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STUDIES IN THE PRODUCTION OF GRAFTED EMBRYOS.

A. J. GOLDFARB.

(*From the Biological Laboratories, College of the City of New York.*)

INTRODUCTION.

It is now generally known that the blastomeres of certain kinds of eggs may, after their complete separation, develop into small though otherwise perfect, larvæ. The reverse experiment of reuniting partially separated or even completely separated blastomeres has also been successfully performed. These experiments demonstrated that it is not only possible to derive two embryos from a single egg, but that two or more blastomeres may be more or less recombined into one organism. These results suggested the possibility of grafting not only the blastomeres, but the eggs themselves. Metchnikoff ('86), Morgan ('95), Zur Strassen ('98), Herbst ('00), discovered and described embryos and larvæ which indicated a grafting of several eggs.

The first successful attempt to graft eggs together was made by Driesch in 1896 with the eggs of various European echinoderms. In the course of several years' experimentation, he perfected the method by which he produced agglutinated and fused eggs, about twenty to the thousand. More recently,

H. V. Wilson ('11), Müller ('11), and others demonstrated that somatic cells could also be fused.

Several investigators have repeated Driesch's experiments with the echinoderms found on this side of the Atlantic but without success. By slightly modifying Driesch's method, I finally succeeded in agglutinating and fusing the eggs of *Arbacia punctulata* at Woods Hole, Mass., in relatively large numbers, ten to forty in every hundred.

In this paper, I propose to give a detailed account of the method used in successfully fusing *Arbacia* eggs, to state briefly the effect of the treatment upon normal development, and to describe some of the agglutinated and fused embryos and larvæ. Since Driesch has given so full, so clear and accurate an account of fused larvæ, I will in this paper emphasize the earlier developmental stages and state but briefly in how far the *Arbacia* larvæ are like or unlike the European fusions described by Driesch.

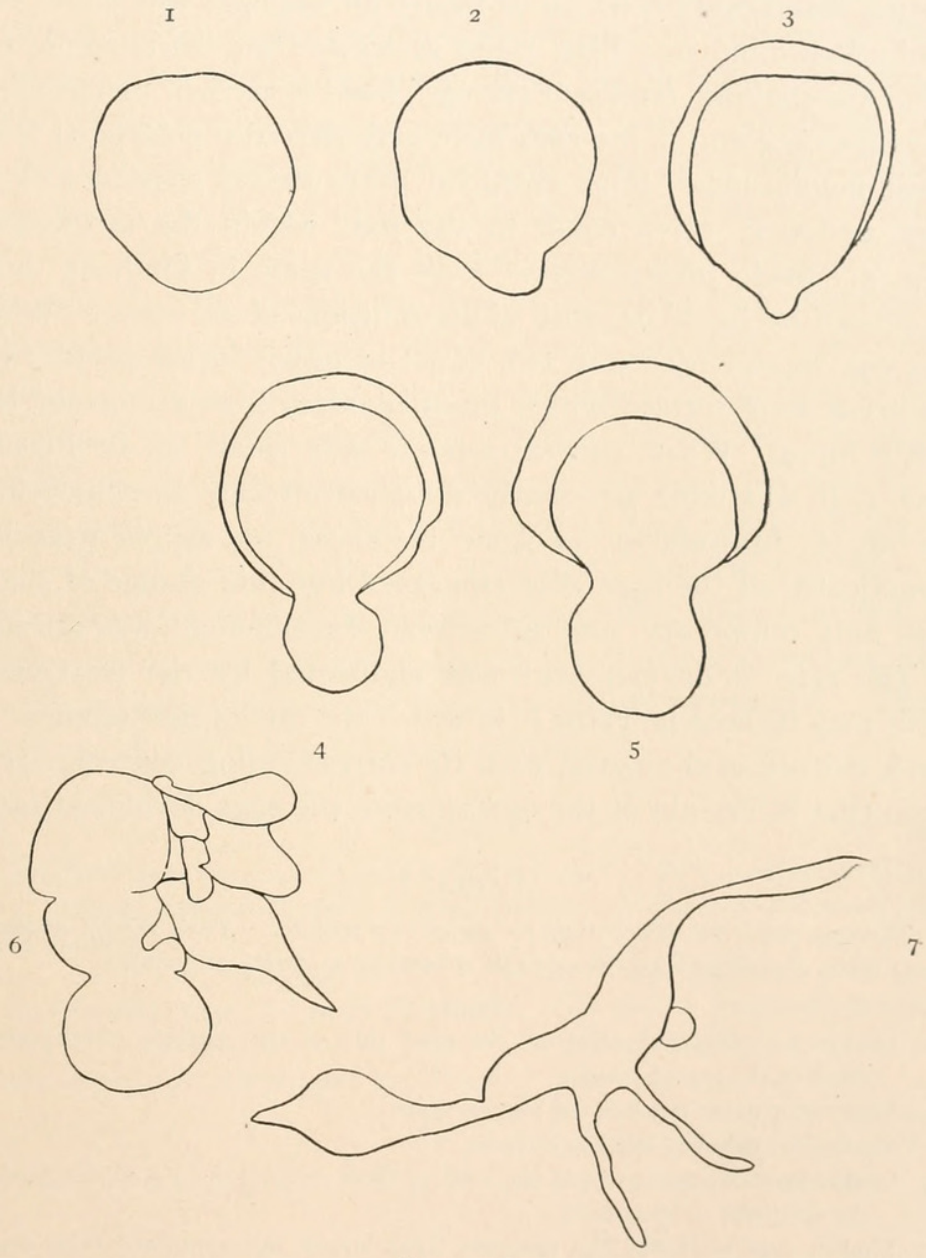
Method Used to Agglutinate Eggs.

The eggs of *Arbacia punctulata* were shaken violently two to three minutes after fertilization so as to remove their fertilization membranes. They were then placed in a calcium-free sea water (van't Hoff formula) prepared with copper or glass distilled water. To this solution four to twelve drops of 0.5 per cent. NaOH were added to every 200 c.c. of the solution. The eggs remained in this alkaline solution for varying periods, as a rule not longer than the first cleavage. Up to this point the method is the one used by Driesch, a method which failed altogether to agglutinate the *Arbacia* eggs. They were then transferred to narrow bore tubes, about 1/8 inch inside diameter, and centrifuged for three to five minutes at about 30 revolutions per minute, and finally placed in sea water. The cultures so treated later contained the agglutinated and fused embryos and larvæ.

As it seemed probable that the violence of the treatment might produce atypic results, a preliminary examination was made to ascertain to what extent anomalies were present in the cultures and how far these were the result of the technique used.

Examination of the Effect of the Treatment upon Development.

Shaking of the Eggs.—When the eggs were shaken about two minutes after fertilization, the fertilization membranes were cast off most of the eggs. When the eggs were shaken four or more minutes after fertilization, the membranes were removed



Figs. 1–7.

with difficulty and the eggs were considerably distorted and otherwise injured. When shaken less than one and a half

minutes after fertilization, many did not segment at all or cleavage was irregular. It was evident that when eggs were shaken about two minutes after fertilization, the fertilization membranes were cast off most readily, and with the least disturbance to subsequent development.

The minimum shaking found necessary to remove the fertilization membranes from all or nearly all the eggs was about one and a half minutes. Figs. 1 and 2, drawn from life immediately after the shaking, represent the eggs freed from their membranes. In Figs. 3, 4 and 5, the eggs have only partially protruded from their membranes. Such extruded parts varied considerably in size, and were often equal to the part within the membrane, Fig. 5. Such "extra-ovates" were the same in kind, as those produced by Loeb' 94, with dilute solutions of sea water. When the egg was divided into two equal or nearly equal parts, twin embryos were formed which often reunited into a more or less single larva. When the extra-ovate was small, as in Figs. 3 and 4, it was most frequently sloughed off and developed into an atypic blastula or, in some instances, an atypic gastrula. Practically all the eggs were elongated but this change of shape was only temporary, and subsequent development was normal.

The rate of development was unaffected by the treatment. This may be seen in Table I. in which the rate of development in each culture is compared with its corresponding control. It is seen that in certain of the experiments the eggs developed more

TABLE I.

The eggs were violently shaken for about two minutes. Their rate of development when compared with the controls showed no material difference.

No.	Result.
20	Shaken lot developed at about the same rate as the controls. Many never reached the pluteus stage.
12*a*	Shaken lot never reached the pluteus stage.
b	Shaken lot behaved like the controls.
37	Shaken eggs developed faster, and more of them reached the pluteus stage, than the controls.
15	Shaken eggs were like the controls, lived longer, but contained more atypic larvæ.
14	Shaken and control lots the same.
25	Shaken and control lots the same. More small-sized larvæ in shaken lot.
34	Shaken and control lots the same. More small-sized larvæ in shaken culture.

slowly or more quickly than its control, but taken altogether the rate of development was clearly unaffected by the shaking; and irregular development was absent, except for the larger number of small atypic blastulæ and gastrulæ arising from the sloughed off small extra-ovates.

Centrifuged Eggs.

When fertilized eggs were centrifuged with or without previous removal of the fertilization membranes, the normality of development depended upon the centrifugal power employed. In Table II., the effect of centrifugalizing from 35 to 90 revolutions per minute, and from 1 to 15 minutes duration, is shown.[1] When centrifuged at the rate of 35 revolutions per minute for ½ to 7½ minutes (in one experiment for 10 minutes), the subsequent development was normal. When centrifuged 60 or more revolutions a minute for even 1 minute considerable numbers of eggs developed but very atypically. When centrifuged for longer periods at this rate, the entire culture was rendered abnormal. At 90 revolutions a minute the embryos were nearly all atypic even though centrifuged ½ to 1 minute. Beyond this rate, or for longer periods of time, the eggs were either killed outright or broken into small fragments, only some of which developed into the atypic blastulæ or atypic gastrulæ already mentioned. These facts are shown in tabular form in Table II.

Table II.

Fertilized eggs were centrifuged at different rates and for varying periods of time. Within well-defined limits of speed and time the embryos and larvæ were quite normal.

No.	Experiment.		Result.
10*e*	Centrifuged and shaken for 24 minutes.		Few alive. Developed to young plutei only.
20–1	Centrifuged 35 × per min. for	6 min.	Developed to gastrulæ only.
		8	Developed to gastrulæ only.
		10	Developed to gastrulæ only.
		12½	Developed to gastrulæ only.
		15	Developed to blastulæ only. Irregular.
25*c*	Centrifuged 35 × per min. for	1	Developed normally.
		3	Developed normally.
		5	Developed normally.
		8	Developed irregularly.

[1] The arms of the centrifuge measured 150 mm. each.

TABLE II.—*Continued.*

No.	Experiment.		Result.
33	Centrifuged 35× per min. for	5	Developed normally. Some irreg.
		7½	Developed normally. Some irreg.
		10	Developed normally. More irreg.
38	Centrifuged 35× per min. for	1	Developed normally. More irreg.
		2	Developed normally.
		2	Developed normally.
		3	Developed normally.
		5	Developed normally. More irreg.
		5½	Developed normally. More irreg.
		6	Developed normally. More irreg.
		7	Developed normally. More irreg.
27*c*	Centrifuged 35× per min. for	6	Developed normally. More irreg.
20–2	Centrifuged 60× per min. for	6	Developed to young plutei only. Many irreg.
		8	Developed same.
		10	Developed same.
		13	Developed irregularly.
25–10	Centrifuged 60× per min. for	½	Developed normally.
		1	Developed increasingly irreg.
		2	Developed increasingly irreg.
		4	Developed increasingly irreg.
		5	Developed increasingly irreg.
38*b*	Centrifuged 60× per min. for	2	Developed to gastrula. Most dead.
18	Centrifuged 60× per min. for	1	Developed normally. Some irreg.
		2	Developed normally. Some irreg.
		4	Developed normally. Some irreg.
		6	Developed normally. Many irreg.
		8	Developed normally. Most irreg.
		10	Developed normally. Most irreg.
25–20	Centrifuged 90× per min. for	½	Developed young plutei only.
		1	Developed increasingly irreg.
		2	Developed increasingly irreg.
		3	Developed increasingly irreg.
		4	Developed increasingly irreg.
		5	Developed increasingly irreg.

In an effort to agglutinate the eggs, they were centrifuged not in urine tubes but in finely drawn glass capillaries, plugged at one end with paraffin, and the eggs liberated a few minutes to 24 hours after centrifuging. This method was unsatisfactory, first, because development ceased altogether within the capillaries, and when liberated, after four or more hours, development was atypical. When liberated, after 18 or more hours in the capillaries, they did not develop at all, Table III. Secondly, in removing the eggs, it was necessary to exert considerable force,

enough to separate the eggs from one another. The difficulty was overcome by using narrow bore tubes whose inside diameter was about 3 mm. Eggs centrifuged in these tubes within the centrifugal limits already mentioned, gave rise to normal embryos and larvæ many of which were agglutinated or fused.

Table III.

The effect of centrifuging eggs in capillary tubes, after they have been fertilized, and had their membranes removed.

No.	Experiment.	Result.
30–2	Not centrifuged.	Kept in capillary ½ hr. Same as control.
3	Not centrifuged.	Kept in capillary 1 hr. Same as control.
4	Not centrifuged.	Kept in capillary 4½ hrs. Same as control. Clusters present.
5	Not centrifuged.	Kept in capillary 18 hrs. Most dead.
25–30	Centrifuged 30 revol. a min. for 1 min.	15 min. Norm. Clusters present.
31	Centrifuged 30 revol. a min. for 1 min.	30 min. Norm. Clusters present.
32	Centrifuged 30 revol. a min. for 1 min.	60 min. Norm. and irregularities.
33	Centrifuged 30 revol. a min. for 1 min.	90 min. Norm. and irregularities.
40	Centrifuged 60 revol. a min. for 1 min.	15 min. Norm. and irregularities.
41	Centrifuged 60 revol. a min. for 1 min.	30 min. Norm. and irregularities.
42	Centrifuged 60 revol. a min. for 1 min.	60 min. Norm. and irregularities.
43	Centrifuged 60 revol. a min. for 1 min.	90 min. Norm. and irregularities.
50	Centrifuged 90 revol. a min. for 1 min.	15 min. Norm. and irregularities.
51	Centrifuged 90 revol. a min. for 1 min.	30 min. Norm. and irregularities.
52	Centrifuged 90 revol. a min. for 1 min.	60 min. Nearly all dead.
53	Centrifuged 90 revol. a min. for 1 min.	90 min. Nearly all dead.
18–29	Not centrifuged.	Kept in capillary 1 hr. Norm.
30	Not centrifuged.	Kept in capillary 2½ hrs. Norm.
31	Not centrifuged.	Kept in capillary 3 hrs. Plutei irreg.
32	Not centrifuged.	Kept in capillary 5 hrs. Few norm. plutei.
33	Not centrifuged.	Kept in capillary 7 hrs. Only gastrula.
34	Not centrifuged.	Kept in capillary 8 hrs. Died early cleavage.
35	Not centrifuged.	Kept in capillary 19 hrs. Died early cleavage.

Calcium-free Sea Water.

When eggs with their membranes removed were placed in calcium-free sea water for half an hour, the blastomeres of the two-cell stage separated from one another either completely,

and the resulting larvæ were perfect though small, or incompletely, and twin or fused plutei were produced as described by Loeb, Morgan and Driesch. When the eggs were kept in the solution for one hour, each of the four quarter blastomeres developed, some into dwarf plutei, others never beyond the gastrula stage. With increasing exposure to the calcium-free sea water, the increasing diminution in the size of the blastomeres, and the probable increasing segregation of formative stuffs, plutei were no longer formed, and abnormal embryos were correspondingly more numerous, most of which died early.

Solutions made with tap water (Woods Hole, Mass.) were highly injurious, for few eggs developed into normal larvæ. With distilled water practically all were normal, provided the eggs were not left in the solution longer than about thirty minutes as shown in Table IV.

TABLE IV.

The effect of calcium-free sea water upon the development of fertilized eggs.

No.		Experiment.		Result.
26*b*	10	Left in solution.	15 min.	Dead. Accident.
	11		30	Same as controls and small plutei.
	12		45	More small plutei and non-developing blastulæ.
	13		60	Only small plutei. Very many irreg.
	14		90	Few small plutei. Most irreg. on bottom, dead.
	15		120	Few small plutei. Most irreg. on bottom, dead.
		Membranes removed.		
	1	Left in solution.	15	Norm. and small plutei.
	2		30	Dead. Accident.
	3		65	Norm. and small plutei.
	4		60	Dead. Accident.
	5		90	Plutei and irregular clusters.
	6		105	Some plutei, most irregular, clusters.
		Distilled Water.		
18	24	Left in solution.	½ hr.	Norm. and small plutei.
	25		1	Small plutei and irregular plutei.
	26		2½	Few small plutei. Most irregular.
	27		6	Most irreg. Nearly all dead.
	28		7	Most irreg. Nearly all dead.
		Tap water.		
	17	Left in solution.	½ hr.	Few norm. plutei.
	18		1	Irregular plutei and irregular gastrulæ.
	19		2½	Only blastulæ irreg.
	20		6	Only blastulæ irreg.
	21		7	Only blastulæ irreg. Few alive.

The Effect of NaOH.

The gelatinization of the egg by the addition of NaOH and other alkalines to sea water aids materially in the agglutination. When 25 drops or more of 0.5 per cent. NaOH solution was added to 200 c.c. of sea water, the gelatinization was so great, that after centrifuging, the eggs were distorted almost beyond recognition. Figs. 6 and 7, on page 75, give an inadequate idea of the degree of distortion. Less NaOH, namely, 4 to 10 drops in the same amount of sea water, gave rise to no abnormalities.

From the foregoing it is clear that within the limits specified, the various steps found necessary in the production of agglutinated eggs, either separately or all together, did not materially effect development, except for the small fragments which gave rise to atypic blastulæ and gastrulæ, already mentioned. Table V. gives a list of experiments in which numerous agglutinated and fused embryos were observed.

Table V.

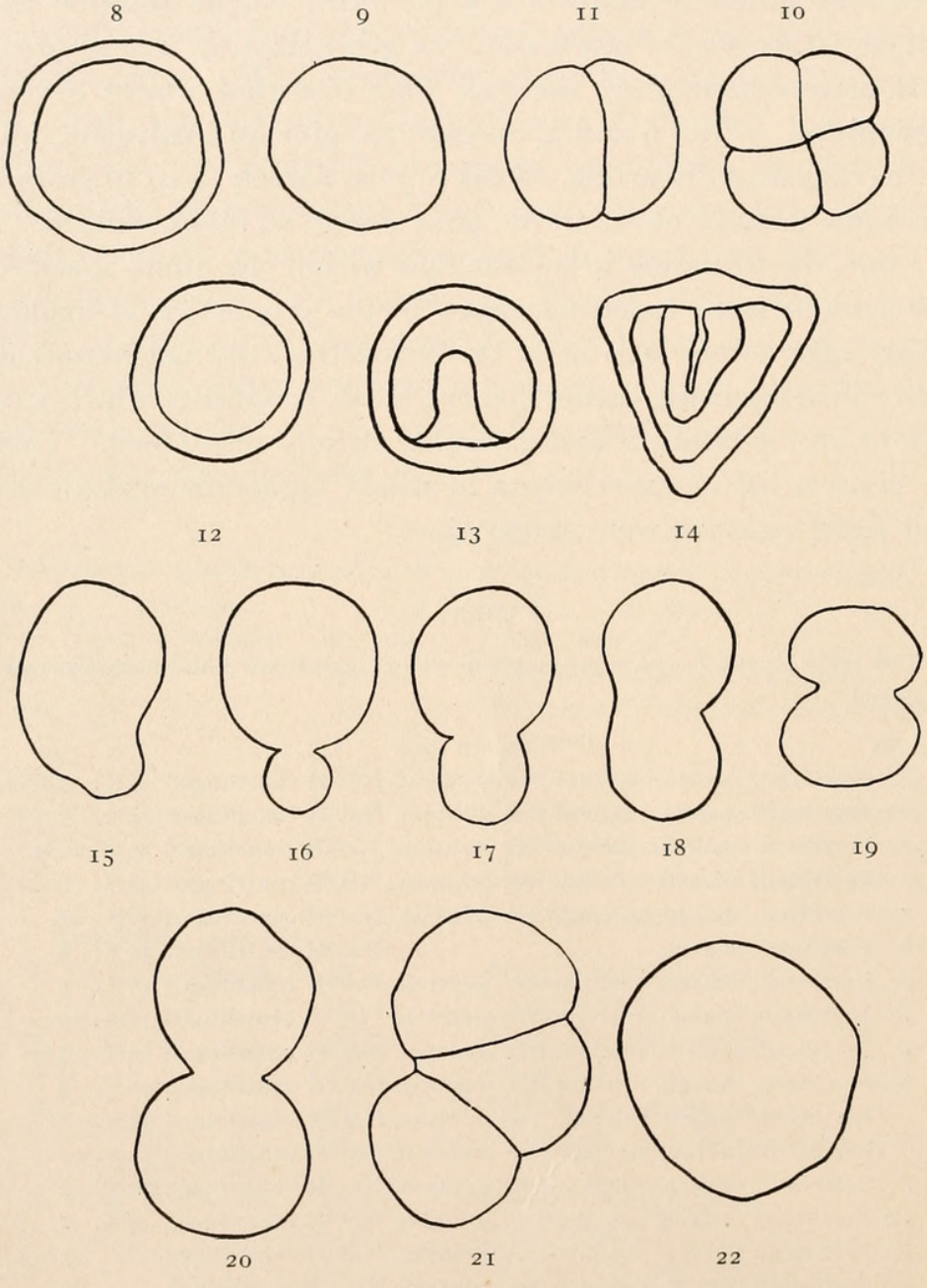
This table gives a list of experiments in which agglutinated and fused eggs were produced.

Exp. No.	Method Used.			
20.3	Fertilized.	shaken,	calcium-free solution,	NaOH centrifuged 35×, 5 min.
3*b*2	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 7½
3*b*4	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 12½
25 *c*5	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 30×, 6
*c*6	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 30×, 8
27 *b*	Fertilized,	shaken,	.....	NaOH centrifuged 35×, 6
ce	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 5
33 *d*	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 30×, 10
34 *d*	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 30×, 4½
37 *b*3	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 3
	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 5
	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 7
*d*1	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 60×, 2
*d*2	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 35×, 2
*d*3	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 3
*d*4	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 5
*d*5	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 7½
*e*2	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 2
38 *b*3	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 2
*d*7	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 5
*d*8	Fertilized,	shaken,	calcium-free solution,	NaOH centrifuged 7

Etc.

The Early Development of Agglutinated Eggs.

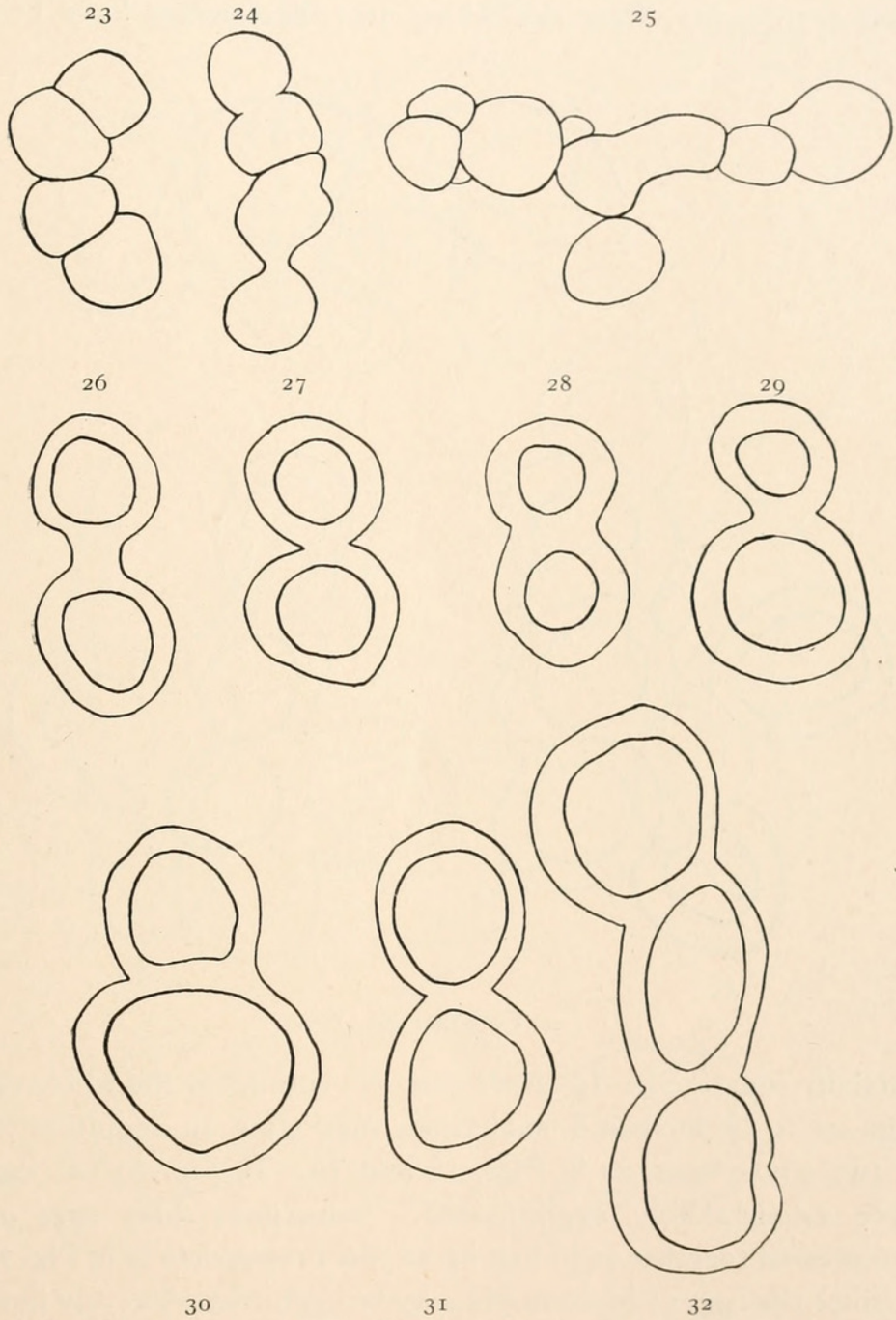
To serve as a basis of comparison, a few typical stages in the development of the normal egg are shown in Figs. 8 to 14. These



Figs. 8–22.

were preserved, magnified and drawn with the camera lucida, to the same scale, as all subsequent ones to be described. In Fig. 8, the egg is enveloped by its fertilization membrane. In Fig. 9 the membrane is not shown. The two and four cell stages

are shown in Figs. 10 and 11. The blastula, gastrula, and young pluteus are shown in Figs. 12, 13 and 14. It need hardly be iterated that the eggs and embryos in the control cultures

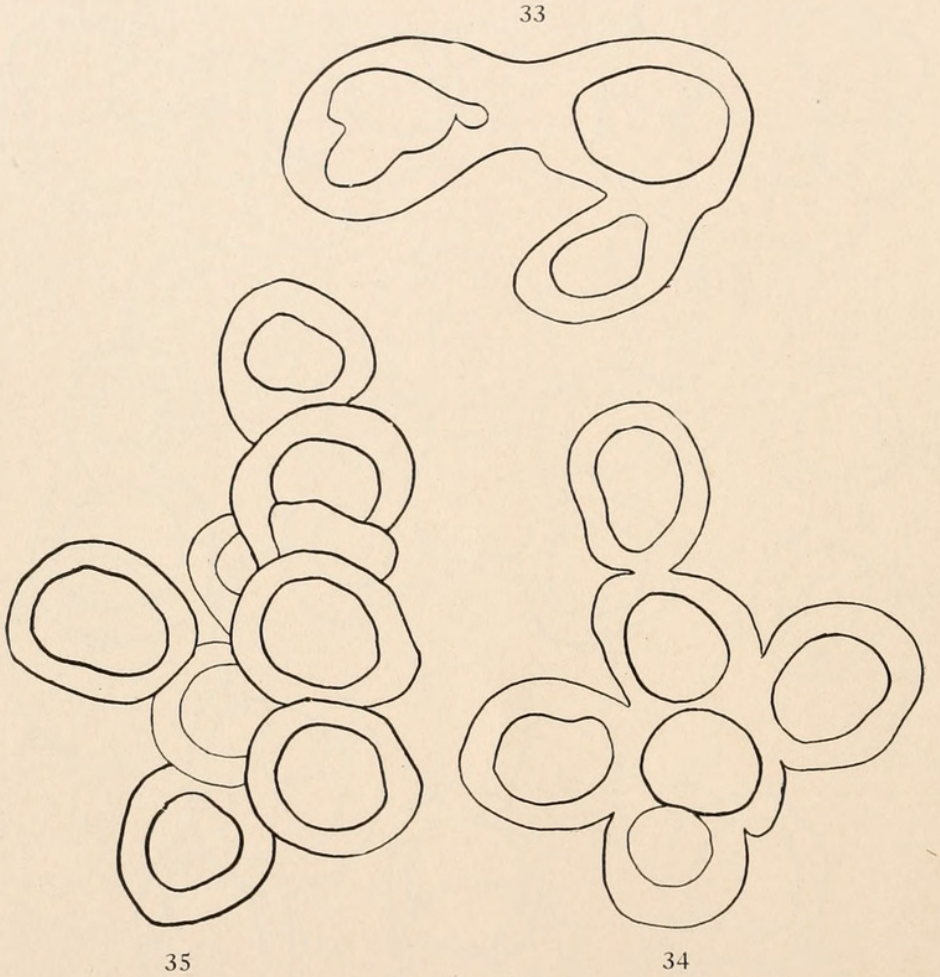


FIGS. 23–32.

varied within certain limits, as carefully worked out by Tennent ('10); the above figures represent typical or average specimens.

The treated eggs differed from the controls, in being elongated

or extra-ovate as seen in Figs. 15, 16 and 17. In Figs. 17, 18 and 19, the two parts of the egg are equal or almost equal. It is sometimes difficult to distinguish between an egg with its equal-sized extra-ovate, from one whose two blastomeres have been

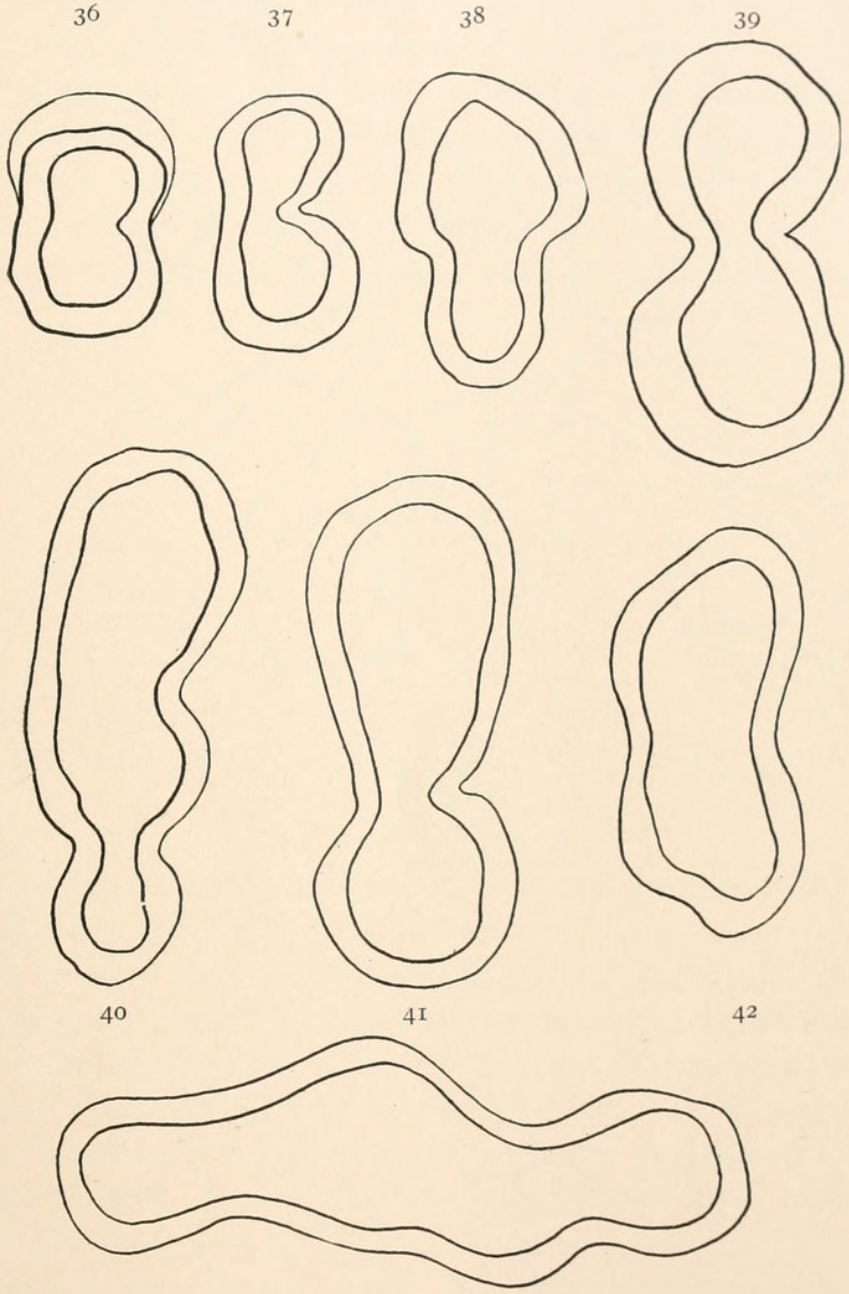


FIGS. 33–35.

partially separated. In either case the volume of the whole egg appears to be increased, sometimes suggesting an agglutination of two whole eggs, as in Figs. 18 and 19. In Fig. 20 two eggs have unmistakably agglutinated. Sometimes three eggs are compressed together as in Fig. 21, or *fused completely* as in Fig. 22.

Since the eggs or blastomeres may be agglutinated at any point of the surface and since the polarity of the egg is unaffected, the cleavage planes may occupy any angle with respect to one another, see Figs. 23, 24 and 25. Unless considerably distorted as in Figs. 6 and 7, each blastomere divided at an angle determined by the angle of agglutination or separation.

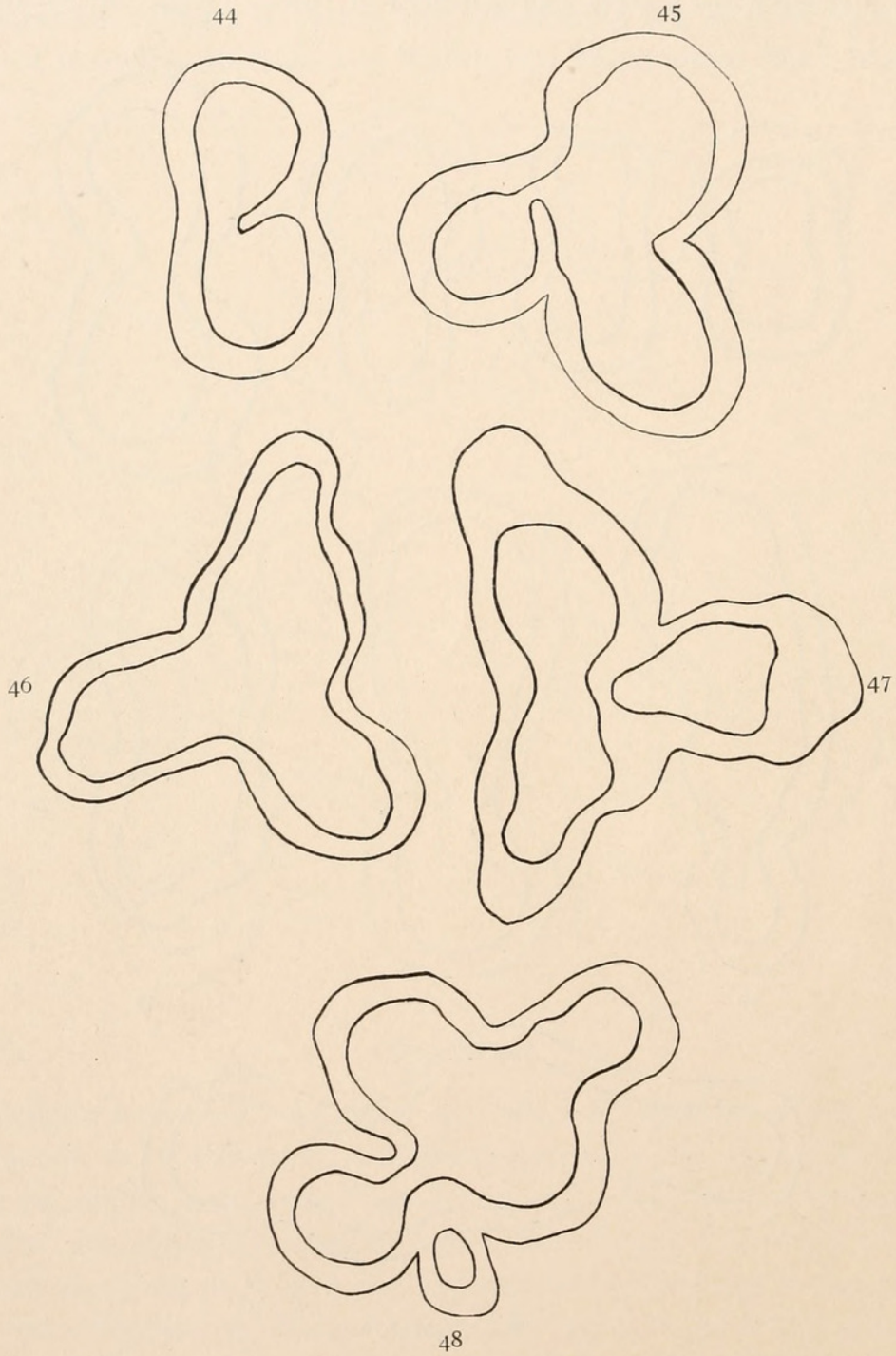
The blastulæ are particularly interesting for they make it possible to determine definitely whether the component members of a cluster are merely agglutinated or fused. Figs. 26, 27 and



FIGS. 36–43.

28 represent half blastomeres in varying degrees of approximation or separation, with their blastocœles entirely separate. Figs. 29 and 30 represent an agglutination of a half and a whole egg,

Fig. 31 an agglutination of two eggs. Three agglutinated eggs are shown in Fig. 32 and two and a half eggs in Fig. 33. Clusters



FIGS. 44–48.

of seven and nine eggs and blastomeres are shown in Figs. 34 and 35.

In a large number of instances a true fusion occurred during the blastula stage, as recognized by the continuous ectoderm and common blastocœle. In Fig. 36 a blastula is shown with a part extruded beyond the fertilization membrane. In Fig. 37 the blastula is pinched together near the middle. A whole and a half egg have been fused in the formation of the single blastula shown in Fig. 38. At least two eggs have united in Fig. 39. In Fig. 40 an egg a half egg and a quarter egg have so fused. Figs. 41, 42 and 43 represent other fusions of two or more eggs into single giant blastulæ. Figs. 44 and 45 are interesting because

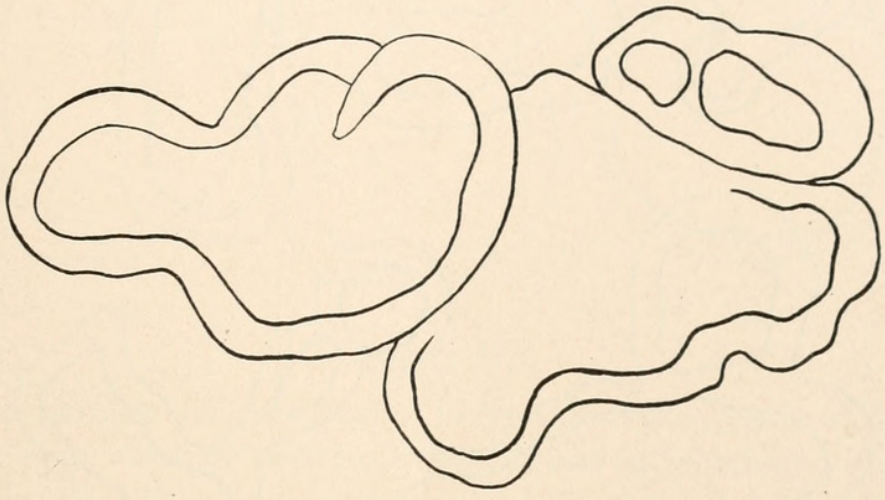


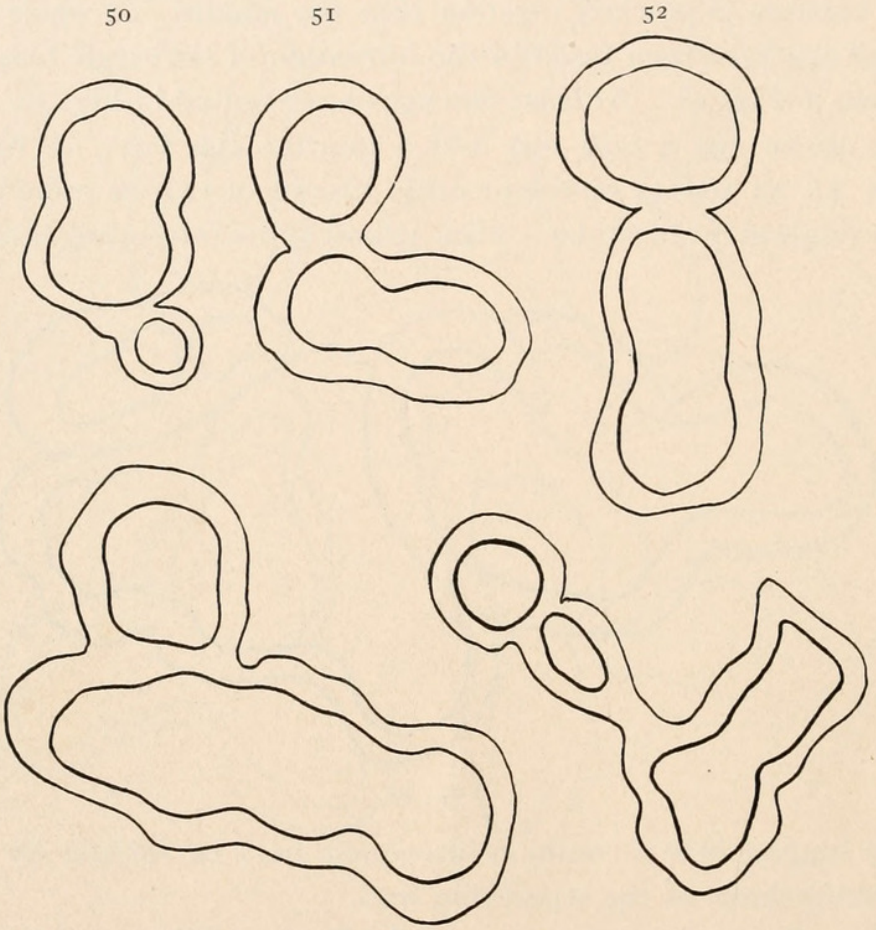
FIG. 49.

they suggest how a common blastocœle may be formed by the breaking down of the separating wall.

The clusters were not necessarily linear. Triangular groups like Figs. 45, 46 and 47 were not uncommon. Other clusters were quite irregular as in Figs. 48 and 49. A large cluster like the one shown in Fig. 49 was more frequently composed of agglutinated eggs or a complex of fused and agglutinated eggs; smaller clusters also included agglutinated and fused members shown in Figs. 50, 51, 52, 53, 54, 47 and 48.

Fused blastulæ tended to lose their individual identity by the continuity of their common layer of cells, by the disappearance of their inner separating walls and by the closer approximation of the separate blastulæ. These processes continued until a spherical or almost spherical giant blastula was produced, in which it was difficult or impossible to distinguish the component members, as in Figs. 55, 56 and 57.

The living clusters of embryos were easily distinguished from the normal ones. The latter swam with a characteristic uniformly rotary motion at or near the surface of the water. Agglutinated or fused blastulæ swam at or near the bottom swaying



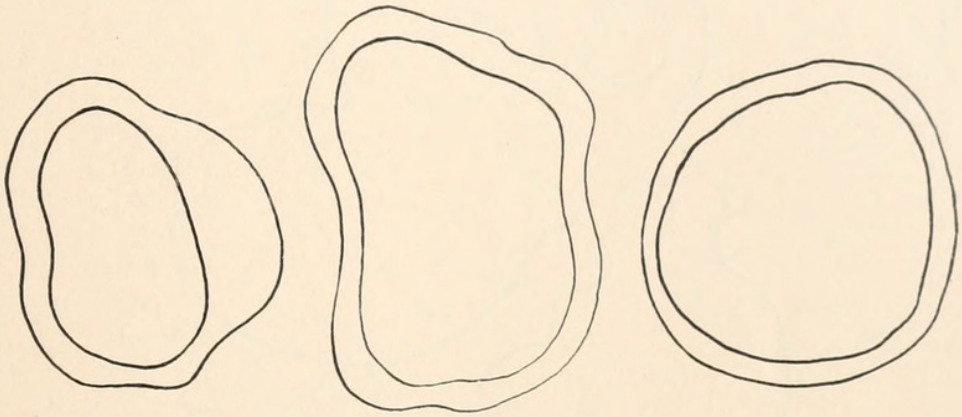
FIGS. 50–54.

irregularly and moving much more slowly. I have counted as many as fourteen full-sized blastulæ, of which Fig. 58 represents a large group of this kind. These are short lived, as already mentioned, and only the smaller clusters continue their development.

THE DEVELOPMENT OF AGGLUTINATED AND FUSED GASTRULÆ.

The differentiation of the archenteron definitely established the axes of the component members. Inasmuch as half eggs developed in precisely the same manner as whole eggs, the following statements apply to both.

The axes of the archenteron in the different gastrulæ of a cluster were found in every possible angle with respect to one another, suggesting that the polarity of the egg was unaffected by the proximity of the other. Two gastrulæ with their oral ends together, and their archentera in the same axis are shown in Fig. 59, and similar gastrulæ with their archentera almost in the same axis are shown in Fig. 60. The apex or aboral end



56 55 57

FIGS. 55–57.

of one gastrula may be attached to the side of the other and their archentera about 90° apart as in Figs. 61 and 64, or almost parallel in Fig. 62. The two gastrulæ may be attached at or near their aboral ends, Fig. 63. In Fig. 65 the oral end of one gastrula is attached to the aboral end of the second, and the archentera lie in an almost continuous line. In Fig. 66 the archentera lie in the same axis, but the gastrulæ are attached by their aboral ends. Many other examples could be cited. They clearly point to the conclusion that at this stage of development the polarity of the gastrulæ need not be changed by the proximity of other.

FUSED GASTRULÆ.

The same conclusion obtains with reference to fused gastrulæ. In Fig. 67, the blastopores are at opposite ends, namely 180° apart and the archentera have grown toward each other in the same axis. In Fig. 68, the blastopores are about 45 degrees apart and the archentera have crossed each other at this angle. Perhaps these two instances suffice as examples of this kind of independent development of the archentera. In other instances however the archentera met and fused.

In Fig. 69, the archentera have approximated but not yet fused into one continuous gut. In Fig. 70 the two have unmistakably united into a single continuous gut with no trace of blastopores.

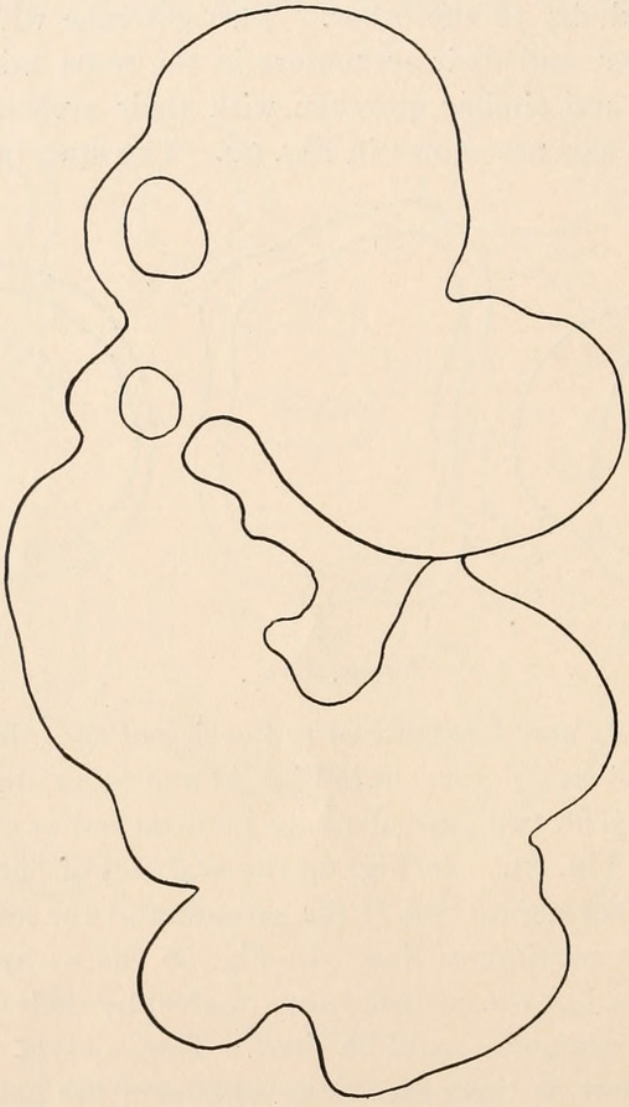
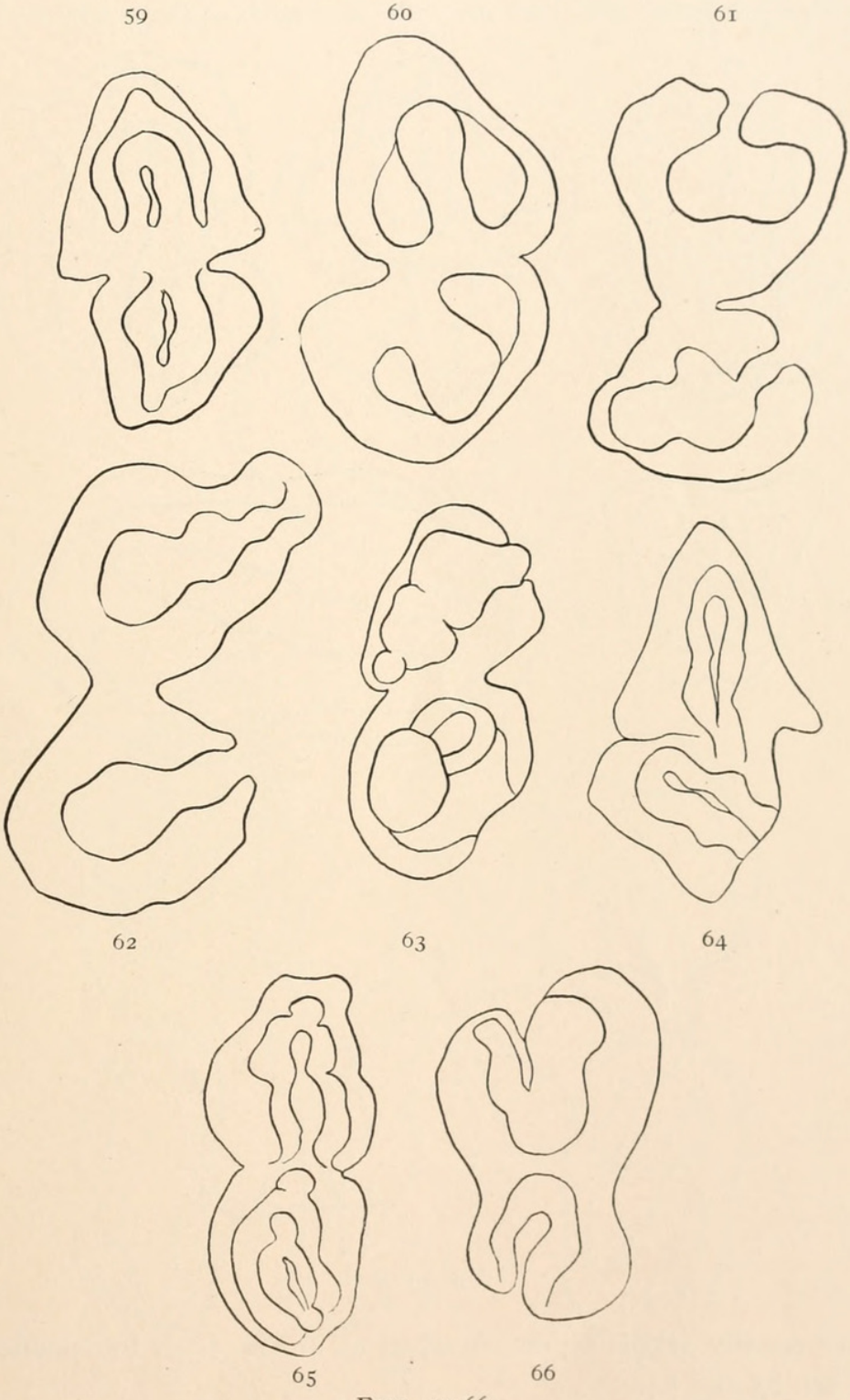


FIG. 58.

A similar gastrula drawn from life and drawn at greater magnification than the rest is shown in Fig. 71. In a cluster of four gastrulæ two have separate guts, two have united ones, Fig. 72.

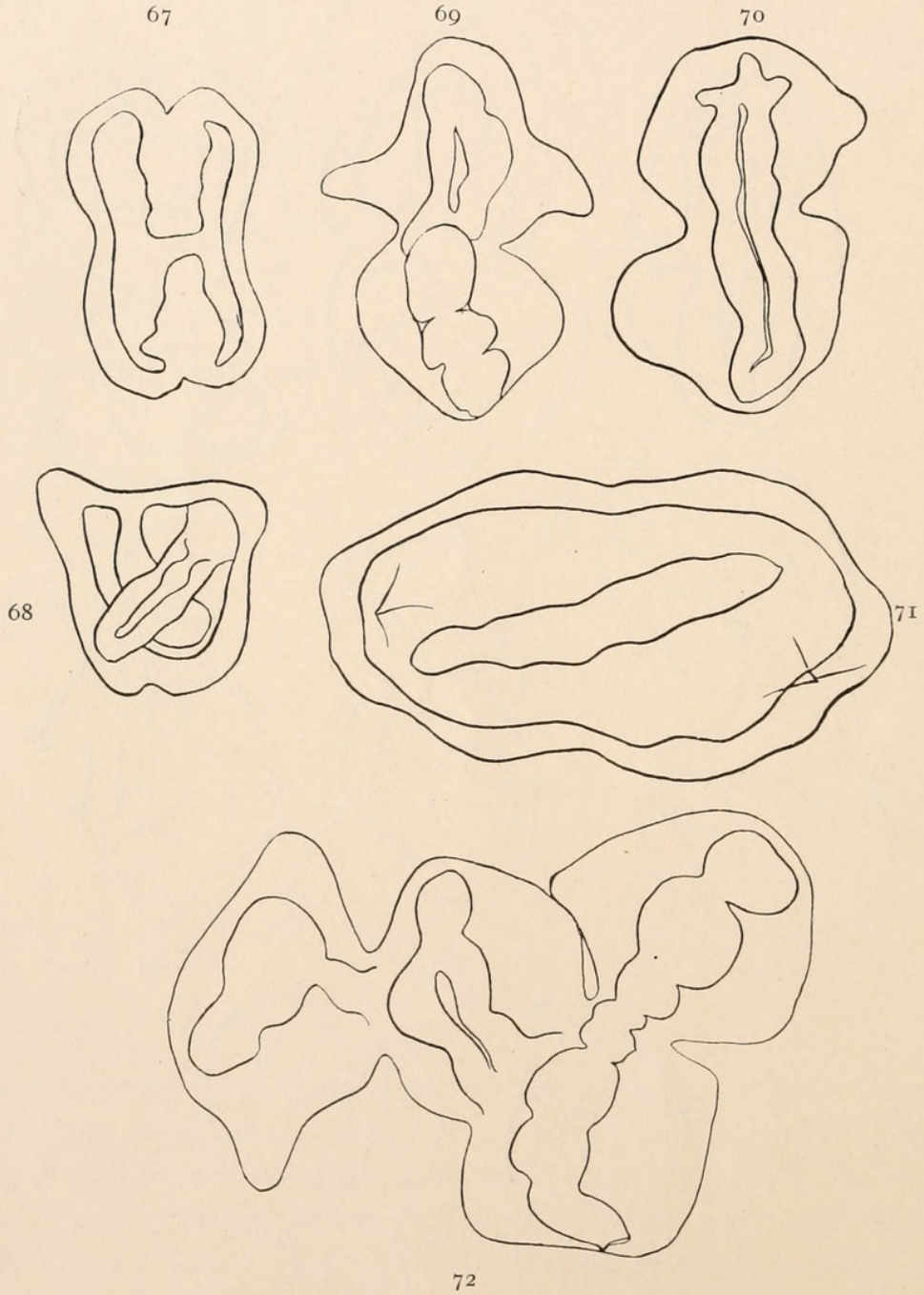
Instead of fusing end to end, the archentera may unite near their middle as in Fig. 73, or the blastopores with or without parts of the archentera may be united as in Figs. 74, 75 and 76. If one were to suppose the fusion of two archentera side to side, as suggested by the preceding figure, one would anticipate the single giant archenteron shown in Figs. 77 and 78.

There were in the different cultures two other kinds of fusion that deserve brief mention. In the one, a gastrula developed in only one of the pair of eggs or blastomeres as shown in Figs.



FIGS. 59–66.

79 and 80. The archenteron in such fusions may grow beyond the boundaries of the blastomere or egg into the blastocœle of its neighbor as seen in Fig. 81. In the second group the archentera

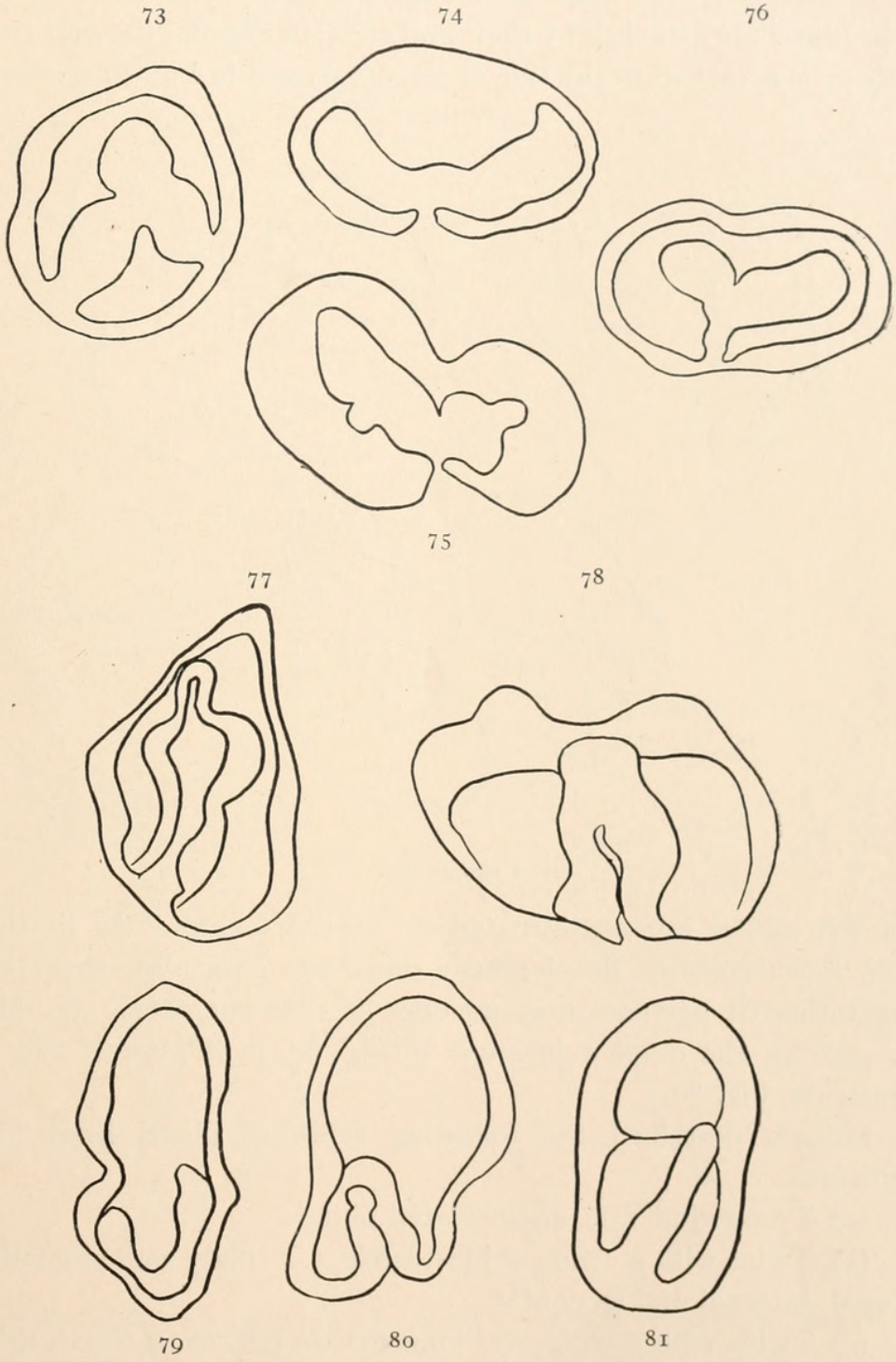


FIGS. 66–72.

are usually atypic either detached as in Fig. 82 or independent, Figs. 83 and 84.

THE DEVELOPMENT OF AGGLUTINATED AND FUSED PLUTEI.

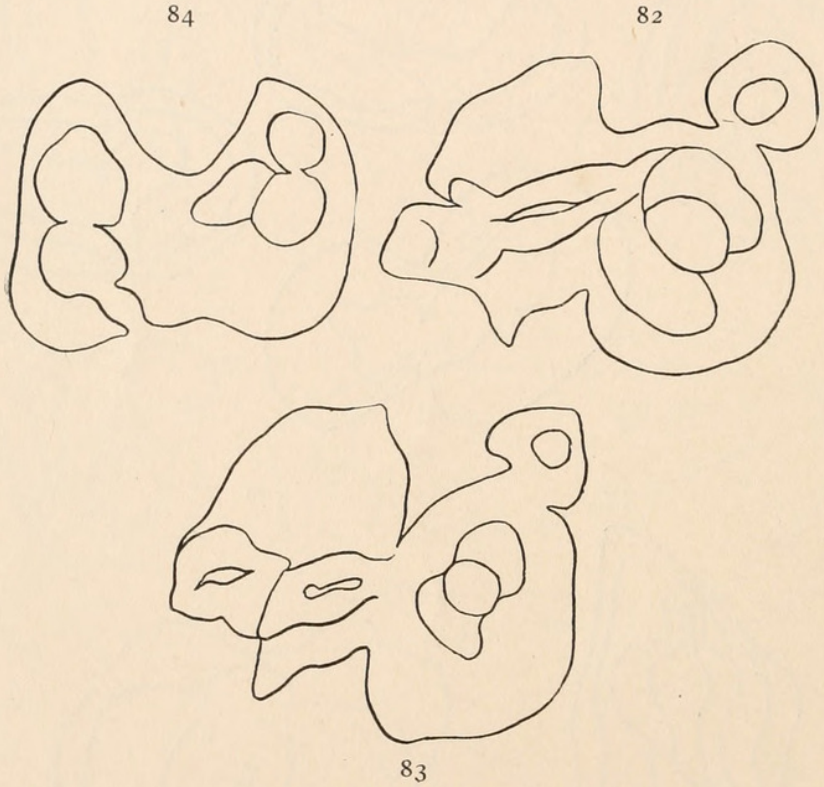
It will be recalled that the agglutinated embryos tended to separate and that this tendency increased during development. When the pluteus stage was reached, very few remained aggluti-



FIGS. 73–81.

nated, and these were in nearly every instance, clusters of two. The separation of the embryos of *Arbacia* occurred in exactly the same manner as described by Driesch.

These permanently agglutinated plutei may be attached to one another at any angle as in Figs. 85, 86 and 87. In Fig. 85 the plutei are attached by their oral ends; in Fig. 86, the oral end of one is attached to the side of the other, and in Fig. 87 a group



FIGS. 82–84.

of five plutei are agglutinated at various angles. As in the previous stages of development one finds here also, that the agglutinated partners may develop at different rates, the one a pluteus, the other a blastula as Fig. 88, or a pluteus and a gastrula, Fig. 89.

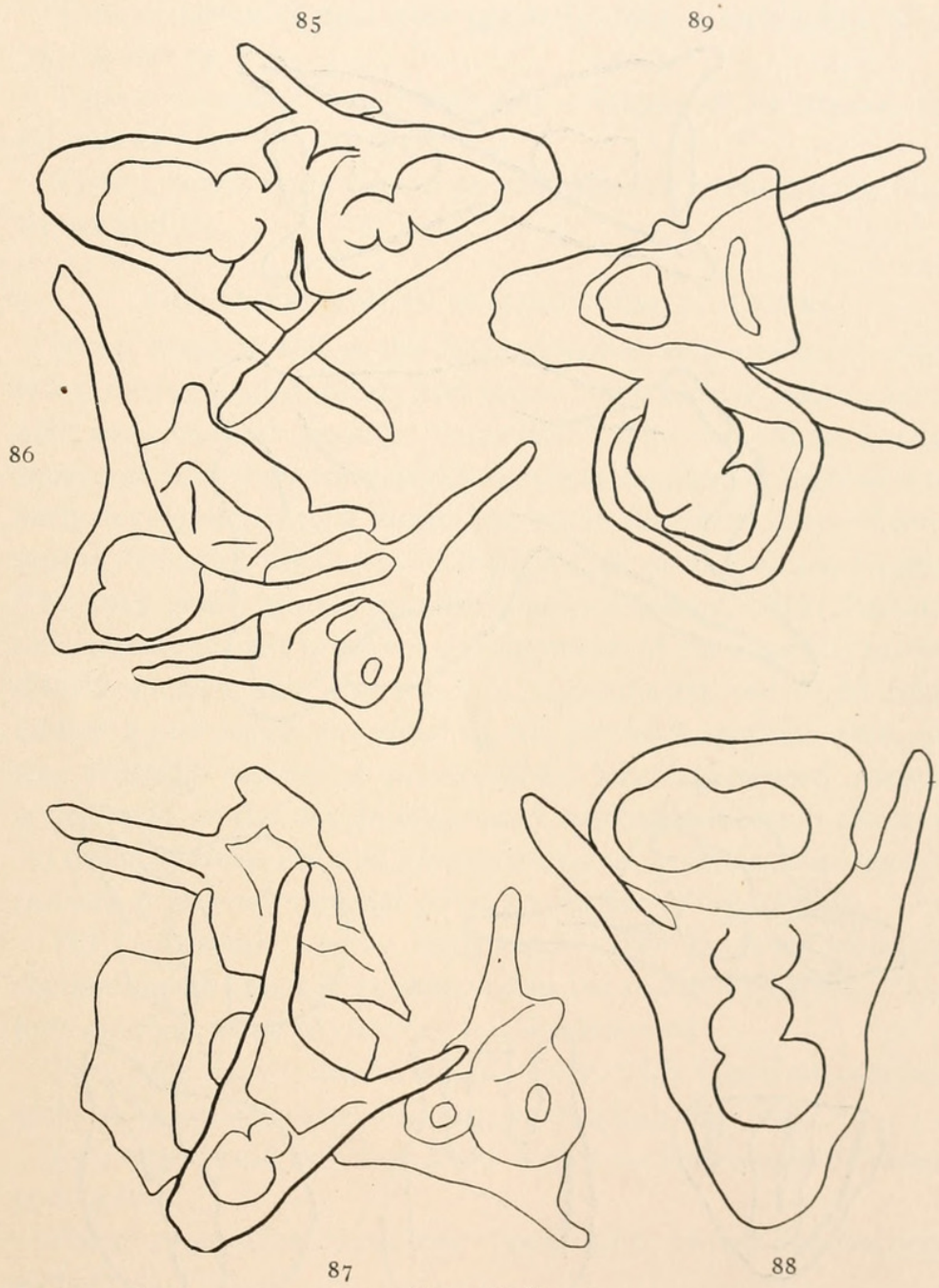
Driesch described the following types of plutei from his cultures:

(*a*) True twins, *i. e.*, agglutinated plutei.

(*b*) Twins with a common blastocœle, *i. e.*, body wall partially fused, internal organs double.

(*c*) Twins with a reciprocal influence on growth, a true fusion with an enlargement of the body.

(*d*) Fusion with partially double archenteron.
(*e*) True fusion with a single set of organs.
(*f*) Single body with a second parasitic archenteron.
Some of these types he obtained from *Echinus microtuber-*

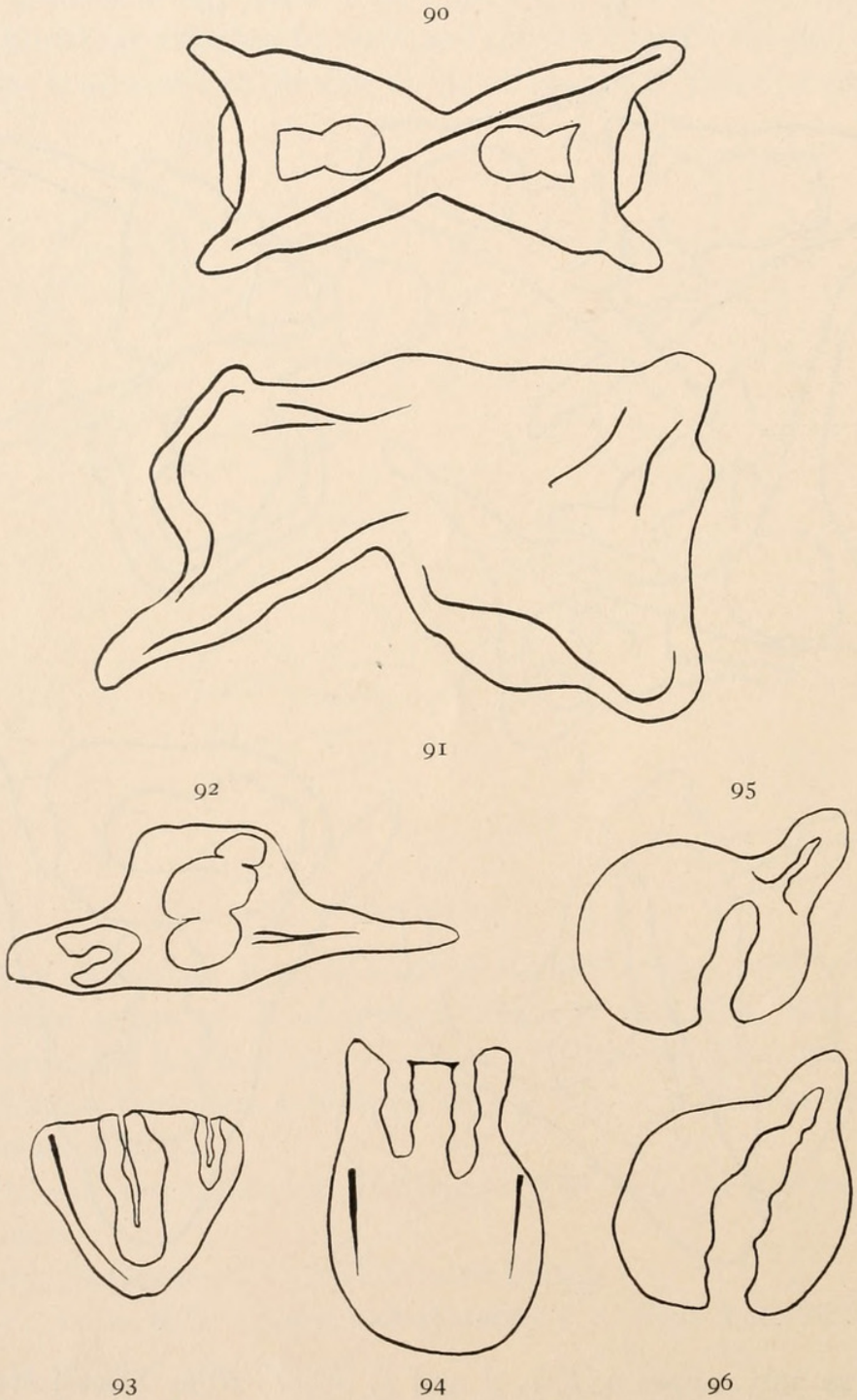


FIGS. 85–89.

culatus only, types *a*, *b*, *c*, *d* and *e*; others from *Sphærichinus granularis*, types *e* and *f*; and some from both of these echinoderms, type *e*.

Since Driesch has so clearly and accurately described these types, there is no need to enter into detail except to mention that with *Arbacia punctulata*, I obtained all of them, namely:

Type *a*, or true twins, Figs. 85, 86 and 87.



FIGS. 90–96.

Type *b*, pair of plutei with a common blastocœle, Fig. 90, drawn from life at greater magnification.

Type *c*, fusion of two, but with double set of organs, very few examples, Figs. 94 and 82.

Type *d*, fusion of two with partially double archentera, Figs. 73, 74 and 75.

Type *e*, complete fusion with but a single set of organs, the pluteus of Figs. 88 and 78.

Type *f*, single body with a second parasitic archenteron, Figs. 84, 92 and 93.

The Development of Individual Clusters.

In an effort to follow the developmental and regulative processes, during agglutination and fusion, each cluster was isolated and examined at periodic intervals. The very large groups separated into their component members, as already pointed out, each developing into a normal larva or the inner ones disintegrated bringing about the disintegration of the entire cluster. The very small groups usually remained intact. Sketches and memoranda made during the development of these small clusters clearly showed that the types of agglutinated and fused eggs, embryos and larvæ described in the previous section, were not the artificial results of preservation, but represented various serial steps in the development and regulation of such clusters. To describe these isolated groups would be to repeat the descriptions of preserved material given in the preceding section.

This statement applies with equal force to fused clusters, though not all types were observed in the isolated clusters. The following are some of the true fusions observed:

Two eggs fused into a single body with two independent archentera equal in size, Fig. 94,[1] or unequal, Fig. 93.

Two eggs fused into one body with two archentera attached end to end.

Three eggs fused into one body with three independent archentera.

Two fused eggs and one egg fused to a blastomere developed independent archentera, Fig. 95, which subsequently came in contact and fused into one very long archenteron, Fig. 94.

[1] Figs. 93 to 95 are drawn from hand sketches.

Two eggs fused into one pluteus with independent archentera, one very much larger than the other.

Two eggs fused completely into one pluteus body with one set of organs.

Other clusters showed distinct retrogressive and involutional changes of which the following are a few examples:

Four blastulæ were agglutinated in a row. The end ones enlarged, the inner ones became small. One of these small blastulæ developed an archenteron which later shrank and disappeared completely. Certain other changes occurred which altered the character of the group so that ultimately three minute blastulæ were crowded together at one point on the periphery of a large blastula.

Two large fused blastulæ were attached to a third blastula very much smaller. The three fused into one body with three independent archentera. The next day the small archenteron disappeared, skeletal rods differentiated on one side of the body constituting a fusion of a pluteus and a gastrula. Two days later the parts had fused more completely, into a single body with a single normal-sized archenteron and a single skeleton.

Two eggs developed into fused gastrulæ and later into fused plutei, attached by their oral surfaces. One of the plutei decreased in size while the other increased correspondingly, the total linear dimensions remaining constant. The smaller pluteus ultimately became less than one quarter its original size.

Summary.

Prior to this work, no one had succeeded in fusing at will the eggs of any animal found on this side of the Atlantic. By a modification of the Herbst-Driesch method, described in the text, it was possible to agglutinate and to fuse relatively large numbers, namely 10 to 40 per cent. of the sea urchin *Arbacia punctulata*.

Such clusters whether studied in mass cultures or in isolated groups, developed into all the types of larvæ described by Driesch for *Echinus microtuberculatus* and *Sphærechinus granularis*, namely:

(*a*) True twins.

(*b*) Twins with a common blastocœle.

(*c*) Twins with a reciprocal influence on their growth.

(*d*) Fusion with partially double archentera.

(*e*) True fusion with a single set of organs.

(*f*) Single body with a second parasitic archenteron.

Since little attention has been given to the early development of such agglutinations and fusions, the text emphasizes the earlier stages summarized as follows:

Clusters of 2 to 20 eggs and blastomeres were successfully agglutinated. The large clusters nearly always disappeared either by the separation of the outer members or by the death and disintegration of the inner ones. Small clusters of 2, 3 or 4 eggs or blastomeres survived and either remained agglutinated or were fused.

The eggs were agglutinated either at the egg stage or during the formation of the blastula. In clusters which remained agglutinated and did not fuse, the members developed independently, *i. e.*, the polarity was not affected by the proximity of the other, and the rate of development was not necessarily different in the individual members of a cluster.

Fusion occurred infrequently at the egg stage, but more commonly took place during the blastula or later stages. Such fusion involved either the body wall, one or more of the internal organs or all of these.

Fusion was frequently determined by the degree of compression of the component eggs or blastulæ, as well as the position and angle of attachment.

Embryos fused end to end frequently developed a single archenteron, about twice the normal size, either with or without blastopores.

Embryos fused with their axes 90 degrees apart, frequently fused in such a manner that their archentera united at the point of contact.

Embryos fused with their axes parallel and close together, frequently fused in such a manner that the two archentera fused into one, along their whole length.

The study of individual clusters served to show that these types of agglutination and fusion represented regulative and

sometimes involutional stages in the development of these clusters.

The evidence supports the view that not only may an egg give rise to several perfect larvæ, but that several eggs may be united so as to constitute a single larva, with or without traces of its duplicate nature.

In conclusion I wish to thank Prof. F. R. Lillie for placing the facilities of the Marine Biological Laboratory at Woods Hole, Mass., at my disposal.

BIBLIOGRAPHY.

Driesch, H.

'91 Entwickelungsmechanic Studien. Part 1. Der Werth der beiden ersten Furchungszellen in der Echinodermen entwickelung. Experimentelle Erzeugung von Theil und Doppelbildungen. Zeit. Wiss. Zool., Vol. 53, 1891, p. 160.

'96 Betrachtungen über die Organization des Eies und ihre Genese. Archiv für Entw., Vol. 4, 1896, p. 75.

'96 Über einige primare und secundare Regulationen in der Entwickelung der Echinodermen. Archiv für Entw., Vol. 4, 1896, p. 247.

'00 Studien über das Regulationsvermogen der Organism. Part 4. Die Verschmelzung der Individualitat bei Echinodermkeimen. Archiv für Entw., Vol. 10, 1900, p. 411.

'00 Die Isolirten Blastomeren des Echinodenkeimes. Arch. für Entw., Vol. 10, 1900, p. 361.

'10 Neue Versuche über die Entwickelung verschmolzener Echinodenkeime. Archiv für Entw., Vol. 30, 1910, p. 8.

Herbst, C.

'00 Über das Auseinandergehen von Furchungs und Gewebezellen in Kalkfreiem Medium. Arch. für Entw., Vol. 9, 1900, p. 425.

Loeb, J.

'94 Über eine einfache Methode, zwei oder mehr zusammengewachsene Embryonen aus einem Ei hervorzubringen. Pfluger's Arch., Vol. 55, 1894, p. 525.

'95 Beitrage zur Entwickelungsmechanic der aus einem Ei enstehenden Doppelbildungen. Arch. für Entw., Vol. 1, 1895, p. 453.

'95 Über die Grenzen der Theilbarkeit der Eisubstanz. Pfluger's Arch., Vol. 59, 1895, p. 379.

'08 Über den Mechanismus der Agglutination. Zeitschrift für Chemie, Vol. 3, 1908.

'09 Über Die Chemischen Bedingungen für die Enstehung Zwillinge beim Seiegel. Arch. für Entw., Vol. 27, 1909, p. 119.

Morgan, T. H.

'95 The Formation of One Embryo from Two Blastulæ. Arch. für Entw., Vol. 2, 1895, p. 65.

'95 Studies of the Partial Larvæ of *Sphærechinus*. Arch. für Entw., Vol. 2, 1895, p. 81.

Muller, Karl.

'11 Reductionserscheinungen bei Süsswasserschwammen. Arch. für Entw., 32, 1911, p. 557.

Tennent, D.

'10 Variations in Echinoid Plutei. Journ. Exp. Zoöl., Vol. 9, 1910, p. 657.

Wilson, H. V.

'11 Behavior of Dissociated Cells. Journ. Exp. Zoöl., Vol. 11, 1911, p. 281.

Zur Strassen, O.

'98 Über die Riesenbildung bei *Ascaris* Eiern. Arch. für Entw., Vol. 7, 1898, p. 642.