# STUDIES ON AN ASCIDIAN OF MADRAS COAST

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UNIVERSITY OF MADRAS

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BY

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# PREFACE

The fact that India lags far behind the rest of the world in zoological research is painfully clear to one interested in the study of Ascidians. There is a wealth of sessile Ascidian fauna in Indian waters, and yet so far only nine papers have been published:—one, by Das ('36) on the Anatomy of *Herdmania pallida* (Heller), in the Indian Zoological Memoirs Series; two, by the present author, 'The development of *H. pallida*' ('53), and the 'Anatomy and larval organisation of Polyclinum sp.' (42); and the remaining six, on the taxonomy of a few forms by Herdman ('91, '06), Oka ('15) and Das ('38, '40 & '45). Even in such a field where the harvest is plentiful and labourers few, during a limited period of three years, one can study only a few aspects of the life of a single Ascidian, if he is to interest the zoologist at large, familiar with the considerable amount of work done on Ascidians of various parts of the world.

Colonies of the Ascidian (*Polyclinum indicum* n.sp.) studied are restricted to a narrow three-mile area of the Madras coast. Since these colonies are found densely crowded together on rocks and thrive in spite of the ceaseless pounding action of the breakers, the author studied the effect of the agitation of water on the rate of metamorphosis, and on the structure of the larva, by subjecting the larvae to a measurable force like the centrifugal force in the laboratory. Secondly, though the phenomenon of asexual reproduction of the Ascidians and the alternation of sexual and asexual phases in the life cycle have been studied with great care by many authors in countries like Europe, Britain and America, where the life cycles in several forms are geared on to the alternation of cold and warm seasons, yet the tropical forms adapted to uniformly warm conditions have not been studied by any so far. Thirdly, the phenomena of regression and regeneration present several unique features in *P. indicum*. The zooids exposed to adverse conditions dedifferentiate but retain the power of recovery till the post-abdomen is affected on the fifth day. Redifferentiation of such colonies, as well as regeneration of mutilated colonies, take place by a process of post-abdominal budding.

In venturing to present these studies the author is only too conscious of the limitations under which they were made. If the city authorities permit temporary structures on the shore opposite to the laboratory, if facilities for bringing necessary quantities of sea-water from different parts of the coast are made available, and if the scheme for circulation of sea-water in the laboratory tanks is completed, several aspects of the life of the Ascidians of Madras coast can be studied, and studied more exhaustively, than has been possible now.

The author wishes to express his grateful thanks to Dr. C. P. Gnanamuthu, Director of the Zoology Research Laboratory, University of Madras, for suggesting the problem, criticism and guidance throughout the course of the work. Thanks are also due to the Syndicate of the University of Madras for awarding me a Research Fellowship.

V. O. SEBASTIAN

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# STUDY OF THE EFFECT OF CENTRIFUGING ON THE LARVAE OF *POLYCLINUM INDICUM*

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## STUDY OF THE EFFECT OF CENTRIFUGING ON THE LARVAE OF POLYCLINUM INDICUM

### A. *Acceleration of metamorphosis of the larvae of polyclinum indicum by centrifuging*

*Introduction.*

As Berrill ('50) observes, "The factors determining the duration of free-swimming life or onset of metamorphosis are not clearly understood although they have been subject of considerable investigation and controversy". Metamorphosis has been found to be influenced by different types of factors by different authors. Firstly, by mechanical injury such as cutting off the tail (Willey '92) or by inflicting injuries on the anterior part of the nervous system of the larva (Zhinkin '38), developmental changes may be induced. Secondly, stimulations through light and shade as well as by mechanical jarrings and agitations have been found by Grave ('36) to influence metamorphosis. Thirdly, Child ('27) has demonstrated that carbon dioxide tension affects metamorphosis, while Berrill ('29) has shown that hydrogen ion concentration of the medium has its effects on metamorphosis. Fourthly, the effect of chemicals such as copper (Grave & Nicoll, '39; Bertholf '45; Glaser & Anslow, '49), isotonic NaCl (Bradway, '36), neutral red (Bradway '36; Zhinkin '38), methylene blue (Zhinkin '38; Bertholf '45), brilliant cresyl blue (Zhinkin '38), Janus green (Bertholf & Mast '44), and a brief exposure to distilled water (Bertholf '45) have been studied. Fifthly, organic compounds like mammalian thyroid glands have been found by Weiss ('28) and Grave ('36) to induce metamorphosis. Tissue extracts of ascidians and other animals have been found to accelerate metamorphosis by Grave ('36) and Grave & Nicoll ('39). Of such a nature may be the results obtained by Grave & Nicoll ('39) who treated the larvae with 'conditioned' sea-water.

From these investigations it is obvious that the rate of metamorphosis depends on a number of physical and chemical factors. In the present study, the factor of disturbance in the water is examined because the larvae of the shore-dwelling forms are al-

ways subjected to the action of the breakers. Colonies of *Polyclinum indicum* Sebastian ('54), found at Madras, are attached to the under-surface of rocks on the shore at tidal level. These colonies are exposed to the pounding action of the waves. The fact that large numbers of these thrive and settle argues that the environment must favour quick metamorphosis, probably through the violent disturbances themselves. A study of the effect of such disturbances on the ascidian tadpoles can be made with advantage in the laboratory by assessing the changes induced in the larvae by a force of a measurable character such as the centrifugal force. The effect after treatment can be graded and its relation with the magnitude and duration of force calculated.

*The Metamorphosis of the Normal Larvae*

The free-swimming period of the larvae of *P. indicum* lasts for 5 to 8 hours. The sea at Madras has an average temperature of 27 to 29°C. It was found that in each batch of lavae liberated metamorphic changes started 5 hours after liberation and were completed in 8 hours. These changes are ushered in when the larvae become inactive and fix on the substratum by means of the anterior adhesive papillae whose secretory cells are protruded out. The tails undergo sudden resorption. After such an initial fixation, the ampullae facilitate the final fixation. Of the numerous changes, the body undergoes a differential growth at the anterior extremity rotating the oral and atrial siphons, the heart commencing to beat after 3 hours, the siphons contracting after 5 hours, the intestine starting to function after 10 hours.

*Experiment*

Larvae used were, just liberated (0 hours growth), 1 hour old, 2 hours old, 3 hours old and 4 hours old ones, and larvae of each category were centrifuged for ½ hour, 1 hour, 2 hours, 3 hours and 6 hours, at a uniform speed of 4000 r.p.m. Experiments were conducted twice a month from August '51 to March '52, each time using about 120 larvae kept inside 4 tubes of sea-water. It was found that more than this number may be too many for the size of the tubes used, as overcrowding may lead to accidental friction which may accelerate metamorphosis as in the overcrowding experiments of Grave ('36), and Grave & Nicoll ('39). A control was maintained by similarly keeping 120 larvae in 4 tubes of the

same size. When the centrifuging was over, the larvae were graded into 5 groups based on the condition of the tail and activity of the larvae; (A) free-swimming larvae, (B) inactive larvae with tails complete, (Fig. 31), (C) inactive ones with tail ¼ resorbed, (Fig. 32), (D) inactive with tail ½ resorbed, (Fig. 33), and (E) inactive, with tail ¾ resorbed, (Fig. 34). After each experiment the percentages of larvae in each group A, B, C, D & E, were calculated, and finaly the averages for all the 16 experiments conducted during the 8 months were taken. It was found from the control that there was no appreciable difference in the time of metamorphosis in any month, all the larvae undergoing tail resorption within a period of 5 to 8 hours.

It was assumed that the centrifugal force affected all the 30 larvae centrifuged together equally because as could be seen after a few revolutions the head becomes directed away from the centre in all larvae, so that the larvae are orientated in this position and not left in different planes. Hence, after a very short time—a few hundreds of revolutions—the centrifugal force acts parallel to the head-tail axis of all larvae alike. Yet, for no accountable reason, the effects vary and the larvae are classified as above. In C, D & E, the secretory cells of the adhesive papillae are shot out and the larvae attach themselves to the bottom of the tube. Once attached, the head-tail axis is maintained in the direction of the centrifugal force. Later still, the adhesive papillae also suffer and are lost, the adhesion being continued by the ampullae being enlarged and pushing the tunic also before them. These features of behaviour of the larvae could be observed in the glass tubes immediately after stopping the centrifuge. When these tubes are left to rest for sometime, the larvae which are not affected continue to swim about.

The radius of the arm of the centrifuge being 15 cms., at a speed of 4000 r.p.m., a force of 2684 G is produced, $G = [r/980\ (n\pi/30)^2]$, where $r$ is the radius of centrifuging, and $n$, revolutions per second. This force being developed in a few seconds and maintained throughout the period in every experiment, the force used was assumed to be constant.

Tables I to V show the results of centrifuging larvae of *P. indicum* of ages 0 to 4 hours, at 4000 r.p.m. for ½, 1, 2, 3 & 6 hours duration.

TABLE I.

The effect of centrifuging larvae of 0, 1, 2, 3 & 4 hours growth at 4000 r.p.m. for ½ hour.

| Age of larvae | Total larvae treated (16 expts.) | Percentage of larvae undergoing resorption of tails. | | | | | Control | Percentage of larvae undergoing resorption of tail. |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | |
| 0 hour | 1945 | 28·53 | 23·80 | 27·12 | 10·74 | 9·81 | 1941 | 0 |
| 1 hour | 1939 | 17·63 | 19·76 | 25·12 | 18·10 | 19·39 | 1937 | 0 |
| 2 hours | 1953 | 6·28 | 9·87 | 19·70 | 29·38 | 34·77 | 1931 | 0 |
| 3 hours | 1931 | — | — | 7·59 | 35·53 | 56·88 | 1937 | 0 |
| 4 hours | 1935 | — | — | — | 7·35 | 92·65 | 1931 | 0 |

After centrifuging for 30 minutes, the effect on the larvae can be assumed to be due to age as well as centrifugal force. Among the batches of 0 hr. (just liberated), 1 hr. old and 2 hrs. old larvae, 28·53%, 17·63% and 6·28% respectively are unaffected after centrifuging. The decrease in the percentages of the free-swimming forms shows that as age advances the effect of the centrifugal force is marked. Similarly, those affected ones, (B, C, D & E groups) show proportionate increase in the amount of resorption of tails. In 3 hrs. old ones all are in C, D & E groups, while in 4 hrs. old ones, only D & E groups are seen. Control shows no change.

TABLE II.

The effect of centrifuging larvae of 0, 1, 2, 3 & 4 hours growth at 4000 r.p.m. for 1 hr.

| Age of larvae | Total larvae treated (16 expts.) | Percentage of larvae undergoing resorption of tails. | | | | | Control | Percentage of larvae undergoing resorption of tail. |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | |
| 0 hour | 1943 | 21·20 | 21·19 | 20·32 | 18·67 | 18·52 | 1942 | 0 |
| 1 hour | 1935 | 10·68 | 17·33 | 26·25 | 23·82 | 21·92 | 1942 | 0 |
| 2 hours | 1940 | — | 7·51 | 23·86 | 33·37 | 35·26 | 1943 | 0 |
| 3 hours | 1934 | — | — | — | 11·89 | 88·11 | 1945 | 0 |
| 4 hours | 1932 | — | — | — | 7·21 | 92·79 | 1928 | 89·10 |

Here, the percentages of free-swimming larvae decrease when compared to the results of centrifuging for ½ hour, and those of larvae with tail resorption increase proportionately. In the control no change is seen except in the 4-hrs.-old larvae which have become 5 hrs. old during the period of centrifuging and hence 89·10% have undergone resorption of tail.

In tables III, IV and V showing the results of centrifuging larvae for 2, 3 and 6 hours respectively, only six experiments were conducted in each case at closer intervals, because it was found from experiments conducted monthly that no appreciable deviations were met with in any of the experiments.

TABLE III.

The effect of centrifuging larvae of 0, 1, 2, 3 & 4 hours growth at 4000 r.p.m. for 2 hours.

| Age of larvae | Total larvae treated (16 expts.) | Percentage of larvae undergoing resorption of tails. | | | | | Control | Percentage of larvae undergoing resorption of tail. |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | |
| 0 hour | 724 | 8·29 | 13·92 | 21·01 | 26·57 | 30·23 | 715 | 0 |
| 1 hour | 727 | 4·27 | 19·12 | 22·03 | 25·58 | 29·00 | 727 | 0 |
| 2 hours | 730 | — | 4·23 | 29·96 | 31·80 | 37·01 | 720 | 0 |
| 3 hours | 723 | — | — | — | 8·15 | 91·85 | 733 | 87·56 |
| 4 hours | 731 | — | — | — | 5·19 | 94·81 | 725 | 91·23 |

The results obtained go to prove that fewer larvae are able to swim freely after centrifuging for 2 hours and more larvae show greater degree of tail resorption. In the control, 3-hour-old larvae have grown to 5 hours old and hence 87·56% have undergone resorption of tail, while the 4-hours-old ones have grown to 6 hours and so 91·23% have undergone metamorphic changes.

TABLE IV.

The effect of centrifuging larvae of 0, 1, 2, 3 & 4 hours growth at 4000 r.p.m. for 3 hours.

| Age of larvae | Total larvae treated (16 expts.) | Percentage of larvae undergoing resorption of tails. | | | | | Control | Percentage of larvae undergoing resorption of tail. |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | |
| 0 hour | 730 | 3·28 | 18·90 | 23·17 | 25·50 | 29·03 | 724 | 0 |
| 1 hour | 731 | — | 3·42 | 27·63 | 31·58 | 37·37 | 722 | 0 |
| 2 hours | 728 | — | — | — | 6·45 | 93·55 | 722 | 88·76 |
| 3 hours | 719 | — | — | — | 5·42 | 94·58 | 721 | 90·45 |
| 4 hours | 731 | — | — | — | 3·27 | 96·73 | 721 | 96·10 |

Here, increase of the duration of centrifuging has resulted in effecting metamorphic changes in a large proportion of larvae, and in their undergoing greater amount of tail resorption. Only in just liberated ones there is 3·28% of free-swimming ones, while in all other cases the larvae are affected and none are free-swimming. The 2, 3 and 4 hours old larvae belong to only D and E groups. In the control 0 and 1 hour old larvae undergo no change after 3 hours, while 2, 3 and 4 hours old ones show metamorphic changes, the proportion increasing according to the increase in age during the duration of centrifuging.

TABLE V.

The effect of centrifuging larvae of 0, 1, 2, 3 & 4 hours growth at 4000 r.p.m. for 6 hours.

| Age of larvae | Total larvae treated (16 expts.) | Percentage of larvae undergoing resorption of tail. | | | | | Control | Percentage of larvae undergoing resorption of tail. |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | | |
| 0 hour | 719 | — | — | — | 5·42 | 94·58 | 721 | 89·45 |
| 1 hour | 731 | — | — | — | 3·27 | 96·73 | 721 | 95·54 |
| 2 hours | 722 | — | — | — | 2·07 | 97·63 | 725 | 100· |
| 3 hours | 728 | — | — | — | — | 100 | 723 | 100· |
| 4 hours | 733 | — | — | — | — | 100 | 723 | 100· |

In this case, as is to be expected in accordance with the results obtained in previous tables, increase in this time of centrifuging has resulted in greater resorption of tails, all falling under D and E groups. In 3 and 4 hours old larvae all have resorbed ¾ of their tails. In the control also there is a proportionate increase in the number of larvae undergoing metamorphosis. All the 3 and 4 hours old larvae undergo metamorphosis. The interesting fact is that there was no casuality in any of the centrifuging experiments.

From the results obtained, calculations were made to express the mean length of resorption of the tail in terms of age of the larvae and duration of centrifuging. For this two-way tables were prepared for length of tail and period of centrifuging, length of tail and age of larvae, and age of larvae and duration of centrifuging, the third factor in each being assumed to be absent, and correlations were calculated. Calculations set forth below were made for experiments on larvae ranging from 0 hour (just liberated) to 3 hours growth, and for duration of treatment from ½ hour to 3 hours. But, the results of centrifuging 4-hour-old larvae for various periods and also the results of centrifuging larvae of various ages for 6 hours were excluded, because the larvae became older and approached metamorphosis.

TABLE VI

Correlation for length of tail and age of larvae

| | Relative length of resorption of tail | | | | | |
|---|---|---|---|---|---|---|
| Age of larva | 0 | ·25 | ·5 | ·75 | Total | |
| 0 hour .. | 77·81 | 91·62 | 81·48 | 87·69 | 338·60 | ($m_1$) |
| 1 hour .. | 59·63 | 101·03 | 99·08 | 107·68 | 367·42 | ($m_2$) |
| 2 hours .. | 21·61 | 70·52 | 101·00 | 200·59 | 393·72 | ($m_3$) |
| 3 hours .. | — | 7·59 | 60·99 | 331·42 | 400· | ($m_4$) |
| Total .. | 159·05 ($n_1$) | 270·76 ($n_2$) | 342·55 ($n_3$) | 727·38 ($n_4$) | 1499·74 | (N) |

$$\text{Arithmetic mean for age of larva} = \frac{0m_1 + 1m_2 + 2m_3 + 3m_4}{N} = 1{\cdot}57$$

$$\text{Arithmetic mean for length of tail} = \frac{0n_1 + {\cdot}25n_2 + {\cdot}5n_3 + {\cdot}75n_4}{N} = {\cdot}524$$

$$\text{Standard deviation for length} = \sqrt{\frac{0^2n_1 + {\cdot}25^2n_2 + {\cdot}5^2n_3 + {\cdot}75^2n_4}{N}} - \text{a.m. length}^2 = {\cdot}26.$$

$$\text{Standard deviation for age} = \sqrt{\frac{0^2m_1 + 1^2m_2 + 2^2m_3 + 3^2m_4}{N}} \text{ a.m. age}^2 - (\text{a.m. length})(\text{a.m. age}) = 1{\cdot}11.$$

$$\text{Product moment} = \frac{\begin{array}{l}0\,(0 \times 77{\cdot}81 + 1 \times 59{\cdot}63 + 2 \times 21{\cdot}61 + 3 \times 0) + \\ {\cdot}25\,(0 \times 91{\cdot}62 + 1 \times 101{\cdot}03 + 2 \times 70{\cdot}52 + 3 \times 7{\cdot}59) + \\ {\cdot}5\,(0 \times 81{\cdot}48 + 1 \times 99{\cdot}08 + 2 \times 101{\cdot}00 + 3 \times 60{\cdot}99) + \\ {\cdot}75\,(0 \times 87{\cdot}69 + 1 \times 107{\cdot}68 + 2 \times 200{\cdot}59 + 3 \times 331{\cdot}42)\end{array}}{N} = {\cdot}14.$$

$$\text{Coefficient of correlation} = \frac{\text{Product moment}}{(\text{S.D. length})(\text{S.D. age})} = {\cdot}48.$$

TABLE VII

Correlation for duration of centrifuging and length of tail

| Duration of centrifuging | Relative length of resorption of tail | | | | |
|---|---|---|---|---|---|
| | 0 | ·25 | ·5 | ·75 | Total |
| ½ hour .. | 53·53 | 79·53 | 93·75 | 120·85 | 347·56 |
| 1 hour .. | 46·03 | 70·43 | 87·75 | 163·91 | 368·12 |
| 2 hours .. | 37·27 | 70·00 | 92·10 | 188·09 | 387·46 |
| 3 hours .. | 22·23 | 50·80 | 68·95 | 254·53 | 396·60 |
| Total .. | 159·05 | 270·76 | 342·55 | 727·38 | 1499·74 |

| | |
|---|---|
| A.M. for duration | .. 1·67 |
| A.M. for length | .. ·524 |
| S.D. for duration | .. ·97 |
| S.D. for length | .. ·05 |
| Coefficient of correlation | .. ·20 |

TABLE VIII

Correlation for age of larva and duration of centrifuging

| | Duration of centrifuging | | | | |
|---|---|---|---|---|---|
| Age of larva | ½ hour | 1 hour | 2 hours | 3 ours | Total |
| 0 hour .. | 71·47 | 78·80 | 91·73 | 96·60 | 338·60 |
| 1 hour .. | 82·37 | 89·32 | 95·73 | 100· | 367·42 |
| 2 hours .. | 93·72 | 100· | 100· | 100· | 393·72 |
| 3 hours .. | 100· | 100· | 100· | 100· | 400· |
| Total .. | 347·56 | 368·12 | 387·46 | 396·60 | 1499·74 |

| | |
|---|---|
| A.M. for age | .. 1·57 |
| A.M. for duration | .. 1·67 |
| S.D. for age | .. 1·11 |
| S.D. for duration | .. ·97 |
| Product moment | .. — ·044 |
| Coefficient of correlation | .. — ·041 |

After this, the partial correlations P12·3 and P12·2 were calculated, i.e., the correlation between age of larvae and relative resorption of tail when the factor of duration of centrifuging is present. From these partial regression coefficient b12·3 and b13·2 were calculated as follows :

| S. D. for length | S. D. for age | S. D. for duration |
|---|---|---|
| $\sigma 1$ | $\sigma 2$ | $\sigma 3$ |
| ·26 | 1·11 | ·97 |

$P12 = \cdot 48$ (age and length correlation)

$P13 = 20$ (duration and length correlation)

$P23 = -\cdot 04$ (age and duration correlation)

$$\sigma 1\cdot 2 = \sigma 1\sqrt{1 - P12^2} = \cdot 26\sqrt{1 - \cdot 48^2} = \cdot 26\sqrt{\cdot 77}$$

$$\sigma 1\cdot 3 = \sigma 1\sqrt{1 - P23^2} = \cdot 26\sqrt{1 - \cdot 20^2} = \cdot 26\sqrt{\cdot 96}$$

$$\sigma 2\cdot 3 = \sigma 2\sqrt{1 - P23^2} = 1\cdot 11\sqrt{1 - (-\cdot 04)^2} = 1\cdot 11\sqrt{\cdot 9984}$$

$$\sigma 3\cdot 2 = \sigma 3\sqrt{1 - P23^2} = \cdot 97\sqrt{1 - (-\cdot 04)^2} = \cdot 97\sqrt{\cdot 9984}$$

$$P12\cdot 3 = \frac{P12 - P13\,P23}{\sqrt{1 - P1^2 3}\sqrt{1 - P2^2 3}} - \frac{\cdot 48 - (\cdot 20 \times -\cdot 04)}{\sqrt{\cdot 96}} = \frac{\cdot 488}{\sqrt{\cdot 96}}$$

$$P13\cdot 2 = \frac{P13 - P12\,P24}{\sqrt{1 - P1^2 2}\sqrt{-P2^2 3}} - \frac{\cdot 20 + (\cdot 48 \times -\cdot 04)}{\sqrt{\cdot 77}\sqrt{\cdot 9984}} = \frac{\cdot 2192}{\sqrt{\cdot 77}}$$

$$b12\cdot 3 = \frac{P12\cdot 3\,\sigma 1\cdot 3}{\sigma 2\cdot 3} - \frac{\frac{\cdot 488}{\sqrt{\cdot 96}} \times \cdot 26\sqrt{\cdot 94}}{1\cdot 11} = \cdot 114$$

$$b13\cdot 2 = \frac{P13\cdot 2\,\sigma 1\cdot 2}{\sigma 3\cdot 2} - \frac{\frac{\cdot 2192}{\sqrt{\cdot 77}} \times \cdot 26\sqrt{\cdot 77}}{\cdot 97} = \cdot 059.$$

The regression of length on duration and age is given by

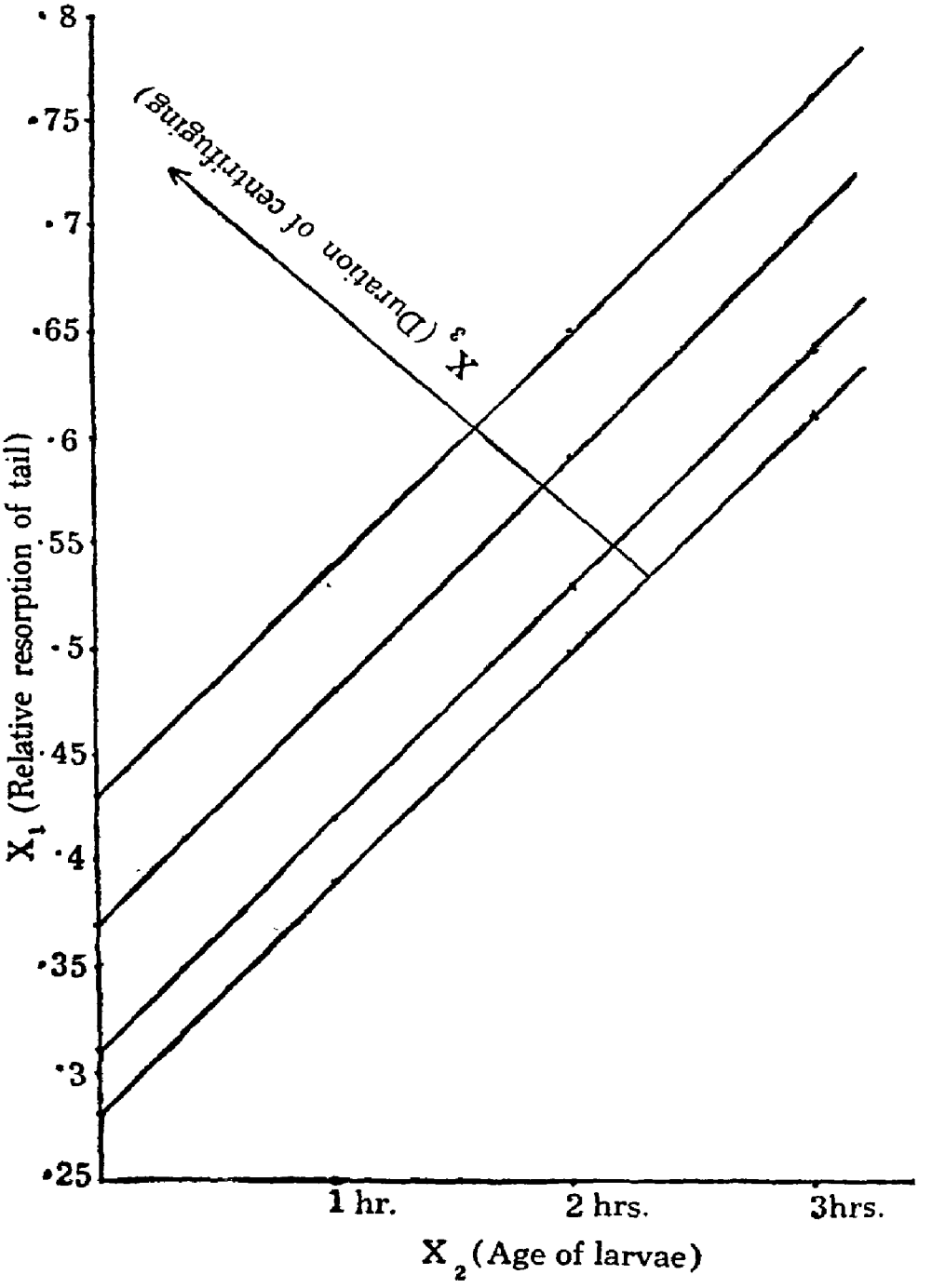
$(x_1 - \cdot 524) = \cdot 059\,(x_3 - 1\cdot 67) + \cdot 114\,(x_2 - 1\cdot 57)$ or

$x_1 = \cdot 11x_2 + \cdot 06x_3 + \cdot 25$ where $x_1$ is the length of tail, $x_2$ is the age of larvae and $x_3$ is the duration of centrifuging. This equation is valid for $0 \leqslant x_2 \leqslant 3$ and $0 < x_3 \leqslant 3$, or $x_3$ (time) can vary between 0 and 3, 3 inclusive and not 0.

The standard error of the estimated value of resorption (from equation) is ·05 (5%).

From the equation it will be seen that the value of the constant term ·25 suggests that there is a sudden resorption in length of the tail as soon as the centrifugal force is applied, and

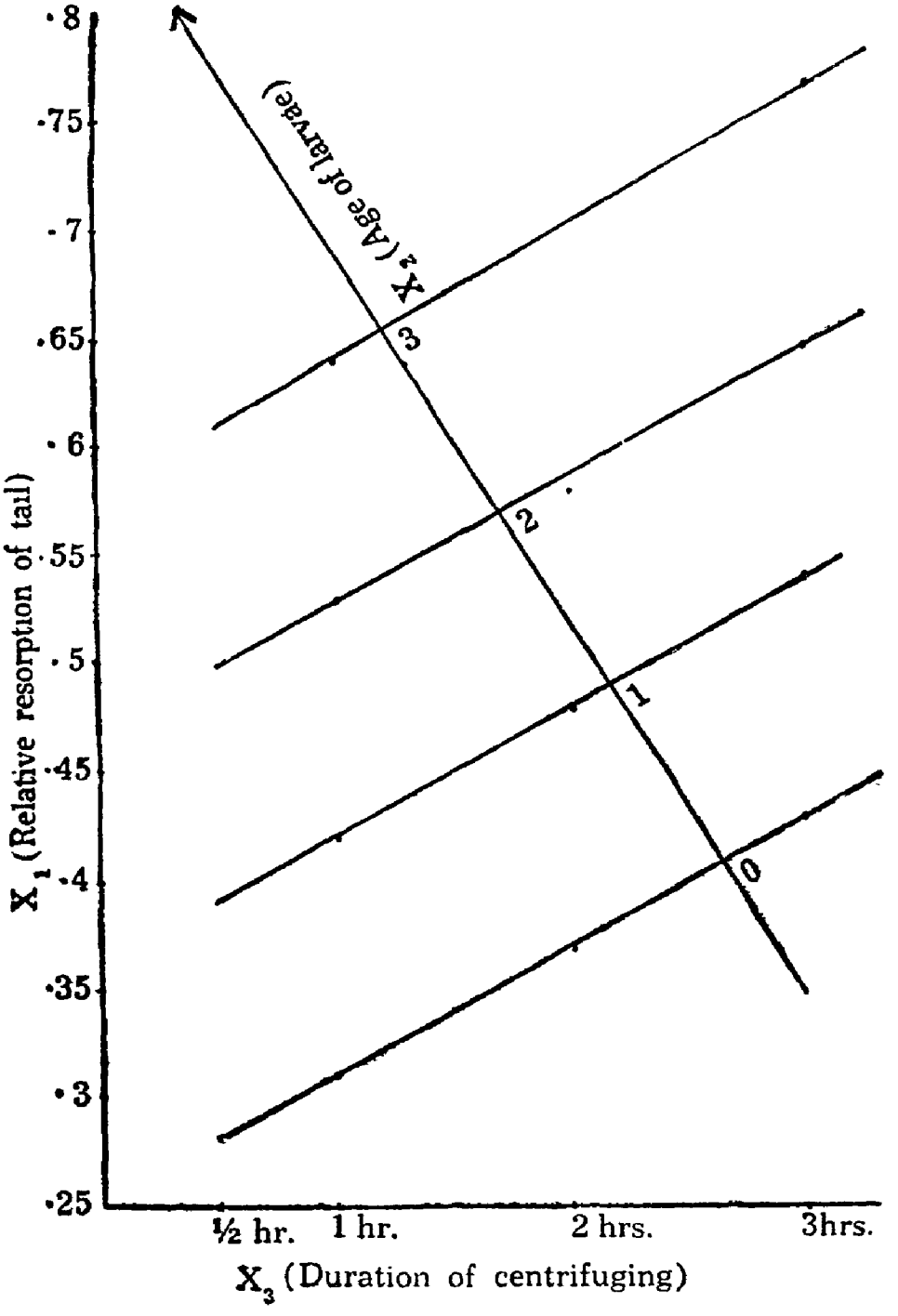
that, the older the age of the larva, the more sudden the resorption, and that the effect of duration of centrifuging on resorption is only about ·06 (6%) of the length of the tail per hour. The results are also illustrated in Graphs 1 and 2.




Graph 1.

The data collected from the centrifuging experiments and the correlations obtained from calculations show that (1) violent agitation (due to a force of 2684 G acting on them) for a period of even six hours does not injure the larvae or make the development atypical, (2) agitation due to centrifugal action accelerates

tail resorption, normally beginning in five hours, to happen within thirty minutes, (3) the resorption of the tail of the larvae subjected to centrifuging is sudden to begin with, and often depends on the age of the larva. Later, it is proportionate to the duration of the period of centrifuging, and the aging of the larva as well.




Graph 2.

It would appear that the acceleration of metamorphosis of the ascidian larvae, may not be only by the influence of light as Grave ('36) has suggested, but also due to mechanical disturbances in sea-water. At any rate, the larvae of *P. indicum,* occurring in

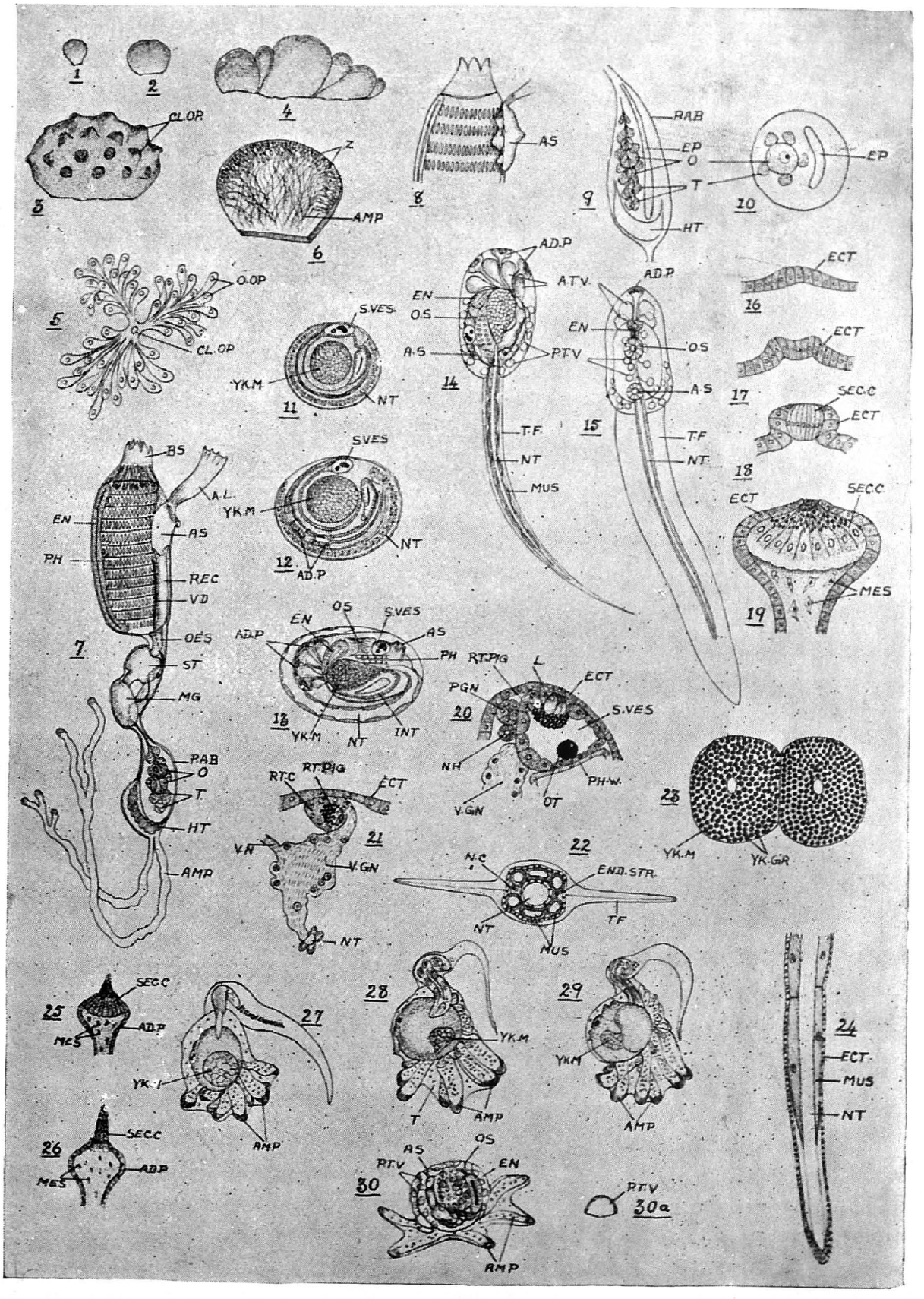



PLATE I: Figures 1 to 30a.

closely crowded colonies in the disturbed areas of a rocky coast suggests that the acceleration of metamorphosis must be a feature of survival. Though the influence of the agitation due to the breakers cannot be directly assessed, the results of the centrifuging experiment show that they are correlated.

B. *The effect of centrifuging on the structure of the larvae*

In the present section the effect of centrifuging the larvae of *P. indicum* on (a) the histological changes leading to the initiation of tail resorption, and (b) on the acceleration of the process of reorientation of the internal structures, are given. Before dealing with these two aspects, a brief account of the structure and post-larval changes of the tadpole of *P. indicum* under normal conditions is given to facilitate comparisons with the changes undergone by the different organs of the centrifuged larvae.

*Structure of the tadpole*

The tadpole (Figs. 14 & 15) measures 1·66 mm. from the adhesive papillae to the tip of the tail fin, the trunk measuring 0·32 mm. and tail 1·34 mm. The tunic covers the whole body and tail of the larva. In the region of the tail, the tunic is expanded into the tail fin, which is horizontal in position due to the rotation of the tail. Test vesicles ("tunic vesicles", Scott '46) are of two types, one directed anteriorly and the other posteriorly. The anterior ones are club-shaped, arranged in a ring of eight in two rows of four each, arising from the anterior ectodermal margin of the body of the larva, and spreading out with a slant towards the dorsal side. The middle two pairs are longer measuring 63·2μ, and the lateral ones are smaller, measuring 47·4μ. The posterior test vesicles are like bunches of grapes, one set dorsal and one ventral in position, with long narrow tubular stalks from which pinnately arranged branches arise ending in round hollow vesicles containing mesenchyme cells. They are of ectodermal origin arising from the anterior margin of the trunk, from the respective dorsal and ventral sides at the level of the origin of the anterior test vesicles. *Adhesive papillae* (Figs. 14, 15 & 19) are three in number, arising from the anterior ectodermal margin of the trunk between anterior vesicles. Each has a long narrow tubular stalk, the distal extremity swelling like a goblet containing secretory cells converging to a point in the central opening. The *mantle* or ectodermal covering of the body and tail, is made up of one layer of cells with distinct nuclei,

containing a large number of yolk granules. The layer covering the trunk has cubical cells and gradually becomes thinner and flattened in the tail region. In the region of the branchial and atrial siphons these cells are columnar.

The *nervous system* (Figs. 20 & 21) consists of the sensory vesicle with the contained ocellus and otolith, visceral ganglion with the visceral nerve, and nerve cord of the larval action system, and the permanent ganglion and hypophysial duct of the adult system. The sensory vesicle is situated between the branchial and atrial siphons to the right side of the median line. The ocellus consists of three lens cells, pigmented optic cup, and associated retinal cells. The otolith is single-celled with a pigmented mass at its distal end, perfectly spherical in shape.

The *digestive tract* (Fig. 14) is a bent tube including the pharynx, oesophagus, intestine and a short rectum, which ends blindly near the level of the oesophagus. There is a middle mass of yolky cells, conical in shape, the narrow portion being connected with the endodermal layer by a short stalk. In transverse sections it is found to be of two squarish portions (Fig. 23), each having a narrow cavity in the centre. The endostyle is placed on the anterior margin of the pharynx, one edge of it reaching the anterior edge of the wolky mass. Pharynx has two rows of stigmata on each side, each row having about eight stigmata. On the ventral side of the yolky mass towards the anterior side is situated the pericardium and heart.

The notochord (Figs. 14, 15 & 24) forms the central core of the tail, having 40 cells placed one behind the other in a row. In a full-grown larva, the boundaries of the notochordal cells are not clearly seen. Due to the twist of the tail through an angle of 90° to the left, the nerve cord and the endodermal strand are found on the left and right sides of the notochord respectively. The muscle bands (Figs. 14 & 24) are situated dorsally and ventrally, and do not extend up to the posterior extremity of the notochord. Each band is formed of three rows of seven muscle cells each. There are only two rows of muscle cells on the sides of the anterior end of the notochord where this enters the body. Each muscle cell has a darkly staining striated cortex, and an inner vacuolated core with cytoplasmic strands and nucleus. The disposition of the striations is oblique, as described by Grave ('21), Conklin ('31), Scott ('46) and Berill ('47). The ectoderm of the tail forms the outer covering of the tissues within.

*Post-larval metamorphosis* (Figs. 27, 28, 29 & 30).

After a period of free-swimming life, the larva fixes on the substratum. During fixation, the secretory cells of the adhesive papillae shoot out (Figs. 25 & 26) through the opening of the goblet-shaped tip exuding the secretory products. Very soon the anterior ampullae grow longer and help further fixation by spreading out in an irregular way, attaining their maximum length of 0·24 mm. in two hours after the initial fixation of the tadpole. The body now undergoes a differential growth at the anterior extremity between the point of fixation and the branchial siphon, rotating the branchial and atrial siphons to the dorsal side. The heart commences to beat after three hours, the siphons contract after the fifth hour, the intestine starts to function after ten hours. At the onset of the metamorphosis, the tail shortens suddenly to three-quarters of its length, but complete resorption of the tail takes a longer time. The posterior ampullae do not disappear immediately after metamorphosis. They swell, and get enlarged in size, and remain all over the surface within the tunic as pyramidal projections, their wall being made up of very thin, unicellular layer of cells (Fig. 30a). The ducts that connect them are not found during this time. The vesicles are clearly visible for a week or more, later disappearing by bursting and releasing the mesenchyme cells lodged within. Under laboratory conditions it has not been possible to keep alive the metamorphosed stages for more than ten to thirteen days.

### (a) *The histological changes leading to the resorption of the tail*

In assessing the effects of centrifuging on the structure of the ascidian tadpole, the resorption of the tail in various stages, and the grouping of centrifuged larvae into A, B, C, D and E, afford enough material for the understanding of the different processes which mark the onset of metamorphosis. Sections were taken through the larvae of all stages and comparisons made. Those falling in Group A (the free-swimming ones), which behave like normal larvae, show no change in histological structure. The sections passing through the tail show the epidermis, muscle and notochordal cells intact, possessing the reserve yolk unchanged. The changes are found in the tails of larvae belonging to the remaining groups.

*Group B.*

(Inactive larvae with tails intact). While most of them undergo no change, some are found to possess a wavy epidermis (Fig. 46), in contrast to the straight ones in normal cases. In these the muscle cells forming a row have been slightly dislocated though the cells themselves have not broken down. The notochord, in some cases becomes slightly wavy in its course, probably because of the dislocation of the myotomes. Sections through the wavy epidermis of the centrifuged larvae show that the epidermal cells contain fewer yolk granules than the normal ones. Hence, a case of partial nutritional exhaustion may set in resulting in a weakening of the cells which may probably account for their loosing normal relations within the tail and becoming wavy.

*Group C.*

(Larvae with quarter of the tail resorbed). Among these larvae one can distinguish three different degrees of the effect of centrifuging, (Figs. 47, 48 & 49). In the first type the unresorbed portion of the tail shows no change in structure. In the second type, the unresorbed portion shows wavy epidermis, and also in some cases, dislodgement of muscles and notochordal tissues, indicating that, the unresorbed portion has been partially affected resulting in slight dislodgement of tissues. The third type shows the different tissues like the muscles and notochord broken down and disorganised in the unresorbed portion of the tail.

A comparison of the reserve yolk contents of the cells of different tissues shows that in the first type there is no reduction in the yolk granules of the different tissues of the unresorbed tail, while in the second type, the yolk granules of the epidermal cells alone appear to have been used up, and in the third type, the cells of all the tissues have lost their yolky granules. This affords direct evidence of the fact that the very first effect of centrifugal disturbance is found in the depletion of the food stored in the cells of the tissues of the tail. This nutritional exhaustion is suggestive of accelerated metabolic activity evoked by the disturbance of the equilibrium.

*Groups D and E.*

(The larvae with half and three-quarters of the tails resorbed). In these, the conditions prevailing after centrifuging can be found

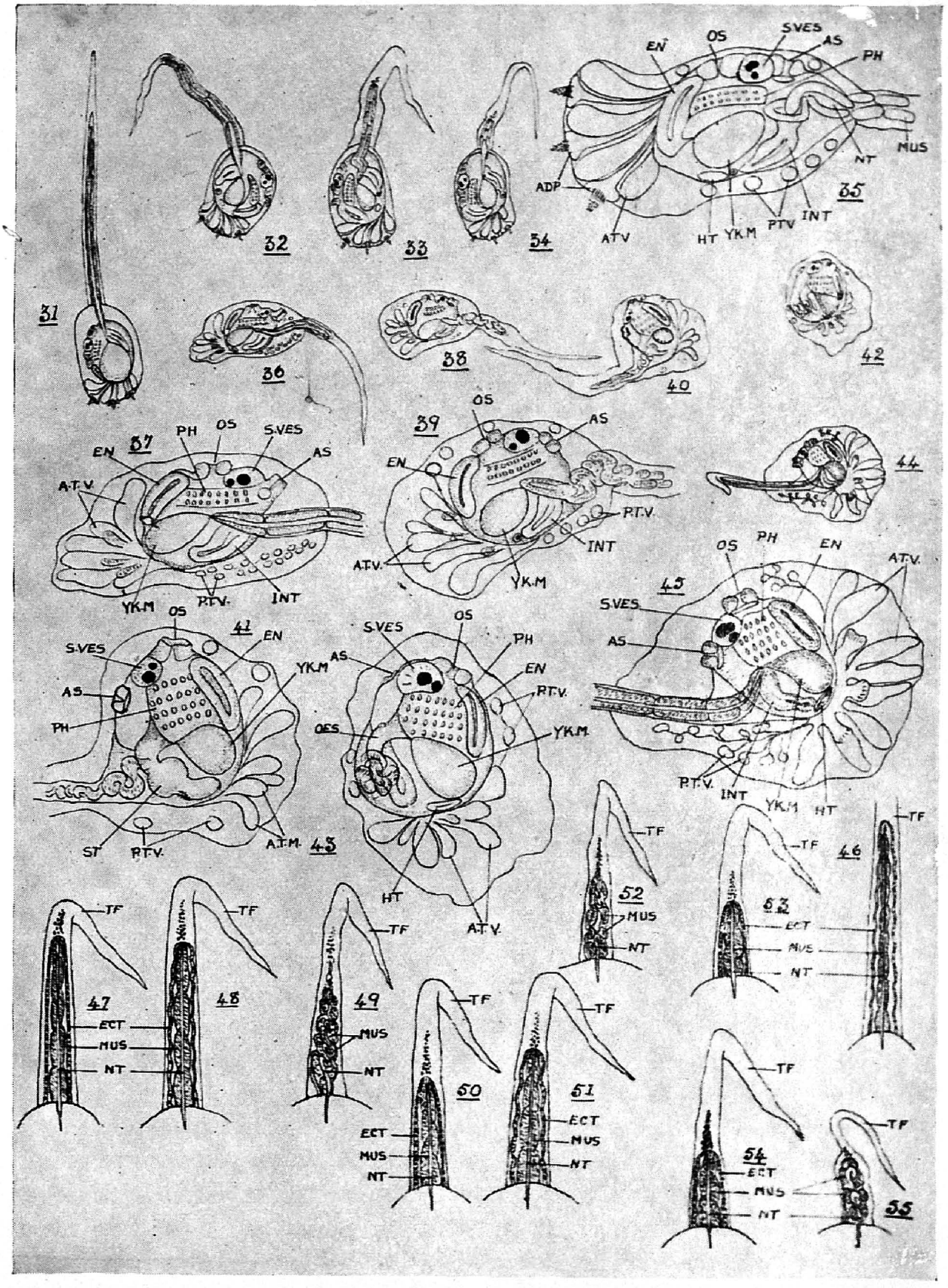



PLATE II: Figures 31-55.

to be the same as described above, (Figs. 50, 51 & 52 for D, and 53, 54 & 55 for E).

From the above facts it will be obvious that the ascidian tadpole whose free-swimming habit is associated with its main locomotory organ, viz., the tail, is affected by centrifugal disturbances to such an extent that, the symptoms of fatigue, aging and histological disintegration are made to appear earlier.

(b) *Changes in the trunk region*

In healthy, free-swimming larvae all the organs inside the trunk are compact as shown in Fig. 14, till they have fixed and undergone metamorphic changes. In most larvae centrifuging causes the organs to spread out as shown in Fig. 35. Wider spaces are found between the endostyle, pharyngeal sac, yolky mass and intestine. The adhesive papillae show the secretory cells protruding out. The anterior test vesicles show separation from one another. These changes are more pronounced in larvae centrifuged for longer periods, probably because they are accentuated by the aging of the larvae. In the larvae 3-hours-old, centrifuged for one hour, and in younger larvae centrifuged for 2, 3, and 6 hours, the trunk ectoderm between the branchial siphon and the substratum becomes accelerated in growth, and this results in a rotation involving reorientation of the organs earlier than would have happened under normal conditions. Among the experimented larvae belonging to different groups, we observe different degrees of these changes constituting metamorphosis. Since among these groups of larvae the changes are related to the different organs, the effect of centrifuging can be discussed with reference to the different organs.

The adhesive papillae which normally help a preliminary fixation of the larva become activated through the secretory clls being shot out suddenly (Fig. 26), and are even completely lost. The anterior ampullae however, come into action earlier than they would under normal circumstances. They become enlarged and spread out and without the aid of a preliminary fixation by the papillae, are able to ensure fixation of the young ascidiozooid, partially assisted by the tunic. Correlated with the accelerated development of the tunic, the posterior ampullae also spread out. These changes relating to the adhesive papillae, ampullae and tunic, which under normal conditions would begin about the fifth hour, are observed much earlier in the centrifuged larvae. One-

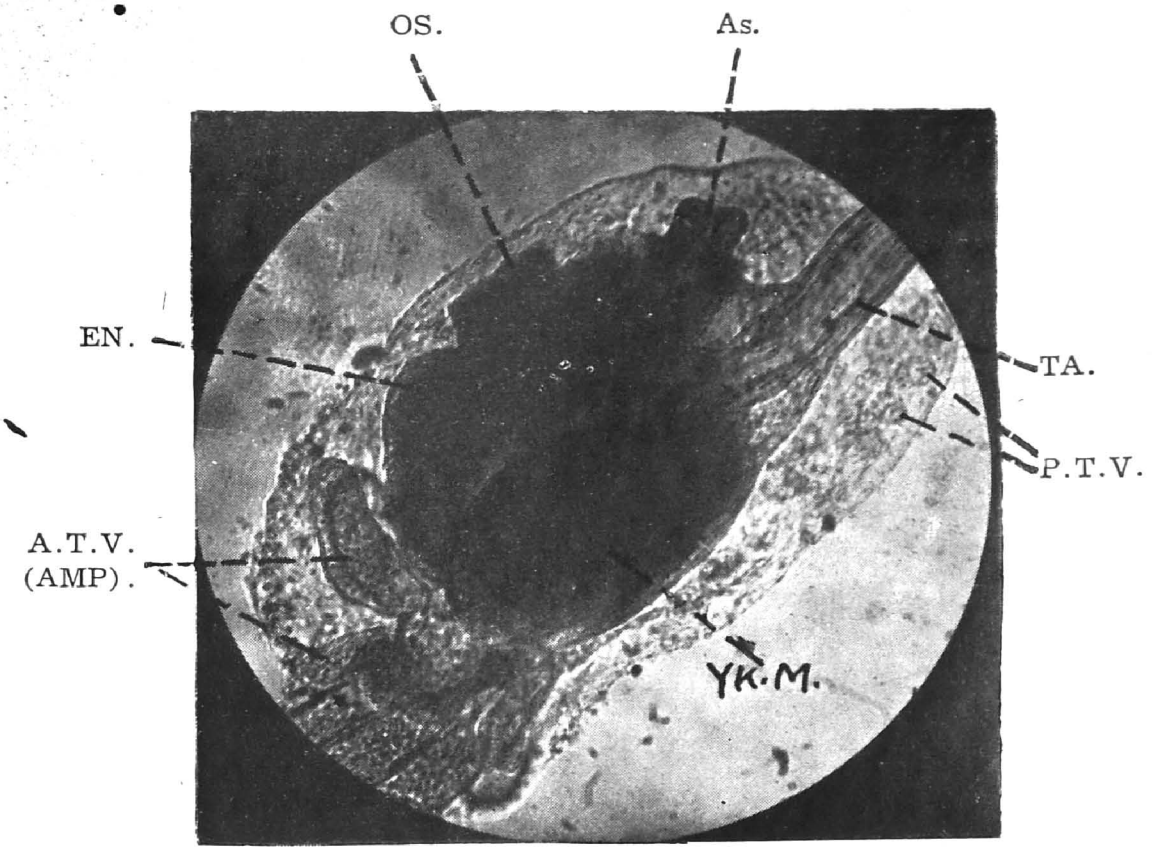
hour-old larvae centrifuged for three hours, or three-hour-old larvae centrifuged for one hour, or other larvae centrifuged for two or three hours are able to show these changes which normally would be completed about the seventh hour. This forward shift of three hours in the sequence of events is therefore an experimental proof of the effect of agitation on metamorphosis.

The endodermal derivatives of the trunk like the pharynx, oesophagus, intestine and rectum undergo all the changes which normally would occur at the eight-hour, even at the fifth hour. This is clearly an acceleration due to centrifugal disturbances. There are no deviations from the normal course of events and no atypical complications in the attainment of the adult form of these organs. However, it may be noted that in the rapid rotation of these organs and the accelerated development they undergo, it seems as if, the quantity of yolk which is normally spent during the extra three hours which these organs would have taken is not expended and therefore the shape of the yolk mass does not change much at the fifth hour of the experiment as it would at the eight-hour of normal development.

The pericardium becomes enlarged to a size which is attained normally only on the eight-hour, but the heart which is formed by the involution from the sides of the pericardium is not as well formed as it would be on the eight-hour. Therefore, centrifuging has not accelerated the formation of the heart and the commencement of its pulsations.

The larval organs associated with the nervous system appear to be unaffected by centrifuging. The sensory vesicle, with its ocellus and otolith, and the visceral ganglion, persist in their original positions. However, due to the shifting and rotation of the trunk organs, the visceral ganglion gets stretched, and after a certain limit it ruptures. It is a significant fact that centrifuging does not accelerate the dissolution of the yolk of the larva, it does not speed up the formation of the heart and the commencement of circulation, nor does it hasten the reorganisation of the larval nervous system. We are forced to conclude that the acceleration of metamorphosis concerns the resorption of the tail and orientation of organs, not their adult differentiation or their functioning earlier.

According to the changes which the organs in the trunk undergo as a result of centrifuging, the centrifuged larvae fall into

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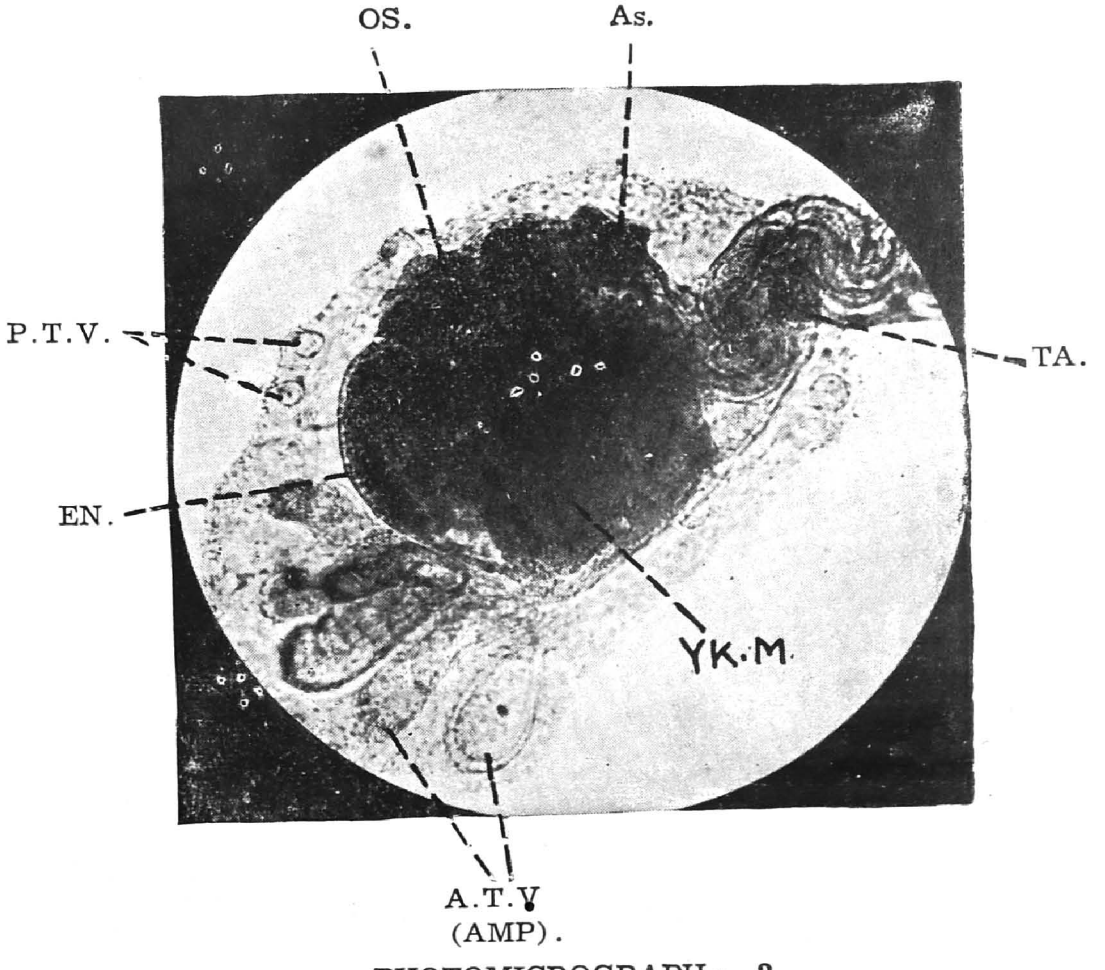



PHOTOMICROGRAPH : 2

three groups. (1) Those in which the epidermis has undergone very rapid growth and the inner organs also have grown in size, (Figs. 36 & 37, and Photomicrograph 1). Correlated with this growth, the mouth and atrial openings have become shifted to a more dorsal position, but the shifting is not complete. This beginning of rotation of the trunk organs is noticed even in larvae which are made inactive by centrifuging, but which have not undergone any shortening of the tail (*Vide* Group B, page 20). (2) In this group of centrifuged larvae, we find the mouth and atrial openings rotated to the dorsal side. The internal organs have undergone a shift in position though they still retain normal compact relationships with each other, (Figs. 38 & 39, and Photomicrograph 2). The pharynx in particular have not expanded and the stigmata have not increased in number. The tail, however, may be resorbed to $\frac{1}{2}$ or $\frac{3}{4}$ the length (as in groups C & D, vide page 20). (3) In these larvae the orientation of the internal organs has been completed, except for the formation of the post-abdomen, and the adult relations have been attained, (Figs. 40 & 41, and Photomicrograph 3). Pharynx is fully developed and is provided with the full complement of stigmata. The tail shortens to half in some, to a quarter in others, and even completely resorbed in still others, (Figs. 42 & 43, and Photomicrograph 4). Several larvae of Group B, with complete tails also fall under this category, (Figs. 44 & 45)

*Discussion.*

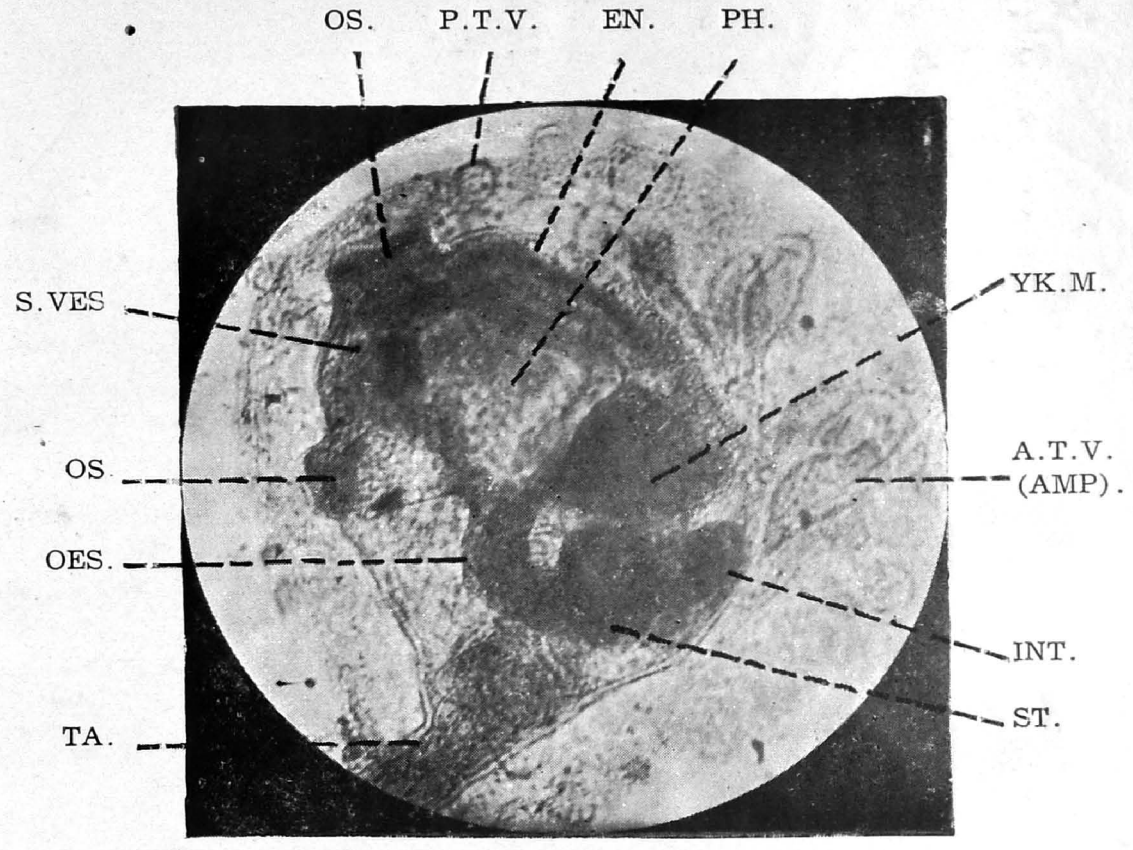
It will be of interest to consider the views of the more recent authors regarding the resorption of the tail. Conklin ('31) was of opinion that absorption of water and swelling of tissues of the tail took place earlier than phagocystosis, and that the tissues were disrupted as a result of autolysis. Grave ('36) however, considered that aging was the chief factor which starts the degenerative changes, and phagocystosis of the tail. Berrill, who first ('29) considered phagocystosis as the primary process, later ('47) entertained the opinion that nutritional exhaustion and aging are the first symptoms. All these authors consider aging as an important factor in the inducement of metamorphic changes and the termination of active, free-swimming period of life of the larva. The explanations put forward by these authors regarding the role of age, and by Berrill ('47) regarding nutritional exhaustion appear supported experimentally by the fact that disturbances of water increases the activity, thus shortening the period of larval free-swimming life and bringing about metamorphosis. The disappear-

ance of yolk granules within the tail tissues is undoubtedly proof of the nutritional exhaustion suggested by Berrill ('47).

The occurrence of larva with tails complete and with trunk organs orientated as in the young ascidiozooids is significant, because, this shows that under violent mechanical disturbances of the environment, fixation, rotation and metamorphosis of the trunk organs can proceed even when the tail has not undergone any visible changss. In spite of the lack of co-ordination of the process of the tail resorption and of the rotation of organs, the abnormal conditions introduced into the environment do not appear to interfere with the typical course of events taking place in each system of organs. This suggests the possibility that metamorphosis of the ascidian larva may be similarly accelerated by the violent mechanical disturbances in the natural habitat of *P. indicum.*

The problem why several sessile animals settle in a gregarious or crowded manner has attracted the attention of several authors like Jeffreys (1863-9), Wilson ('39), Cole & Knight-Jones ('39, '49), Thorson ('46), Burton ('49), Knight-Jones & Stephenson ('50), especially with reference to *Pecten maximus, Ostrea edulls, Sabellaria, Balanus,* etc. These authors were repeatedly driven to the suggestion of there being a chemical complex set up in any crowded habitat which tend to "keep the children at home" (Berrill '50). The experiments performed by Grave & Nicoll ('39) in this connection are very valuable. They demonstrated that certain secretions were thrown out by the free-swimming larvae, and that if the sea-water were conditioned by the accumulation of such secretions, metamorphosis may be accelerated before the larvae were scattered far away from the rest of the settlement.

From the centrifuging experiments it is obvious that violent disturbance of the equilibrium accelerates the metamorphosis of the ascidian larvae so effectively that it is just possible this happens in nature as well. This ascidian is found in shallow sea-shore north of Madras, attached to the rocky cobbles that are heaped along a length of about 3 miles to prevent erosion of earth. In this area the breakers are so ceaseless and violent that nothing floating or swimming within a distance of five to ten yards from the shore is ever allowed to scatter back into the sea. Owing to this ecological limitation, the habitat of the ascidian is well-defined. Within this area of 10 yards from the shore the larvae are so violently disturbed that they settle in groups on the surface of rocks.

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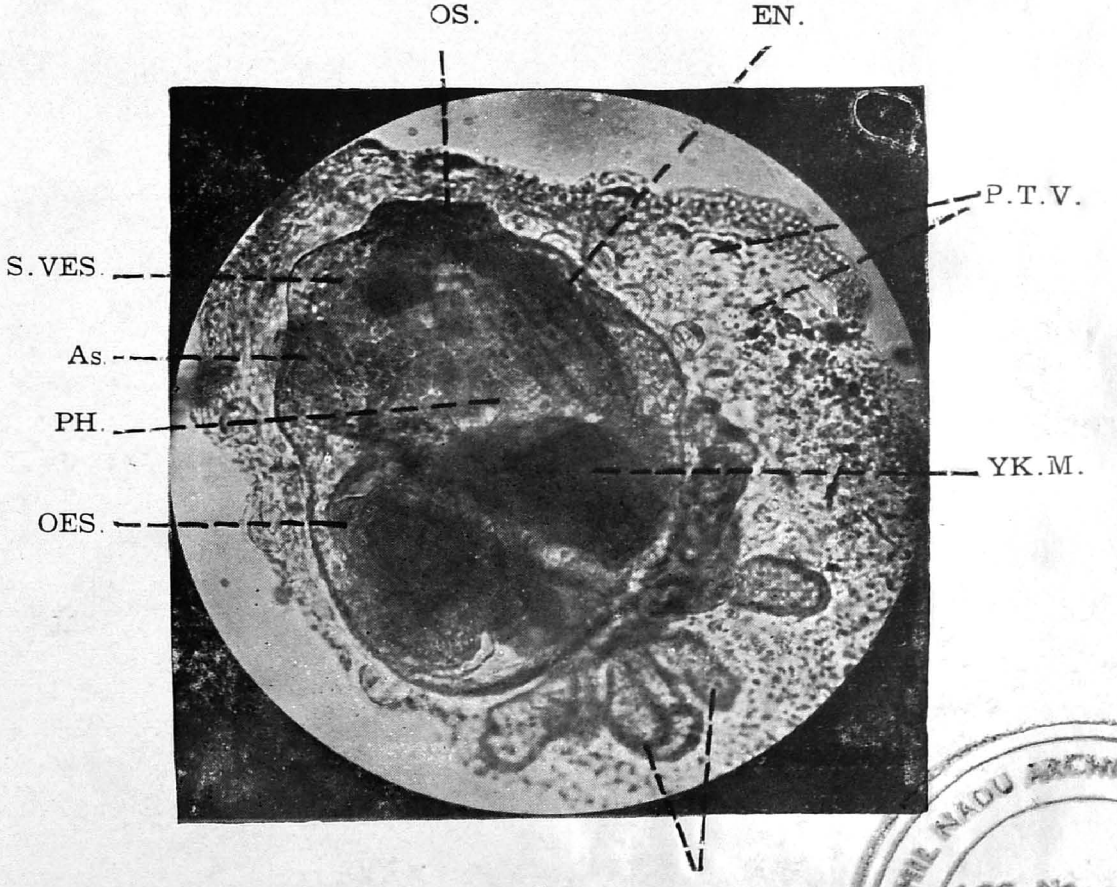


PHOTOMICROGRAPH : 4

# ASEXUAL REPRODUCTION IN *POLYCLINUM INDICUM*

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## ASEXUAL REPRODUCTION IN POLYCLINUM INDICUM

*Introduction.*

Asexual reproduction or budding in Polyclinidae is characterised by post-abdominal strobilation. Kowalewsky (1874) has described the budding of a Polyclinid, *Amaroucium proliferum,* wherein the typical post-abdominal strobilation is seen. A departure from the usual post-abdominal strobilation is found in another Polyclinid, *Aplidium zostericola* (*A. pallidum*), whose budding was studied by Brien ('25—27). In this the abdomen as a whole lengthens, and is included in the process of strobilation. In *Euherdmania,* (Berrill '35), the post-abdomen is responsible for budding, though there appears to be a longitudinal splitting of the epicardium as well. Brien ('37) found that in *Sydnium turbinatum* the zooids of a previous generation, and buds in various degrees of development of regressed zooids occur together in the same colony. Asexual reproduction has not been studied by any in tropical ascidians.

The alternation of sexual and asexual reproduction in different seasons has been observed in several ascidians of temperate waters by different authors. These have shown that they reproduce sexually during spring and summer, while reproducing asexually in autumn and winter. The fall in temperature was considered to be the reason for the production of small winter buds. This view was strengthened by the experiments of Orton ('21) on *Clavelina* who found that, raising the temperature, induced the small winter buds to grow up. Huxley ('21, '26) held the view that regression of sexual zooids and formation of asexual buds may be correlated with the fluctuations in the food supply, the larger zooids regressing when the nutritional exhaustion sets in, and the smaller buds become more important in the formation of the colony. Berrill ('51) speaks of winter budding as due to the rigours of the climate when ordinary life activities are made impossible, and hence sexual and asexual reproductions are geared on to the alternation of the seasons. Brien ('53) is of the opinion that both temperature and food supply may bring about physiological conditions which are responsible for sexual reproduction.

The present author felt that fluctuations in temperature and food supply related to the marked changes in the seasons obtained

in temperate countries not being characteristic of the tropics, a study of the two types of reproduction in the tropical form *P. indicum* would throw further light on the factors associated with the alternation of sexual and asexual phases. The results of the study are presented in three sections; (A) The process of budding and reorganisation, (B) the loss of sexual phase in sea-water different from that of the natural habitat of *P. indicum,* and (C) an analysis of the probable factors inducing sexual reproduction.

## A. *The Process of Budding and Reorganisation*

### *Material and Method.*

Observations were made on colonies of *P. indicum* freshly collected from their natural habitat, the rocky shores of Royapuram area of the Madras coast. As fresh collections were possible in all months of the year there was enough material to follow the changes in the zooids over a fairly long period, and observations were repeated whenever necessary, in order to confirm the results. Fresh colonies were cut through and the zooids which were undergoing regression, and buds were sketched. Later, the regressing zooids and buds were dissected on a slide and the gross anatomy studied. Details of histology were studied by paraffin embedding and sectioning. Bouin's fluid was found a suitable fixative. Staining with Heidenhain's haematoxylin and Mallory's triple was found sufficient. It was thus possible to follow the changes in the external and internal structure of the zooids especially those which were reproducing and liberating larvae and those which were regressing, and also those which were asexual in character.

### *The isolation and structure of buds.*

The buds are formed from the post-abdomen. The post-abdomen, at the time of budding elongates to 2½ to 3 times the length of the thorax and abdomen put together, (Figs. 56, 57 and 58). When the post-abdomen thus elongates the nutritional trophocyte cells enter into it from the region of the thorax. After this the thorax and abdomen regress and autolyse, and the post-abdomen is isolated. The anterior third of the post-abdomen swells, (Figs. 59) and at the posterior border of this region the epidermis constricts (Fig. 60) until it reaches

the epicardium and cuts into it. Thus the bud becomes free (Fig. 61a). A longitudinal section passing through this region of constriction reveals the details as shown in figure 63. In a similar way the middle third of the post-abdomen also swells and breaks away due to epidermal strobilation, (Fig. 61b) thus setting free the remaining two buds (Figs. 62 a, b & c). Since all the three buds are formed by the strobilation of the same stock, they are similar, except for some difference in the gonads.

The outer covering of each bud is the epidermal layer of the parental post-abdomen. In the centre, longitudinally placed is the epicardium, which also belongs to the original zooid. Surrounding the epicardium inside the outer epidermis is found the trophocyte cells which migrated into the elongating post-abdomen. These cells form the nutritive reserve for the future reorganisation of the blastozooid. While the epidermis, epicardium and trophocytes are found alike in all the three buds, the gonad is found to vary in the different buds. In the anterior bud (Fig. 62a), the gonad loses its shape, gets disorganised and finally disappears. In the middle bud (Fig. 62b) most cells of the gonad lose their shape and disintegrate, but a few remains unchanged. Here, the trophocyte cells are less densely packed than in the anterior bud. In the posterior bud (Fig. 62c) the entire gonad remains unchanged, and only a few trophocyte cells are found.

A cross-section of the bud (Fig. 64) shows an outer layer of epidermis, composed of a single layer of rectangular cells. In the middlde is the thin-walled epicardium, which is syncytial, the cells being distinguishable only by the darkly-staining nuclei. Closely adjoining the inner margin of the outer epidermis is found a mass of thickly packed cells, the trophocytes. These cells are circular in shape with irregular border, and with fine glanular bodies inside. Between the epicardial wall and the inner boundary of the trophocyte cells there is a vacant space, clearly visible in the anterior bud, but occupied by the sperm bunches in the middle and posterior buds (Fig. 65).

### *Development of Organs in Reorganisation*

(a) *Pharynx and Peribranchial cavities.*

These organs are first differentiated from a swelling of the anterior end of the epicardium (Fig. 66), which later shows a division into three sacs by constriction and folding (Figs. 67 and

68). Thus a median and two lateral cavities are formed. Of these, the middle one is the rudiment of the pharynx, and the lateral ones, the rudiments of the peribranchial sacs. The stigmata are formed by the constriction and breaking through of the branchial wall at regular intervals (Figs. 70 and 72). In the beginning four to five rows of stigmata are thus formed. The ventral wall of the pharyngeal sac gets thickened and finally grooved along its whole length, to form the endostyle. The two peribranchial walls grow in size, encircle the branchial sac, and finally their distal extremities touch and fuse, thus forming the perivisceral cavity.

(b) *Nervous system.*

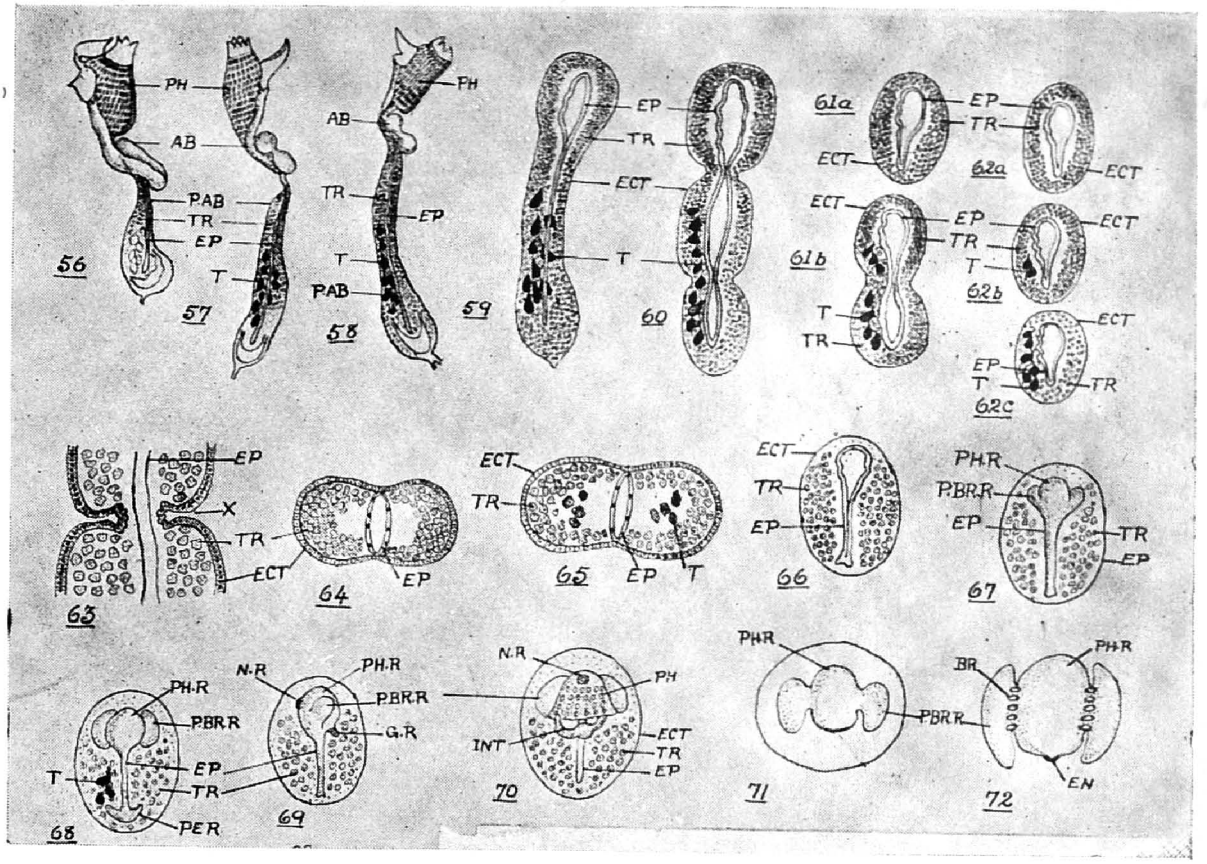
In *P. indicum,* a small protuberance is produced from the antero-dorsal side of the pharynx, and this opens later as the ciliated funnel. From the dorsal side, the defenitive ganglion, and from the ventral side, the neural gland are formed by proliferation. This process is similar to what occurs in *Glossoforum* and *Circinalium,* (Pizon 1892).

(c) *The abdominal structures.*

As is usual in Polyclinids where the abdomen is not included in the post-abdominal strobilation, the abdomen develops as a small outpushing (Fig. 69) from the postero-ventral margin of the pharyngeal bag. This elongates further into a tube which later becomes differentiated into distinct regions—a proximal oesophangus, a middle bulging stomach and a distal hind-gut. The abdomen curves and finally assumes the form of a loop characteristic of the adult zooid (Fig. 70).

(d) *Pericardium and heart.*

While the pharyngeal and peribranchial sacs, nervous system and abdomen are being formed from the anterior region of the epicardium, the pericardium and heart are formed from its posterior extremity. The formation of the pericardium and heart resembles what has been described in the case of *A. pallidum* by Brien ('25). The posterior extremity of the epicardium dilates, and extends towards both sides (Fig. 68). These extensions grow and join posteriorly, and finally the pericardium severs its connection completely, with the epicardium. This is the pericardium of the blastozooid. The heart is formed by the invagination of the pericardium. The whole structure later takes a curved shape.



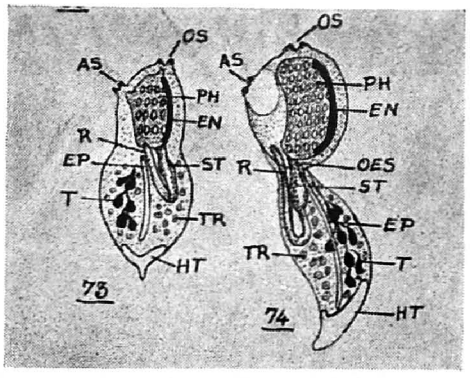




PLATE III : Figures 56 to 74.

(e) *Gonads.*

While the other parts are being laid down, clusters of sperm bunches can be seen in the spaces between the epicardial wall and the epidermis, clearly distinguishable from the trophocyte cells which have become considerably depleted by this time. In the anterior bud the reproductive organs are reorganised by the proliferation of the cells of the epicardium. In the middle bud the part of the gonad left proliferates the rest of the gonads. In the posterior bud the parental gonad persists intact. The presence of a large number of trophocytes in the anterior bud is obviously to provide the nutrition required for it to develop the entire gonad from its epicardium. This fact is obvious from the fewer trophocytes in the middle bud which has already a part of the gonad of the parent—and there being least in the posterior bud which has all its gonad from the parent.

(f) *The branchial and atrial siphons.*

When all the organs of the blastozooids have been formed, the anterior extremity of the pharynx adjacent to the invagination of the ectoderm sends an outpushing to meet it. At the place of contact the walls break through and the branchial siphon is thus formed. In a similar way the atrial siphon is formed by an outpushing of the peribranchial wall meeting an invagination of the outer ectoderm. Figs. 73 and 74 show stages in the growth of the bud, leading to the final shape.

The zooids of a colony do not regress and form buds together, some zooids systems may regress while others remain active and may regress later. The temporal difference in the regression of the zooids, however, is not so marked as in *Sydnium turbinatum* studied by Brien ('37) where some zooids may remain active even when buds have been formed by the others. Though a normal zooid produces usually three buds, it also happens that some small-sized zooids produce only two or even one bud. Due to cramping of the individual zooids for space within the confines of a colony, there are considerable differences in size of the entire zooid, as well as that of the thorax, abdomen and post-abdomen. The reduction in the number of buds is invariably associated with a shortening of the length of the post-abdomen. Generally such reduction in the length of the post-abdomen happens in the zooids which are situated farthest away from the common cloacal opening,

along the outer periphery of the system. In cases where only two buds are formed both contain gonads, and the posterior bud contains more of the gonads and has fewer trophocytes than the anterior bud. When only one bud is formed, the sperm bunches remain practically unchanged, and contains all the trophocytes which entered the post-abdomen. Though the types of buds thus differ, their growth and differentiation into zooids are typical and similar.

*Discussion.*

It is significant that in most of the details of differentiation of tissues and organs, growth, and attainment of form and symmetry, the process of asexual reproduction in this tropical form resembles that of the forms of the temperate region. Of the few features of difference, the number of buds and the changes in the gonads are notable. While in the Polyclinids studied by Kowalewsky ('74), Brien ('25, '27, '37), Berrill ('35, '50) a row of 8 to 10 buds is formed by ectodermal strobilation of the post-abdomen, in *P.indicum,* the number is reduced to three or even less. All the buds formed by *Sydnium turbinatum* contain a portion of the ovotestis, whereas the anterior of the three buds formed by *P.indicum* has no gonad, and has to proliferate the reproductive cells from the epicardium. The reduction in the number of buds formed in the present form, is undoubtedly correlated with the fact that reproduction goes on throughout the year without a winter break, while *S.turbinatum* which produces more buds does so only during spring and summer and is unproductive during winter. The autolysis of the gonad in one of the three buds formed by *P.indicum* is more difficult to account for. If, however, the asexual phase of the ascidian is considered as a period of recovery after sexual reproduction, the autolysis of a part of the gonad exhausted of its nutritional reserve and vitality is understandable—especially in view of the frequent repetitions of sexual reproduction.

B. *Loss of sexual phase in sea-water different from that of the natural habitat of P.indicum*

When colonies which live only in the Royapuram area of the Madras coast were reared in the laboratory in sea-water procured from Chepauk area, the colonies did not thrive for more than four months. Normally, in nature there would have

been four alternations of sexual and asexual generations, in the course of four months. But, in the laboratory, the four sexual generations were suppressed and only the four asexual generations were gone through. The repetition of only asexual generations of reproduction under these artificial conditions and their culmination in death suggested there being certain abnormal conditions. It was felt that a careful study of the conditions which may be responsible for the suppression of the sexual phase would be of interest.

In order to understand these factors the structural changes undergone by the colony and zooids were first studied in the laboratory. Colonies which were collected from their natural habitat and which were found to liberate larvae were kept in troughs in the laboratory. The zooids liberated larvae in large numbers on the first day, less on the second day and in stray numbers on the third day. Even after liberation of larvae is over, the zooids remain with their oral and cloacal openings opened to the exterior. The white rims of the openings were visible, arranged in patterns on the outer surface. This condition is seen for two weeks, after which both types of apertures begin to disappear. Along with the disappearance of these openings, the surface of the colony becomes smooth all over. During this time the suspended particles and debris settle on the surface to the extent of giving the colony the appearance of being decayed. Wiping with a fine camel hair brush restores the real healthy colour. After nearly two weeks the oral apertures appear again on the surface in pattern formation, and also the cloacal apertures in between the zooid systems. About two weeks later these newly formed cloacal apertures disappear, and the surface of the colony becomes smooth as before. This appearance and disappearance of the apertures is repeated fortnightly.

To observe the changes taking place in the interior of the colony, the colonies were cut open and examined everyday. During the first week there is no change in the size or shape of the zooids. Changes begin from the 8th day onwards.

Eighth day: The post-abdomen becomes longer. No change is visible in the thorax and abdomen, or any other organ of the zooid.

Ninth day: The post-abdomen grows longer still. A few trophocyte cells have wandered into the post-abdomen, making the anterior portion opaque.

Tenth day: The post-abdomen increases to nearly twice the original length, the trophocyte cells crowd farther inside the post-abdomen, thus increasing the opaque area.

Eleventh day: The length of the post-abdomen increases still more and reaches the maximum length which is at least three times the length of the branchial sac and abdomen put together. The trophocyte cells have descended far down into the post-abdomen reaching very nearly the heart. The testicular vesicles of the posterior extremity are visible.

Twelfth day: Except a small portion of the posterior extremity the rest of the post-abdomen has been rendered opaque by the descent of the trophocytes.

Thirteenth day: More trophocytes enter the post-abdomen, and the posterior extremity also becomes more opaque. The heart continues to pulsate.

Fourteenth day: The heart stops beating. The thorax begins to show signs of regression, autolysis of the different structures takes place, so that the bounding lines between different organs becomes indistinguishable. It is during this time that the outer surface of the colony becomes smooth because of the disappearance of the oral and cloacal openings.

Fifteenth day: The thorax has undergone almost complete autolysis, and some very young embryos that are lodged inside the pharynx are found to lie within the matrix, which later disintegrate and disappear.

Sixteenth day: The abdominal loop loses its definite form and becomes thick and opaque.

Seventeenth day: The abdominal loop has completely disappeared leaving only the opaque post-abdomen. This is now narrow anteriorly and bulges posteriorly. The course of the epicardium within the opaque post-abdomen can be seen as a narrow clear streak running through the whole length.

Eighteenth day: The process of strobilation begins, which shows an antero-posterior gradient. The narrow anterior part of the post-abdomen which forms a third of the length, swells while the rest of the portion remains as before.

Nineteenth day: The swelling of the anterior part of the post-abdomen reaches its maximum size and a constriction behind

this rudimentary bud cuts through. Simultaneously the next region of the post-abdomen, forming the middle third of the length swells and is marked off behind by a constriction.

Twentieth day: The anterior bud breaks away while the middle one also is ready to constrict off.

Twentyfirst day: The middle one also breaks away making the third also free. The three buds formed shift slightly from the end to end arrangement so that each bud has its anterior end free and is able to move forwards without colliding with one another. Each bud is oval in shape and shows the anterior end of the epicardium to be swollen.

Twentysecond to twentyfifth day: Reorganisation of internal organs of zooids is accelerated. Different organs are being laid down inside the space of the bud. The bud also shifts towards the surface of the colony.

Twentysixth day: As growth proceeds, the zooids move nearer to the surface of the colony. The size of buds has increased.

Twentyseventh to twentyeighth day: The buds now have become like the zooids in appearance. The post-abdomen shows signs of descending. Some of the zooids have almost touched the outer surface of the colony.

Twentyninth to thirtieth day: All the zooids are in contact with the outer border of the colony. The appearance of zooids is marked by the establishment of the oral and atrial openings.

Further observations were made on the changes in the zooids during the asexual and sexual phase of the colony. The first generation (after the sexual phase) of blastozooids, again underwent regression, reappearing again on the surface, but within 25 to 26 days, showing thereby a hastening of the asexual phase. The noteworthy feature is that the first generation of the blastozooids did not liberate larvae prior to their regression. In due course, the second generation of blastozooids also underwent regression without liberating larvae, and the reorganised buds reappeared in about twenty-one to twenty-three days, showing a further hastening of the whole process. A fourth generation of blastozooids was obtained by asexual budding of the third generation after about three weeks. Beyond the fourth generation the colony showed definite senescent changes culminating in death. All colonies

were not able to undergo four generations of asexual cycle. Sixty per cent went through only two generations, thirty per cent reproduced asexually for three generations, and only five per cent for four generations of consecutive asexual reproduction. About five per cent survived and underwent regression but decayed finally.

In all generations of blastozooids the number of buds and their reorganisation was typically similar to the normal process But the parent being an asexual blastozooid, the daughter buds did not receive any gonad from the parent, nor did they develop any.

### C. *An analysis of the factors inducing sexual reproduction*

#### 1. *Introduction.*

Though in all the colonies brought from the natural habitat to the laboratory and studied by sections there is ample evidence that sexual generation alternates with asexual, yet in order to follow it in the same individuals a detailed study was made as follows. A number of colonies of blastozooids which developed from the post-abdominal buds of asexually reproducing colony in laboratory tanks, and which had established connections with the outer surface were kept in cages made of wire gauze tied to rocks and immersed among the breakers of Royapuram area. Thus the colonies were restored to their natural habitat. After six days the colonies were taken from the cages and brought to the laboratory and were examined, and it was found that gonads had developed inside the post-abdomen. This showed that alternation of generations is a regular feature and that there are factors in nature which induce development of sexual organs but which have not been provided in the laboratory. In order to investigate the factors which induce sexual reproduction in nature, the following experiments were performed.

#### 2. *Experiments.*

a. *Supply of additional nutrition.* Since it has been suggested by Huxley ('21) and Brien ('53) that nutritional exhaustion may be responsible for the induction of asexual phase and that the abundance of food supply in spring may be correlated with the onset of sexual reproduction in the forms they have studied, in all the experiments performed by the author, care was taken to see

that there was no lack of food supply. Further, a few healthy colonies were kept in a large trough of water, to which a fresh collection of townet water was added, soon after changing the sea-water in the trough each day. In spite of such additions of extra food, the colonies failed to produce sexually.

b. *Crowding of sexual and asexual colonies.* Since the colonies of *P.indicum* are gregarious in their natural habitat, and since it is usual to find some of the mature ones liberating larvae, while others are in the process of asexual budding, it was felt that probably those reproducing sexually may have an 'influence' of a chemical character not unlike that which Grave & Nicoll ('39) suggested for hastening metamorphosis.

Two colonies with asexually developed blastozooids were left in fresh sea-water contained in troughs 9″ in diameter and 4″ high. To these six colonies which were liberating larvae in swarms were added. Nearly five hundred to six hundred larvae were liberated in the course of fifteen minutes. Eight hours after, when the larvae would have metamorphosed, the two colonies of blastozooids were removed to bigger vessels of water and kept under observation. In spite of thus providing this "influence" of sexually reproducing colonies, the blastozooids of the two colonies regressed and continued to reproduce asexually.

c. *Supply of 'conditioned' sea-water.* In this experiment, the 'influence' of sexually producing form was increased several fold by "conditioning" the water in the way described by Grave & Nicoll ('39) and the asexual zooids were reared in it.

One set of 200 larvae were kept in 20 c.c. of sea-water, and after five hours they showed signs of metamorphosis. They were removed and another set of 200 larvae were placed in the same water. These larvae showed signs of metamorphosis earlier, and after removing them, a third set was introduced. By about the fifth batch, the concentration of substances in the sea water became so high as to be lethal to the larvae. Therefore, for purposes of this present experiment only three sets of larvae were used to "condition" the water, in 100 cc. of which, a colony of blastozooids was left. After three hours it was removed to a bigger trough of water. This was repeated in order to make sure that the colony was acted on by "conditioned" water. In spite of this, the zooids never liberated larvae, but proceeded to bud asexually.

d. *Supply of sea-water of higher oxygen concentration.* The samples of sea-water taken from the Chepauk area opposite to the laboratory and used for the experiment, and the samples of the sea-water from the rocky Royapuram area, four miles north of the laboratory, where *P.indicum* occurs, were analysed. The results are tabulated in Table IX. It was found that where the ascidians live, the sea-water is richer in oxygen, obviously due to the breakers dashing against the rocks and dissolving the air to a greater extent than near the laboratory where the shore is sandy and level.

TABLE IX

| Sea water at Chepauk. | | | Sea water at Royapuram. | | |
|---|---|---|---|---|---|
| pH. | Salinity $0/_{00}$. | $0_2$ cc. per litre. | pH. | Salinity $0/_{00}$. | $0_2$ cc. per litre. |
| 8·2 | 33·00 | 3·99 | 8·2 | 34·01 | 4·21 |
| 8·2 | 33·98 | 3·80 | 8·0 | 33·2 | 4·99 |
| 8·2 | 30·48 | 3·90 | 8·1 | 30·2 | 4·92 |
| 8·1 | 29·49 | 3·90 | 8·0 | 29·4 | 4·49 |
| 8·1 | 27·39 | 3·90 | 8·15 | 27·1 | 4·98 |
| 8·2 | 27·8 | 3·6 | 8·0 | 27·0 | 4·89 |
| 8·15 | 27·4 | 3·7 | 8·1 | 27·0 | 4·99 |

Based on this finding the following experiments were performed to see if sexual phase could be induced in the laboratory as in the sea by i) oxygenating the sea-water in the laboratory troughs, ii) by transferring colonies which have regressed to the natural environment.

i. *Oxygenating sea-water in laboratory troughs*

Pure air from an aspirator was passed through a mixture of equal volumes of Conc. KOH and NaOH and was bubbled through 15 litres of sea-water taken from the Chepauk area. The bubbling was adjusted in such a way that in about 1½ hours 2000 c.c. of $CO_2$ — free-air was let in and $O_2$ concentration was

increased from 3·99 cc. to 5 cc. per litre, as was found in the natural habitat of the ascidian.

Colonies of ascidians containing first generation, second generation, third generation and fourth generation of blastozooids obtained (*vide* Section B pages 34-37) by keeping them in sea-water obtained from Chepauk were transferred to troughs containing the oxygenated sea-water. The water was changed twice every day and the reactions of the zooids were observed. Within three to six days sexual glands were found to develop inside the post-abdomen and in ten to twelve days larvae were liberated showing thereby that, in a medium of optimum temperature and nutrition, a higher $O_2$ concentration was necessary for the development of sexual organs and normal life cycle. Observations made after 10 - 12 days are recorded in Table X.

TABLE X

| Generation of blasto-zooids. | No. of colonies observed. | Approx. No. of zooids in each. | Number of zooids with | | | Total No. of zooids becoming sexually mature. |
|---|---|---|---|---|---|---|
| | | | gonad developed. | mature eggs and embryos. | mature larvae. | |
| First | 23 | 150-300 | 30-40 | 20-30 | 20-25 | 70-95 |
| Second | 20 | 100-150 | 25-30 | 20-25 | 15-20 | 60-75 |
| Third | 12 | 90-100 | 20-25 | 20-25 | 10-15 | 50-65 |
| Fourth | 8 | 90-100 | 15-20 | 15-20 | 10-15 | 40-65 |

ii. *Transferring colonies with blastozooids to natural habitat*

As can be seen from Table IX, the pH, salinity and $O_2$ concentration of the sea-water samples from the Chepauk station, and from the natural habitat, Royapuram, of ascidians, differ considerably. Even when the $O_2$ concentration is higher in the latter station, the pH is the same as in the former station, and even when the pH is higher in the former station the $O_2$ concentration is far lower when compared with the latter station. Hence, it was considered that the sea-water of these two areas of the Madras coast are different not only in $O_2$ concentration but also in pH

range and therefore, probably in other chemical complexes. In order to test if the induction of the sexual phase in the previous experiment is only because of higher concentration or percentage of $O_2$, colonies with blastozooids, and which had passed through first, second, third and fourth generations were kept in gauze cages, immersed in water on the sea-shore of Royapuram tied on to rocks, and observations made. The results are tabulated in Table XI after a period of eight to ten days.

TABLE XI

| Generation of blasto-zooids. | No. of colonies observed. | Approx. No. of Zooids in each. | Number of zooids with | | | Total No. of zooids becoming sexually mature. |
|---|---|---|---|---|---|---|
| | | | gonad developed. | mature eggs and embryos. | mature larvae. | |
| First | 22 | 150-300 | 60-80 | 35-50 | 30-40 | 125-175 |
| Second | 19 | 100-150 | 40-60 | 25-30 | 20-30 | 85-120 |
| Third | 13 | 90-100 | 35-40 | 20-25 | 20-25 | 75-90 |
| Fourth | 9 | 90-100 | 20-30 | 15-20 | 15-20 | 50-70 |

In this experiment also as in the previous experiment, the colonies which have more or less suppressed the sexual generation owing to the adverse conditions in the laboratory begin to show the revival of the sexual activity within two days of their being restored to their natural habitat. This revival of sexual activity is more obvious in the first two types of colonies rather than in the latter two types, which have had their sexual phase suppressed for three and four generations. There was no difference in this respect between the colonies restored to the natural habitat and those which received oxygenated sea-water in the laboratory. Meanwhile the colonies kept under control, i.e., (in sea-water from the Chepauk station without additional oxygenation) did not show the onset of the sexual phase, but exhibited symptoms of regression preparatory to another asexual generation. The colonies which are in the third and fourth generations of asexual reproduction take proportionately a longer time for the revival of sexual phase. Hence, as can be seen from the tables, the

numbers of zooids which showed sexual activity after a period of ten to twelve days in the first experiment, and in a period of eight to ten days in the second experiment are seen to be fewer in the third and fourth category of colonies. The data presented in Table X show a total of seventy to ninety-five zooids indicating a sexual phase after ten to twelve days of the first generation, whereas over 125-175 zooids appear to have recovered their sexual activity when left in natural habitat (See table XI) even within 8-10 days. In a similar way zooids of the generation which had suppressed the sexual activity during one or more generations show a corresponding difference in recovery when treated to more $O_2$ in the laboratory, and when restored back to natural habitat. It must also be noted that the recovery of a larger number of zooids within eight to ten days in the natural habitat, and a smaller number over a longer period of ten to twelve days in the laboratory must be explained as due to the difference in the artificial conditions presented in the laboratory.

From the above we can conclude (1) that the zooids are able to revive their sexual activity when supplied with more $O_2$ in the laboratory as obtained in the natural habitat; (2) that since treatment with sea-water of lower $O_2$ concentration leads to a loss of the sexual phase and a repitition of the asexual phase for even four generations, it is clear that the insufficiency of $O_2$ affects *P.indicum* like other adverse conditions in bringing about regression and dedifferentiation and formation of buds. (3) that the repetition of asexual reproduction alone without an alternation of sexual phase leads to senescence and death. (4) that colonies of *P. indicum* occur in the rocky Royapuram area and not the sandy Chepauk area of the Madras coast must be related (among other factors) to the fact that sea-water has a higher concentration of $O_2$ in the former station than in the latter.

*Discussion*

A close study of the phenomenon of asexual reproduction in *P.indicum* and a collation of data obtained from different experiments performed in connection with the repetition of asexual reproduction without the alternation of sexual phase, as well as a review of the observations made by Orton, Huxley and Brien on the alternation of generations will show that we are dealing with not only features of the normal physiology of marine animals in close harmony with their environment, but also with factors

responsible for the metagenetic character of the life cycle, and that therefore it is difficult to isolate one or two factors as being solely responsible.

Orton ('20, '21) dealing with factors of breeding of marine animals disregards the factor of abundance of food and of differences in salinity, but considers temperature variations as the chief factor determining the onset of breeding. This conclusion that there is a temperature constant which applies to most marine species and that when this condition is provided breeding proceeds, will not explain the onset of the sexual phase in a tropical form like *P. indicum,* since in Madras the temperature of the sea ranges between 27-29° C. near the sea-shore throughout the year and this temperature range does not allow any degree of stimulus for the evocation of the asexual or the sexual phase. Orton's experimentally inducing the winter buds of *Clavelina* to sexual activity by raising the temperature does not shed any light on the problem relating to *P. indicum.* His using water different from that of the natural habitat may import conditions other than temperature as well, as has been found by the present author in rearing *P. indicum.* When we consider the fact that in the laboratory tanks the temperature of the sea-water was maintained at 27-29° C, (the temperature of the sea), and the fact that a rise of temperature to 32°C proves lethal, and yet the sexual phase was suppressed for four generations, we may legitimately conclude that rise of temperature may not be responsible for the induction of sexual phase, as claimed by Orton for *Clavelina.*

Berrill ('51) suggests that a period of nutritional exhaustion which follows after a sexual phase is responsible for a phase of asexual budding. This appears supported by Huxley's ('21) findings. He found that in *Perophora,* the sexual zooids regress when there is a scarcity of food and that the asexual stolons produce buds tiding over the adverse period. While this may be true in a temperate region where an abundance of food is noted with the advent of warm spring, in a tropical area like Madras, however, the amount of food available never varies to any marked extent. The experiments described on page 43, supplying additional planktonic food to asexually reproducing zooids of *P.indicum* did not induce a revival of sexual activity. Hence it is difficult to conclude that nutrition by itself or taken together with temperature as suggested by Brien ('53) can account for the onset of sexual activity or its suppression in *P.indicum.*

If, as Orton ('21) suggested, there are no "recondite chemical complexes" responsible for the induction of sexual phase, it may be assumed that (as Grave and Nicoll '39) found in stimulating larvae to accelerate their metamorphosis) these complexes must have been introduced into the sea-water by a large number of sexually reproducing forms in the same locality. As the experiment (b) described on page 39 shows, the crowding together of different colonies with a large number of zooids either in the sexual or asexual phase, does not appear to be responsible for the induction of sexual activity of *P.indicum.*

The solution to the problem, however, must be sought in the physico-chemical condition of the sea-water in the natural habitat and elsewhere. Observation shows that the colonies of *P. indicum* occur only in the three-mile stretch of the rocky part of the coast and nowhere else. Analysis of the sea-water shows that in this area the $O_2$ concentration is distinctly higher, on doubt due to the breakers crashing on the rocks. The experiments show that in sea-water from other areas, *P.indicum* shows a repetition of asexual reproduction to the exclusion of the sexual phase which can be however induced by oxygenating the water. Therefore it is legitimate to conclude among other factors contributing to the normal life of the ascidian and the regular alternation of sexual and asexual generations of tropical forms like *P.indicum,* physico-chemical factors peculiar to the natural habitat play an important part.

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## EXPLANATION OF FIGURES

Fig. 1 & 2. Small colonies of *Polyclinum indicum*.

„ 3. A full-grown colony of *P. indicum*.

„ 4. A group of colonies of *P. indicum* of various sizes.

„ 5. A zooid pattern on the surface of a colony.

„ 6. A solid section of a colony showing disposition of zooids and ampullae.

„ 7. An ascidiozooid.

„ 8. Pharyngeal region of an ascidiozooid showing the nature of the atrial siphon.

„ 9. Post-abdomen of an ascidiozooid.

„ 10. T. S. post-abdomen.

„ 11. 12 & 13. Embryos of *P. indicum* in various stages of development.

„ 14. Tadpole larva of *P. indicum* (side view).

„ 15. Tadpole larva of *P. indicum* (dorsal view).

„ 16, 17 & 18. Stages in the development of adhesive papillae of larva of *P. indicum*.

„ 19. L. S. adhesive papillae.

„ 20. Section passing through the nervous system of the larva of *P. indicum*.

„ 21. Section passing through the visceral ganglion of the larva of *P. indicum*.

„ 22. T. S. tail of the larva of *P. indicum*.

„ 23. T. S. yolky mass of the larva of *P. indicum*.

„ 24. L. S. posterior extremity of the tail of the larva of *P. indicum*. (tail fin is excluded).

„ 25. Adhesive papillae of the larva in which secretory cells are protruding out prior to fixation.

„ 26. Adhesive papillae showing the shooting out of the secretory cells as a result of centrifuging.

„ 27, 28 & 29. Stages in the metamorphosis of the larvae of *P. indicum*.

„ 30. A fixed young ascidiozooid after metamorphosis.

„ 30a. A single posterior ampulla.

„ 31. Type B tadpole larva of *P. indicum* after centrifuging.

„ 32. Type C tadpole larva of *P. indicum* after centrifuging.

„ 33. Type D tadpole larva of *P. indicum* after centrifuging.

„ 34. Type E tadpole larva of *P. indicum* after centrifuging.

„ 35. Magnified view of the trunk of larva after centrifuging.

„ 36. Group A of trunk of centrifuged larva.

Fig. 37. Same enlarged.

„ 38. Group B of trunk of centrifuged larva.

„ 39. Same enlarged.

„ 40. Group C of trunk of centrifuged larva.

„ 41. Same enlarged.

„ 42. Group C of the trunk of contrifuged larva, but tail completely resorbed.

„ 43. Same enlarged.

„ 44. Group C of the trunk of larva with tail intact.

„ 45. Same enlarged.

„ 46. Tail of Type B tadpole after centrifuging, showing wavy epidermis.

„ 47. & 48. ¼ resorbed tails after centrifuging, showing straight and wavy epidermis.

„ 49. Same with all tissues broken down.

„ 50 & 51. ½ resorbed tails after contrifuging, showing straight and wavy epidermis.

„ 52. Same with all tissues broken down.

„ 53 & 54. ¾ resorbed tails after centrifuging, showing straight and wavy epidermis.

„ 55. Same with all tissues broken down.

„ 56, 57 & 58. Various stages in the development of the post-abdomen of *P. indicum* during asexual budding.

„ 59. Post-abdomen, after regression of thorax and abdomen, showing swelling of anterior end.

„ 60. Same showing the way of constiction of buds.

„ 61a. Anterior bud which has been severed.

„ 61b. The middle and posterior buds in the acts of separation.

„ 62a. Anterior bud.

„ 62b. Middle bud.

„ 62c. Posterior bud.

„ 63. L. S. showing the way of constriction of the epidermis prior to to final severance of bud.

„ 64. T. S. anterior bud.

„ 65. T. S. Middle or posterior bud.

„ 66. Bud with anterior swelling or epicardium.

„ 67. Buds with rudiments of pharynx and peribranchial invaginations

„ 68. Bud showing further development of pharynx, peribranchial invagination, pericardium and testis.

Fig. 69. Side view of bud showing anteriorly the pharyngeal rudiment, peribranchial invagination, and rudiments of the nervous system and gut.

„ 70. Bud showing more advanced development of pharynx, peribranchial sacs and gut.

„ 71. Diagramatic view of the rudiments of pharynx and peribranchial sacs.

„ 72. Diagramatic view of the development of gills and endostyle.

„ 73 & 74. Blastozooids undergrowing more advanced growth.

## EXPLANATION OF PHOTOMICROGRAPHS

1. The trunk of the larva of *P. indicum* of Group 1, after centrifuging.
2. The trunk of the larva of *P. indicum* of Group 2, after centrifuging.
3. The trunk of the larva of *P. indicum* of Group 3, after centrifuging.
4. The trunk of the larva of *P. indicum* of Group 3, after centrifuging, showing complete resorption of tail.

## KEY TO LETTERING

| | |
|---|---|
| AB. | Abdomen. |
| ADP. | Adhesive papillae. |
| AMP. | Ampullae. |
| AS. | Atrial siphon. |
| AMP. | Ampullae. |
| BR. | Gill. |
| CL. OP. | Common cloacal opening. |
| ECT. | Ectoderm. |
| EN. | Endostyle. |
| END.STR. | Endodermal strand. |
| EP. | Epicardium. |
| G.R. | Rudiment of gut. |
| HT. | Heart. |
| INT. | Intestine. |
| MES. | Mesenchyme cells. |
| MUS. | Tail muscles. |
| N.R. | Rudiment of nervous system. |
| NT. | Notochord. |
| O. | Ovary. |
| O.OP. | Oral opening. |
| OS. | Oral siphon. |
| P.AB. | Post-abdomen. |
| P.BR.R. | Rudiment of peribranchial sacs. |
| PER. | Pericardium. |
| PH. | Pharynx. |
| PH.R. | Rudiment of pharynx. |
| PH.W. | Pharyngeal wall. |

| | |
|---|---|
| P.T.V. | Posterior test vesicles. |
| R. | Rectum. |
| REC. | Rectum. |
| RT.PIG. | Retinal pigment. |
| SEC.C. | Secretory cells. |
| ST. | Stomach. |
| S.VES. | Sensory vesicle. |
| T. | Testis. |
| TF. | Tail fin. |
| TR. | Trophocytes. |
| V.GN. | Visceral ganglion. |
| V.N. | Visceral nerve. |
| X. | Place of strobilation. |
| YK.M. | Yolky mass. |
| Z. | Zooids. |
| TA. | Tail |
| A.T.V. | Anteriortest vesicles. |