Studies on dwarf larvae developed from isolated blastomeres of the starfish, *Asterina pectinifera*

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SUMMARY

Not only a whole denuded egg, but also blastomeres isolated from 2-, 4- and 8-cell starfish embryos developed into morphologically normal, but dwarf bipinnariae, the sizes of which were roughly proportionate to that of the respective original blastomeres. Some of the blastomeres isolated from the 16-cell stage were also capable of developing into the larval stage. All isolated blastomeres divided in good synchrony with the control embryos. Blastulae of all groups gastrulated within quite a short range of time, around 14.5 h after insemination at 20 ± 1 °C, although one-third of the 1/8-blastula missed this chance but gastrulated by 19.5 h. The number of constituent cells of the 1/8-gastrula was counted to be about 560, which corresponds roughly to one-half of that of the 1/4-, one-fourth of the 1/2- and one-eighth of the 1/1-gastrula. This ratio also fitted roughly for the total cell volume.

The results are compared with those of other invertebrate species, as well as of some vertebrates, and are discussed in connexion not only with the concepts of 'regulative' and 'mosaic' eggs, but also with a criterion that does not fit into either of these; the developmental system of the mammals.

INTRODUCTION

Driesch, in his historic experiments of 1892 and 1900, reported the capacity of isolated 2- and 4-cell sea urchin blastomeres to develop into normal plutei, although the resultant larvae are smaller and their rate of development slower than normal. His conclusion was that all parts of an egg are 'equipotential' and, therefore, are capable of replacing any missing parts (Driesch, 1900, 1908). He referred to such eggs as 'regulative eggs'. Although much evidence indicates that there is no egg, in reality, that is a completely regulative egg in Driesch's sense (Waddington, 1956), it is true that Driesch's experiments greatly influenced the scientific thought of the investigators who followed him.

Morgan (1895) was to demonstrate that 1/2- and 1/4-larvae of the sea urchin contain only a half and a fourth, respectively, of the number of cells of a whole larva. Tyler's proposal (1933) with respect to the slower rate of development of a dwarf embryo was that it is due to the extra time required to supply the amount of energy necessary for the developmental processes. Hörstadius, on

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the other hand, has established the ‘two-gradient theory’ of echinoderm eggs through laborious experimentation in which he isolated and recombined blastomeres of early cleavage stages (see Hörstadius, 1973).

Isolation experiments were resumed recently by Hagström & Lönning (1965), who reared isolated blastomeres of 16-cell sea urchin eggs. In their experimental system, the isolated blastomeres cleaved at about the same rate as those in situ. Isolated blastomeres of other invertebrate species, as well as of some lower vertebrates, were also shown to develop into morphologically normal embryos (see Wilson, 1925; Huxley & DeBeer, 1934; Reverberi, 1971). The regulative capacity of isolated blastomeres of several mammalian species also has become a current subject in this field (see Wilson & Stern, 1975).

The aim of the present study is to make clear the developmental capacity of the isolated blastomeres, the time course of their development and the cellular composition (the number of cells and their total volume) of the resultant larvae. The starfish makes a good material for the present purposes, since the cells undergoing early development are bound only loosely together, even within the fertilization membrane, because of the lack of a functional hyaline layer (Fig. 1 a–f). This situation places the cells, when stripped of the fertilization membrane, in a state much closer to normal than those of other animal species. What is more, no further experimental conditions, such as a cation-free environment, are required in order to isolate the early blastomeres. Normal early development and the behaviour of early cells and cell clusters of this species have been reported briefly, elsewhere (Dan-Sohkawa, 1976).

It will be shown, in this report, that all eight blastomeres isolated from an 8-cell embryo are capable of developing into small bipinnariae, while at least some of the blastomeres obtained from a 16-cell embryo are able to develop into young bipinnariae. The schedule of cleavage, time of hatching and the onset of gastrulation were checked and the number of constituent cells of the gastrula was examined. Furthermore, the values obtained for the 1/2-, 1/4- and 1/8-embryo, as well as that of the denuded egg, were compared with that of the normal embryo.

The results, indicating a strong inclination of the starfish eggs towards the ‘regulative’ type, enabled us to interpret the concept of both ‘regulative’ and ‘mosaic’ eggs in terms of only two factors, i.e. the number of cells participating in the process of blastulation and the capacity of the members to promote the

**Figure 1**

Successive cleavage stages of normal, pre-hatching development in the starfish, Asterinapectinifera. (a) Fertilized egg, 1½ h after insemination. Polar bodies showing. (b) 2-cell stage, 2 h after insemination; (c) 4-cell, 2½ h; (d) 8-cell, 3 h; (e) 16-cell, 3½ h; (f) 32 (2³)-cell, 4½ h; (g) 64 (2⁴)-cell, 4½ h; (h) 2⁵-cell, 5½ h; (i) 2⁶-cell, 6½ h; (j) 2⁷-cell, 6¾ h; (k) 2⁸-cell, 8½ h; (l) 2⁹-cell, 9¼ h; (m) Rotation, 11½ h. Thickening of the vegetal wall is clear.
Development of starfish dwarf larvae

(a)  

(b)  

(c)  

(d)  

(e)  

(f)  

(g)  

(h)  

(i)  

(j)  

(k)  

(l)  

(m)
process of further development. On the other hand, an additional factor was necessary to explain the developmental mechanism of the mammals, which concerns the regulation of size of the cell population as a whole.

MATERIALS AND METHODS

Mature eggs of the starfish, \textit{Asterina pectinifera}, were obtained by the method reported previously (Dan-Sohkawa, 1976), in which a whole fresh ovary was treated for 20 min with $10^{-6}$ M 1-methyladenine in sea water (Kanatani, 1969). Eggs were inseminated within about 20 min after being shed. Spermatozoa were obtained by making several cuts in a testis laid on a dry Petri dish. They were kept in a refrigerator and used for the day.

Stripping off of the fertilization membranes. Eggs were denuded of their fertilization membrane also by the method used before (Dan-Sohkawa, 1976, 1977). That is, the fertilized eggs were subjected to three brief washes with 1 M urea, as soon as the fertilization membrane began to rise. Care was taken to use a sperm suspension dilute enough so that only about 60\% of the control eggs were fertilized. This concentration was sufficient to cause 100\% fertilization in denuded eggs and, at the same time, to decrease the ratio of abnormal cleavage due probably to polyspermy. Denuded eggs were left undisturbed until the onset of the 1st cleavage, after being spread gently on the bottom of a 1\% agar-lined Petri dish containing millipore-filtered (pore size 0.45 $\mu$m) sea water. Abnormally dividing eggs, or those missing the cleavage schedule, were removed with a fine pipette (tip diam. ca. 0.5 mm) during the 1st cleavage.

Isolation of the blastomeres. Single blastomeres of 2-, 4- and 8-cell embryos, which were separated quite easily from each other by a gentle agitation of the water, were collected during the later half of the respective cleavages with the same pipette. They were transferred into each of the micro-wells of a plastic microtest culture plate containing millipore-filtered sea water.

Since the blastomeres began to acquire some adhesiveness to each other from the 16-cell stage on, more care was required in isolating the 1/16-blastomeres. Eight-cell embryos were collected and the cells were spread on another agar-lined Petri dish. After allowing them to divide once, the doublets were separated carefully by a gentle pipetting. Only a group of which all the members divided synchronously and all the doublets separated relatively easily was used for further observations. A group of 16 was collected and transferred to three or four micro-wells. Special care was taken to check that none of the cells would lie too close to others, since the descendant cell clusters will easily join into one, once they come into contact (Dan-Sohkawa, 1977). The number of cell clusters was checked at intervals until hatching.

Time-lapse cinematography. Time-lapse cinematographs (16 mm) were taken with the aid of a Nikon CFMA equipped with a Bolex H-16 camera, and analysed with a Nac Dynamic Frame Analyser. The processes of cleavage
Development of starfish dwarf larvae

of the whole egg and isolated blastomeres was filmed with a frame interval of 18 sec. This film was used to analyse the schedule of cleavage and time of hatching. Swimming embryos were filmed with 24 frames per sec, and the film was subjected to an analysis of the time of initiation of gastrulation. The total cell volume of the embryo was estimated, also using these films, on the basis of the cross-sectional areas measured by planimetry. This was considered to give the best and most easily measured estimate of the volume, considering that the embryos are of an irregular shape.

Squash preparations. The number of constituent cells of the dwarf embryos were examined by a modified method of Matsumoto & Okazaki (1977). Normal and dwarf gastrulae were fixed, at 21.5 h after insemination, for 1 h at room temperature in a mixture of acetic acid and absolute alcohol (1:3). They were stained with aceto-carmine for more than 30 min and squashed on a slide glass, and the numbers of nuclei were counted on photographs.

RESULTS

(I) Normal early development

The mature ova are spheroidal, translucent, brownish orange in colour, and about 170 \( \mu m \) in diameter. They are heavier than sea water. The fertilization membrane starts to elevate about 2-3 min after insemination, and its separation is completed within about 45 sec; it then rises gradually to form a perivitelline space of 30 \( \mu m \) or more (Fig. 1a). The first and the second polar bodies are emitted around 60 and 90 min after 1-methyladenine treatment, respectively. The egg surface was observed to become depressed in parts about 10 min prior to the appearance of either of the polar bodies; the spherical form was recovered soon after their emission.

The cleavage is of the holoblastic, radial type. The first cleavage, through the animal-vegetal axis, took place at about 100 min after insemination at 20 ± 1 °C (Fig. 1b). The schedule of succeeding cleavages was as summarized in Table 1. Blastomeres divided quite synchronously until the 10th cleavage, the intervals between cleavages being roughly 35 min up to the 5th cleavage. Eggs developed into early coeloblastulae during the 5th interphase, i.e. the interphase after the 5th cleavage, but the adherence between the cells appeared still to be weak (Fig. 1f). The blastomeres became tightly packed during the 8th interphase, as the result of increasing adherence between adjacent cells (Dan-Sohkawa & Fujisawa, unpublished). The intervals between cleavages grew gradually longer after the 6th cleavage, until they reached 70 min between the 9th and the 10th. The blastulae began to rotate at about 4 h after the 10th cleavage (Fig. 1m).

At least two asynchronous divisions were observed during the period from the end of the 10th cleavage to the initiation of rotation. An 11th (asynchronous) cleavage was observed in all the blastulae, usually at about 1.5 h after the 10th.
Fig. 2. Post-hatching development of the starfish, Asterina pectinifera. (a) Onset of gastrulation, 15 h after insemination. (b) Mid-gastrulae, 22 h. Mesenchymal cells are leaving the roof of the archenteron. (c) Late gastrulae, 30 h. Long filopodia projected from mesenchymal cells. (d) Bipinnariae, 3 days after insemination. Large mouth, oesophagus, round stomach and intestine are visible.

Time-lapse cinematography revealed that the blastomeres divided once more after this cleavage, but this was not always ascertained. They hatched out as free-swimming blastulae at about 1.5 h after rotation. Thickening of the vegetal wall was observed during late rotation (Fig. 1m). Most of the embryos, in the present study, did not form wrinkled blastulae (Komatsu, 1972).

Gastrulation was initiated at the vegetal pole at about 1 h after hatching (Fig. 2a). While invagination of the archenteron proceeded, mesenchymal cells were gradually set free into the blastocoel from the bulging tip of the archenteron (Fig. 2b). When the length of the archenteron nearly reached the maximum value, at about 30 h after insemination, mesenchymal cells on the invaginating tip were observed to protrude long filopodia (Fig. 2c). The mouth opened through at the side, around 48 h after insemination. The stage of young bipinnaria (Fig. 2d) was attained 3 days after insemination.
(II) Developmental capacity of isolated blastomeres

Regardless of the stage of isolation up to the 16-cell stage, the developmental features of isolated blastomeres were essentially the same as that of a whole denuded egg, reported previously (Dan-Sohkawa, 1976).

Hardly any technical problems arose in isolating 1/2- and 1/4-blastomeres as an intact set, and in bringing them up as identical twin- and quadruplet-bipinnariae (Fig. 3a), respectively. Identical octaplets, on the other hand, were rather difficult to obtain (Fig. 3b), because of their lighter weight and of the stronger adhesiveness to the glass surface, i.e. to the inside wall of the pipette, which characterizes the blastomeres at this stage. It is not, however, so difficult to isolate and rear 1/8-blastomeres, if they do not have to be in a whole set.

As has already been mentioned, 1/16-blastomeres were far more difficult to isolate than 1/8-blastomeres. In spite of all our efforts, no complete set of 16 identical dwarfs has been obtained successfully, yet. The occurrence of gastrulation in seven out of 16 is the highest rate of success obtained so far (Fig. 3c). All of the 1/16-embryos which succeeded in initiating gastrulation developed into young bipinnariae (Fig. 3c, insertion).

A single example of an 1/32-gastrula was also obtained.

Since no effort was made to feed any of these dwarfs, their fates, especially with respect to their ability to metamorphose, are unknown at present.

(III) Time course of development of the dwarf embryos

The schedules of cleavages of 1/1-, 1/2-, 1/4- and 1/8-blastomeres are summarized in Table 1. As clearly shown in this table, isolated blastomeres maintained good synchrony with the normal eggs all through the cleavage stages.

Cells which had been spreading randomly over the substratum during the cleavage stages (Fig. 4a, b) began to adhere very closely together during the 8th interphase. The free edges of these cell clusters started to curl up after the 9th cleavage (Fig. 4c). Before the clusters closed into irregularly shaped hollow blastulae (Fig. 4d), the 10th, synchronous cleavage took place. Dwarfs and denuded embryos started to swim slightly earlier than the initiation of rotation in the control.

The time of the onset of gastrulation in the dwarfs, as well as in the 1/1- and normal embryos, is summarized in Table 2. Most of the normal, 1/1-, 1/2- and 1/4-embryos started to gastrulate by 14.5 h after insemination (ca. 1.5 h after hatching), while one-third of the 1/8-embryos failed to do so. Even these embryos, however, started to gastrulate by 19.5 h.

(IV) Number and total volume of constituent cells of the dwarfs

The number of constituent cells of early gastrulae was determined in squash preparation; the results are summarized in Table 3. Although there was con-
Fig. 3. Dwarf bipinnariae developed from isolated blastomeres. (a) Identical quadruplets. (b) Identical octaplets. (c) Bipinnariae developed from 1/16-blastomeres.
Table 1. *Intervals between cleavages, and time of hatching in dwarf embryos of the starfish, Asterina pectinifera*

<table>
<thead>
<tr>
<th>Embryos</th>
<th>No. of embryos</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>8th</th>
<th>9th</th>
<th>10th</th>
<th>Rotation† (swimming)</th>
<th>Total</th>
<th>Hatching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal embryo</td>
<td>7</td>
<td>36</td>
<td>33</td>
<td>33</td>
<td>34</td>
<td>36</td>
<td>41</td>
<td>49</td>
<td>70</td>
<td></td>
<td></td>
<td>(642)</td>
<td></td>
<td>83 (725)</td>
</tr>
<tr>
<td>1/1-embryo</td>
<td>4</td>
<td>36*</td>
<td>33</td>
<td>33</td>
<td>34</td>
<td>35</td>
<td>36</td>
<td>42</td>
<td>57</td>
<td>78</td>
<td></td>
<td>(627)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2-embryo</td>
<td>4</td>
<td>36*</td>
<td>32</td>
<td>31</td>
<td>32</td>
<td>35</td>
<td>37</td>
<td>39</td>
<td>45</td>
<td>60</td>
<td></td>
<td>(575)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4-embryo</td>
<td>4</td>
<td>36*</td>
<td>33*</td>
<td>33*</td>
<td>36</td>
<td>37</td>
<td>38</td>
<td>44</td>
<td>50</td>
<td>65</td>
<td></td>
<td>(599)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/8-embryo</td>
<td>4</td>
<td>36*</td>
<td>33*</td>
<td>33*</td>
<td>38</td>
<td>35</td>
<td>37</td>
<td>43</td>
<td>50</td>
<td>63</td>
<td></td>
<td>(628)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Since the embryos, at these stages, are still in preparation for observation, the values of the upper row(s) were averaged and applied. This is allowed since embryos of all groups cleaved in good synchrony.

† Rotation corresponds to swimming in denuded embryos.
Table 2. Time of initiation of gastrulation in dwarf embryos of the starfish, Asterina pectinifera

<table>
<thead>
<tr>
<th>Embryos</th>
<th>No. of embryos</th>
<th>13-0</th>
<th>13-5</th>
<th>14-0</th>
<th>14-5</th>
<th>15-0</th>
<th>15-5</th>
<th>19-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal embryo</td>
<td>120</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>1/1-embryo</td>
<td>64</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>63</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>1/2-embryo</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>54</td>
<td>71</td>
<td>73</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>1/4-embryo</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>1/8-embryo</td>
<td>42</td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>28</td>
<td>28</td>
<td>31</td>
<td>42</td>
</tr>
</tbody>
</table>

The total volume of the constituent cells of the embryo was estimated on the basis of the difference between the total volume of the embryo and that of the blastocoel. The results are shown in Table 4. The comparative total cell volumes of 1/2-, 1/4- and 1/8-embryos were about 53%, 29% and 15%, respectively, of that of the 1/1-embryo. These values remained constant at least from hatching to the early gastrula stage.

**DISCUSSION**

The synchrony of division observed between isolated blastomeres, whole denuded eggs and control eggs, up to the hatching stage, strongly suggests that all the isolated blastomeres and denuded eggs were behaving essentially in the same manner as in situ. This also applies to the further development, as judged by the short range of time in which most of these embryos started to gastrulate. The reason for the delay observed in one-third of the 1/8-embryos is not known.

The facts that (1) the numbers of constituent cells of the 1/2-, 1/4- and 1/8-embryo were about 52%, 23% and 13%, respectively, of the 1/1-embryo, and that (2) the total volume of the constituent cells of these embryos remained constant at least from hatching to the early gastrula stage.
<table>
<thead>
<tr>
<th>Embryos</th>
<th>No. of gastrulae examined</th>
<th>Average</th>
<th>Individual counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal gastrula</td>
<td>14</td>
<td>4908</td>
<td>4132 4454 4551 4564 4661 4675 4791</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4969 5117 5166 5325 5358 5436 5505</td>
</tr>
<tr>
<td>1/1-gastrula</td>
<td>14</td>
<td>4207</td>
<td>3356 3830 3840 3963 4027 4086 4163</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4185 4432 4437 4468 4600 4743 4766</td>
</tr>
<tr>
<td>1/2-gastrula</td>
<td>17</td>
<td>2168</td>
<td>1650 1851 1945 1957 1957 2038 2045</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2145 2148 2158 2180 2237 2305 2407</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2457 2582 2784</td>
</tr>
<tr>
<td>1/4-gastrula</td>
<td>18</td>
<td>980</td>
<td>744 817 835 838 846 847 852</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>901 925 954 986 994 1032 1108</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1153 1167 1263 1379</td>
</tr>
<tr>
<td>1/8-gastrula</td>
<td>19</td>
<td>555</td>
<td>390 434 439 445 446 504 508</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>523 547 548 571 581 634 635</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>653 659 666 672 686</td>
</tr>
</tbody>
</table>
constant at about 53%, 29% and 15% of that of the 1/1-embryo throughout the period examined, i.e. up to the early gastrula stage, are considered to indicate the absence of a special kind of regulation, either in the rate of cell division or in the size of the cells. We do not know the reason why the cell number of the 1/1-embryo fell about 17% below that of the normal, but, since the 1/1-embryo develops in perfect synchrony with the normal embryo and forms a completely normal bipinnaria (Dan-Sohkawa, 1976), we consider it not an essential defect of the experimental system.

The identical twin-, quadruplet- and octaplet-bipinnariae obtained in the present investigation, on the other hand, clearly indicate the totipotent nature of the starfish blastomeres up to, at least, the 8-cell stage. This means that early blastomeres are much closer, in nature, to the fertilized egg, i.e. a single independent, diploid cell, than to the highly cooperative cells of a multicellular system, such as a swimming blastula. This fact, along with the case with which these blastomeres separate from each other, therefore, strongly supports the idea that early embryos should be comprehended as a multisystemic collection of individual cells, which, eventually, will develop into a single system consisting of highly cooperative cells (Dan-Sohkawa, 1976, 1977). We only know, at present, that at least seven out of the 16 blastomeres are totipotent. Nothing is known as to the location of these blastomeres in the animal or vegetal hemisphere of the original egg, or whether this is a real limitation due to a restriction in the quality of the developmental potentialities, or a limitation reflecting a quantitative restriction in the size of the developing body.

Thanks to the strong inclination of the starfish eggs toward the ‘regulative’ type, we can explain the phenomenon of a ‘regulative egg’ (e.g. Driesch, 1892; Hörstadius, 1937; Lutz, 1949) by means of only two factors: namely, (1) the size of the population of cells participating in the process of blastulation, and (2) the capacity of all the members to promote the normal process of development of the species, in the morphological sense. As to the factor (1), it is clear that there is no limitation to the number of cells that can participate in the blastulation process. The size of the cell cluster can be much larger than the normal, as in the case of joined larvae (Dan-Sohkawa, 1977) or much smaller, as in the present cases, the actual size depending on the number of cells which

<table>
<thead>
<tr>
<th>Embryos</th>
<th>No. of embryos</th>
<th>Comparative total cell volumes (%) (means with standard deviations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1-gastrula</td>
<td>10</td>
<td>100±4.2</td>
</tr>
<tr>
<td>1/2-gastrula</td>
<td>10</td>
<td>53.2±1.3</td>
</tr>
<tr>
<td>1/4-gastrula</td>
<td>10</td>
<td>29.3±1.4</td>
</tr>
<tr>
<td>1/8-gastrula</td>
<td>10</td>
<td>14.7±1.6</td>
</tr>
</tbody>
</table>
happened to be in contact with each other at the time of initiation of the process. And, as to the factor (2), since it is well established that all the cells of a body are equipped with genetic information concerning every aspect of development, the ability or inability of early blastomeres to develop into harmonized dwarfs should be a matter of an uneven distribution, both in space and in time, of cytoplasmic factor(s) supporting or restricting the expression of certain bits of information. The phenomenon of a ‘mosaic egg’ (e.g. Costello, 1945; Cohen & Berrill, 1936), therefore, can be explained as a variation of ‘regulative egg’, or vice versa, in connexion with the second factor (see also Wilson, 1925).

Compared with either of the two types of developmental mechanism, the descendants of isolated blastomeres of mammalian species behave in quite a different manner (Tarkowski, 1959; Seidel, 1960; Moore, Adams & Rowson, 1968; Hoppe & Whitten, 1972). The size of these embryos at the blastocyst stage varies roughly in proportion to the original size of the respective isolated blastomeres (Tarkowski & Wróblewska, 1967), whose size, however, will be regulated back to normal during the earlier half of pregnancy (Tarkowski, 1959). The same is to be said in the case of embryos formed by a fusion of two or more early embryos (Mintz, 1965; Buehr & McLaren, 1974). In order to explain the developmental mechanism of mammals, a third factor, namely, regulation in the size of the cell population to that of the normal, has to be introduced in addition to the two factors already mentioned. Although nothing is known about the role of this capacity for regulating the size of the cell population in normal development, it is no doubt a matter of an increased versatility gained by evolutionary processes, and will make an interesting problem for the future.

The present experimental system is thought to be valid not only for considering the meaning of the size of a normal egg, but also for analysing the minimum size necessary for various aspects of development. Experiments to explore whether all the 16 blastomeres show the same developmental behaviour and observations on the further development of the dwarf embryos, including their growth and metamorphosis, are contemplated.

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Development of starfish dwarf larvae


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