- Fig. 54. Fusing of yolk-lobe; $\frac{2}{4}$ embryo; from side.
 Fig. 55. Resting $\frac{2}{4}$ embryo; from side.
 Fig. 56. $\frac{4}{8}$ embryo (apparently $\frac{4}{4}$); from above.
 Fig. 57. Early $\frac{8}{16}$ ($\frac{8}{8}$) embryo; from above.
 Figs. 58—63. Cleavage of larger $\frac{1}{2}$ blastomere of unequally-dividing cooled eggs.
 Fig. 58. Undivided round $\frac{1}{2}$ blastomere.
 Fig. 59. Formation of a yolk-lobe; from side.
 Fig. 60. Fusing of yolk-lobe; early $\frac{2}{4}$ embryo; from side.
 Fig. 61. $\frac{2}{4}$ embryo.
 Fig. 62. $\frac{4}{8}$ embryo (apparently $\frac{4}{4}$ embryo); from above.
 Fig. 63. Early $\frac{8}{16}$ ($\frac{8}{8}$) stage; from above.
 Figs. 64—67. Cleavage of $\frac{1}{2}$ blastomere of equally-dividing cooled eggs. First (partial) form.
 Fig. 64. Undivided round $\frac{1}{2}$ blastomere.
 Fig. 65. $\frac{2}{4}$ embryo.
 Fig. 66. $\frac{4}{8}$ embryo; from side.
 Fig. 67. $\frac{6}{12}$ embryo; from above.
 Figs. 68—72. Cleavage of $\frac{1}{2}$ blastomere of equally-dividing cooled eggs. Second (total) form.
 Fig. 68. Undivided round $\frac{1}{2}$ blastomere.
 Fig. 69. Passage to trefoil stage; from side.
 Fig. 70. Early $\frac{2}{4}$ stage; from side.
 Fig. 71. Resting $\frac{2}{4}$ stage; from side.
 Fig. 72. $\frac{4}{8}$ embryo (apparently $\frac{4}{4}$); from above.

Appendix.

On Cleavage and Mosaic-Work.

By

Edmund B. Wilson.

Recent discussions in embryology have emphasized the need of experimental studies on the mosaic-like type of cleavage occurring in annelids and mollusks where the cell-lineage is accurately known, and many attempts have in fact been made by the writer to study the development of isolated blastomeres in *Nereis* and other annelids. Practical difficulties have thus far rendered these attempts fruitless, but Mr. CRAMPTON has had better success with the gasteropod egg. His results are based on oft-repeated

experiments, and while showing some gaps especially in the precise study of the later stages, they nevertheless supply many valuable data and I am able to vouch for their accuracy by personal observation of most of the forms he has described.

The results stand in the most striking contrast to those reached from the study of separated blastomeres in the Echinoderm, Medusa, Teleost and Amphioxus. Apart from a few unimportant details blastomeres of the 2-cell or 4-cell stage, whether isolated singly or in groups ($\frac{1}{2}$, $\frac{1}{4}$, $\frac{2}{4}$, $\frac{3}{4}$), segment precisely as if the missing portions of the egg were present and the resulting larval fragment is completely devoid not only of the power of a regulatory rearrangement of its material (*»regulatorische Umbildung«*, DRIESCH), but also of regenerative or postgenerative power. The cleavage is thus demonstrated by experiment to be in the gasteropod precisely what I have asserted that of *Nereis* to be, viz.: *»a visible mosaic-work, not one ideally conceived by a mental projection of the adult characteristics back upon the cleavage-stages. The principle of »organbildende Keimbezirke« has here a real meaning and value«*¹). It is true that the larvae arising from the isolated blastomeres are not in all cases strict partial larvae, since mesoblast-bands are never produced from the cell (*»d'«*) which represents the mesoblast pole-cell. This however is owing merely to a cessation of development on the part of this cell and it is important to observe that the cell itself is formed (from the large left-hand posterior macromere *D*) in the normal manner and divides into two symmetrical halves as in the complete embryo. With this exception, however, the partial larvae remain actual fragments of the normal forms as far as their development can be accurately traced. The gasteropod egg is thus a strict extreme, forming the last term in a series of which the Medusa, Amphioxus, the Teleost and the Echinoderm form the earlier terms, while the frog and the ctenophore occupy intermediate positions²). It is now clearly apparent that the wide divergence of opinion that appears in recent discussions on the nature of development has arisen largely through differences in the forms investigated. The study of the gasteropod alone would lead to a mosaic theory even stricter than the original hypothesis of ROUX. The study of Amphioxus or the Medusa alone would

¹) Amphioxus, pag. 613. Journ. of Morph. Vol. VIII, 3. 1893.

²) DRIESCH's and MORGAN's paper (this Archiv. II, 2. 1895) was received after the completion of Mr. CRAMPTON's work.

lead to as complete a denial of the entire principle as that to which HERRWIG was led. Both these extremes must however be brought under a common point of view; for it is certain that development must be fundamentally of the same nature throughout

Figs. 1—4. Embryos of *Nereis*.

(Roman numerals designate cleavage-planes. The circles are oil-drops.)

Fig. 1. Normal 4-cell stage. Fig. 2. Normal 8-cell stage; first quartet of micromeres above. Fig. 3. Young trochophore showing the four entomeres, each containing a large oil-drop, and the twelve cells of the prototroch. Fig. 4. Eight-cell stage produced under pressure. Dotted lines show the outlines of the micromeres formed after release from pressure.

the series, and the differences between the various forms must be of secondary moment. In returning once more to this subject¹⁾ I desire to add a few experimental data to those contained in Mr.

¹⁾ vide *Amphioxus*. Journ. of Morph. Vol. VIII, 3. 1893. pag. 604—615; also Wood's *Holl Biological Lectures*. II. 1894.

CRAMPTON's paper and to seek a clearer definition of the problems that they suggest.

DRIESCH's pressure-experiments have proved that in the echi-
noder-egg the immediate causes of differentiation are to be sought
not in the nucleus but in the cytoplasm. The same is shown in a
very convincing manner by similar experiments on the annelid egg.
In the normal development of *Nereis* the archenteron arises from
four large cells or macromeres (entomeres) which remain after the
successive formation of three quartets of micromeres (ectomeres) and
the parent-cell of the mesoblast. After the primary differentiation
of the germ-layers the four entomeres do not divide again until a
very late period (free-swimming trochophore), and their substance
always retains a characteristic appearance, differing from that of
the other blastomeres in its pale non-granular character and in the
presence of large oil-drops. If unsegmented eggs be subjected to
pressure, as in DRIESCH's echinoderm experiments, they segment in
a flat plate, all of the cleavages being vertical. In this way are
formed 8-celled plates in which all of the cells contain oil-drops
(Fig. 4). If they are now released from the pressure each of the
cells divides in a plane approximately horizontal, a smaller granular
micromere being formed above, leaving below a larger clear macro-
mere in which the oil-drops remain. The 16-cell stage there-
fore consists of eight deutoplasm-laden macromeres and eight
protoplasmic micromeres (instead of four macromeres and twelve
micromeres, as in the usual development). These embryos de-
veloped into free-swimming trochophores containing eight
instead of four macromeres which have the typical clear
protoplasm containing oil-drops¹). In this case there can be
no doubt whatever that four of the entoblastic nuclei were normally
destined for the first quartet of micromeres (Fig. 2), from which
arise the apical ganglia, and the prototroch. Under the conditions
of the experiment however they have given rise to the nuclei of
cells which differ in no wise from the other entoderm-cells. The
immediate cause of differentiation must therefore be sought not in
the distribution of the nuclei or their mode of division, but in con-

¹) This experiment was only once successfully performed and I have had
no opportunity to repeat it. Unfortunately no drawings were obtained of the
later stages, nor was the ultimate fate of the larvae determined. They had
however precisely the appearance of normal trochophores except in regard to
the entoblast.

ditions existing in the cytoplasm — i. e. in the »Plasmabau« as maintained by DRIESCH¹⁾, and here again the principle of »Organbildende Keimbezirke« has a real meaning (WILSON, l. c.), as DRIESCH in his later writings has admitted²⁾.

We are thus led anew to the view which has been urged both by HERTWIG and by DRIESCH that the various degrees of partial development beginning with the echinoderm-egg and culminating in the gasteropod may be due to varying conditions of the egg-cytoplasm in the different forms. DRIESCH in his earlier works³⁾ ascribed partial development to purely mechanical causes — namely, to an inability on the part of the partial embryo to assume the normal form of the complete embryo (blastula, etc.) due to a lack of movements among the blastomeres (»Gleiten«), to physical pressure caused by presence of the injured blastomere (frog) or to the presence

¹⁾ DRIESCH is in error in attributing to me the view that the nucleus is the immediate cause of differentiation (Analyt. Theorie d. Entwicklung. pag. 104, 105), for I expressly stated (*Amphioxus*. Journ. of Morph. VIII. pag. 607) that the location of the »idioplasm« was left »an open question«. DRIESCH not only misses the entire point of my discussion of the first unequal division of the *Nereis*-egg, but states (at pag. 104 of the Analyt. Theorie) the exact opposite of what I really said as may be seen from the following quotations:

WILSON.

»The cause of the inequality must lie in the undivided ovum The conclusion seems unavoidable that the differentiation in size is caused by a specific form of activity in the cytoplasm (or archoplasm) occurring prior to cell-division.« (Biological Lectures, Wood's Holl. II. pag. 12.)

DRIESCH.

»Da nun im Bau des Eies durchaus keine Differenz in Richtung dieser zu sehen ist so soll die Eistruktur hier nicht für die ungleiche Theilung verantwortlich gemacht werden können, sondern diese eben vom Kern, wie nach ROUX und WEISMANN, ausgehen.« (Analyt. Theorie. pag. 104.)

The misrepresentation was of course unintentional, but since DRIESCH rightly considers the case my »Hauptargument« it is unfortunate that he did not read more carefully. I regret my use of the words »idioplasm« and »germplasm«, since they were understood by DRIESCH to be synonymous with nuclear substance. The context shows however that this meaning was not intended, the words being used merely to designate the specifically organized egg-substance (whether nuclear or cytoplasmic) characteristic of the species, without any implication as to its location. This was intentionally left in doubt.

²⁾ »Gilt also für sie« (i. e. the ectomeres of *Nereis*) »wohl HERTWIG's Satz, dass sich ja naturgemäß jedes Organ rückschreitend bei genauer Betrachtung auf Furchungszellen zurückführen ließe, so gewinnt dagegen bei allen vom Stoffbau des Eies abhängigen Erscheinungen das His'sche Princip der Keimbezirke einen tieferen Sinn.« (Analyt. Theorie d. Entwicklung. pag. 101.)

³⁾ Cf. Zur Theorie der thierischen Formbildung. Biologisches Centralblatt. XIII. pag. 305.

of deutoplasm (Ctenophore). That this explanation is inadequate is proved by the case of *Ilyanassa*, where the isolated blastomere assumes a perfectly spherical form like that of the original egg, yet exhibits a strict partial development. It is still more conclusively proved by my observations on *Amphioxus* and those of O. SCHULTZE on the frog, where a complete half-sized embryo may arise from each blastomere of the 2-cell stage, even when they remain in close contact and without disturbance of their form. DRIESCH himself recognizes this in his later works, and in the extremely interesting papers on the Ctenophore egg, published jointly with MORGAN, partial development is ascribed to a »Lokalisation der ersten Formbildungsfaktoren« in the egg-cytoplasm and an inability on the part of the egg-substance (cytoplasm) to reassume the arrangement characteristic of the entire ovum. In the last paper¹⁾ the very significant admission is made that the degree of localization of the formative factors (»Grad der Lokalisation der ersten Formbildungsfaktoren«) varies considerably in different animals, the ctenophore differing in this respect »ziemlich erheblich« from the sea-urchin. This is a full recognition of the principle on which I have endeavored to lay stress in several papers, namely, that »The segmentation of the egg presents more or less of a mosaic-like character according to the period at which differentiation appears and the rate at which it proceeds as expressed in limitations of the power of development in the individual blastomeres and their differences in size and structure«²⁾. This statement is certainly inadequate and stops far short of DRIESCH's exhaustive analysis with the final results of which as expressed by himself and MORGAN at pag. 222 of their second paper³⁾ I entirely agree. I must admit, furthermore, that HERTWIG's views regarding the interaction of blastomeres, as such, which I formerly accepted, must be considerably modified; for the evidence is now overwhelmingly strong (teste *Ilyanassa* and the pressure-experiments) that cell-formation, though intimately related with differentiation, cannot be regarded as its primary cause.

The admission that the degree of differentiation or localization in the egg-substance at the beginning of development varies in different forms is one of fundamental importance, for upon it turns

¹⁾ Von der Entwicklung ungeführter Eier mit Protoplasma defekten. Archiv f. Entwicklungsmechanik. II, 2. 1895. pag. 222.

²⁾ »The Mosaic Theory of Development«. Biological Lectures. II. 1894.

³⁾ op. cit.

the entire theory of embryonic development. WHITMAN, in his brilliant essay on the »Inadequacy of the cell-theory of Development«¹⁾, has denied that such differences of degree exist and maintains, if I understand him correctly, that the egg-substance (cytoplasm) is definitely preorganized in all eggs, even before cleavage begins (l. c., pag. 109). It is not entirely easy to gather from this and subsequent essays WHITMAN's precise conception, but it seems clear that his views are only superficially similar to those of DRIESCH. For DRIESCH the specific cytoplasmic egg-organization (»Eibau«) is essentially a new formation — »der Bau des Eies ist eine Neubildung« (Analyt. Theorie, pag. 124). He does not enter specifically upon the nature of the process by which the primary »Eibau« arises, and here, as it seems to me, is a distinct gap in his general theory of development which is hardly filled by the brief statement at pag. 122 (op. cit.) that it arises »im Auflösungswege durch einen Elementarprozess wie jedes andere«. It is however a fundamental assumption of DRIESCH's theory, as far as I understand it, that cytoplasmic organization, while affording the immediate conditions of developments is itself a result, in the last analysis, of the nature of the nuclear substance which represents by its inherent composition the totality of heritable potency²⁾. Logically carried out this view inevitably involves the conclusion that the specific plasma-structure of the egg is acquired during its early history — i. e., ovarian maturation —, and I for one do not hesitate to accept this conclusion. We need not assume, for example, that the polarity of egg-cytoplasm is primordial, but that it, like every other localization of the formative factors, is acquired during ovarian development — indeed my observations on sea-urchin eggs seem to show that the establishment of polarity may in this particular case actually be deferred until after fertilization³⁾. That a pre-organization (i. e. localization of the formative factors) of the cytoplasm cannot be regarded as the primary factor in heredity is, I think, conclusively shown by the old argument based on inheritance from the father through the sperm-nucleus, an argument which has become especially convincing in the light of recent researches on the fertilization of the egg showing that the centrosome (like the remaining cytoplasm) is, in some cases at any rate, derived from one sex only. How this fact

¹⁾ Journ. of Morph. VIII, 3. 1893.

²⁾ Analyt. Theorie. pag. 81, etc.

³⁾ Journ. of Morph. X, 1. 1895.

is to be reconciled with the view that inheritance is effected by direct continuity of cytoplasmic organization from one generation to another I am at a loss to imagine.

As regards the question of partial development, MORGAN's beautiful results on the frog's egg¹⁾ following the earlier work of BORN and O. SCHULTZE, show that besides cytoplasmic localization there is an important factor to be considered in the physical consistency of the blastomeres. In some cases this is such as to admit of a rearrangement of the cytoplasmic elements upon isolation of the blastomeres, under the influence of gravity and the like (frog), so that even in the same species either whole development or partial development may occur according to circumstances. In other cases the cytoplasm is more rigid so that no rearrangement ordinarily takes place, and partial development is here the rule, as in *Ilyanassa*. Even in this case, however, as CRAMPTON shows, unusual conditions (cold) may so affect the protoplasm that the isolated blastomere may evince a slight tendency in its first stages to segment like the entire ovum. As both DRIESCH and MORGAN have insisted, however, partial development cannot be ascribed to any single cause, and the problem is not a simple one. The factors by which it is determined probably vary much in different cases and must be specially analyzed in each case.

Oct. 20, 1895.

¹⁾ Anatom. Anzeiger. X, 19. 1895.