can point out the followings : 1) the new pharynx actually grows towards the middle of the test piece during the process of regeneration, 2) the rate of regeneration differs between the anterior and the posterior cut surface, 3) the morphological regulation occurs by morphallaxis throughout the whole regenerating piece.

The first possibility is evidently observed, but not so strong as to affect the measurement value. If the second is probable, the regeneration in the prepharyngeal pieces has to proceed more rapidly or to continue longer at the posterior cut surface than at the anterior one, and *vice versa* in the postpharyngeal piece.

Measurements of the postpharyngeal pieces on the third or the fourth day of regeneration are favourable to the view of this *regeneration speed difference* at the cut surfaces, but those of the prepharyngeal pieces are not necessarily favourable (cf. Table 1). H and T measurements are impossible later, but it is likely that the duration of regeneration differs between the anterior and posterior cut surfaces. Concerning the third possiblity, most of the investigators who studied microscopically Planarian regeneration demonstrated that the embryonic cells migrate from the old parenchymatous tissue to the cut end to form a blastema. The migration of such cells is not only favourable to the blastema formation, but also to the remoulding of the regenerating piece.

As is shown in Table 2, the site of the pharynx indicated in terms of the ratio M/L at fourteen days after cutting shows gradual difference in every group, i. e., it lies the more posteriorly in the piece taken from the more anterior level of the worm.

In the piece from the pharyngeal region, it was found that the regeneration of the new pharynx proceeds exclusively from the atrial wall. While in the pre- and postpharyngeal pieces the new pharynx was established from the material other than atrial. What is the source of the new pharynx ? Child and Watanabe ('35) claimed that the new pharynx is formed by the rejuvenized cells in the old part of the piece. On the other hand, Stevens ('07) and others emphasized that the pharynx is formed by the mesenchymal reserve cells. Further, Okada and Sugino ('37) and Okada and Kido ('43) have pointed out in their transplantation experiments that the new tissue regenerated between the graft and the host is intimately related to the formation of the new pharynx. The present experiment can contribute nothing to this point of dispute. It still remains to be proved.

Summary

1. Headless body of Planarian *Dugesia gonocephala* was cut into seven pieces, and a

comparison of regeneration was made in terms of the length of the following parts measured at the time when the new pharynx first appeared, or on the third or the fourth day after cutting; 1) total length of the piece (L), 2) distance from the tip of the new head to the middle of the new pharynx (M), 3) length of the new head (H) and 4) length of the new tail (T).

2. There is a distinct difference in the ratio M/L between the pre- and postpharyngeal pieces, but no difference was found so far as the piece comes from the same pre- or postpharyngeal region. It is a rule that the new pharynx appears always at the middle point of the posterior border line betwen the new and old tissues in the prepharyngeal piece, while it occurs exclusively in the old tissue behind the regenerating new head in the postpharyngeal piece. In the pharyngeal piece, it is derived from the celles of the old atrial wall.

3. Measurements at fourteen days after cutting indicate that the morphological regulation occurs in due course with differential rate along the anteroposterior axis of the original body.

References

Buchanan, F. W,. 1927. Jour. Exp. Zool., 49, 69.
Child, C. M., 1906. Arch. EntwMech. Org., 20, 380.
Child, C. M., 1912. Jour. Exp. Zool., 13, 103.
Child C. M. 1913. Arch. EntwMech. Org., 37, 108.
Child, C. M. and Y. Watanabe., 1935. Phys. Zool., 8, 1.
Kido, T., 1952. Ann. Zool. Jap., 25, 383.
Morgan, T. H., 1900. Arch. EntwMech. Org., 10, 48.
Okada, Y. K. and H. Sugino., 1937. Jap. Jour. Zool., 7, 373.
Okada, Y. K. and T. Kido., 1943. Jour. Fac. Sci. Tokyo Univ., 4, 601.
Stevens, M. M. ,1907. Arch. EntwMech. Org., 24, 350.
Teshirogi, W., 1955. Zool. Mag. (in japanese), 64, 294.