# CLEAVAGE RATE, OXYGEN CONSUMPTION AND RIBOSE NUCLEIC ACID CONTENT OF SEA URCHIN EGGS

by

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## I. INTRODUCTION

At a given temperature the cleavage rates of the eggs of various sea urchin species may differ widely from one another. The nature of the determination of these differences in cleavage rate is not only of general interest but has also practical importance when it is recalled that genetically determined growth rate differences within a single species are reflected in the cleavage rates of the embryonic cells (c.f. PAINTER[1]).

A number of investigators have studied the cleavage rate of sea urchins' eggs, this material offering many special facilities for experimental attack. The early work of DELAGE, GODLEWSKI, NEWMAN, TENNENT and others, which gave results conflicting in many particulars, has been reviewed by MOORE[2], and the reader is referred to his account.

One line of experiment was the study, within a species, of the relative contributions made by egg cytoplasm, egg nucleus and sperm nucleus to the determination of cleavage rate.

DELAGE[3] cut virgin eggs of *Strongylocentrotus lividus* into nucleated and non-nucleated fragments: when such fragments were subsequently fertilized, the diploid fragment was found to cleave somewhat more slowly than whole egg controls, and the haploid fragment still more slowly.

WHITAKER[4], working with eggs of the starfish *Patiria miniata*, repeated DELAGE's experiment and confirmed his results. He suggested, however, that the effect might be due to inequalities of yolk distribution in nucleated and non-nucleated fragments rather than directly associated with the nuclear differences. TENNENT, TAYLOR, AND WHITAKER[5] also repeated DELAGE's experiments. In this case the material consisted of eggs of *Lytechinus variegatus*, and once more DELAGE's results were confirmed.

WHITAKER[7] went on to study the rate of cleavage of egg fragments of *Arbacia* which, prior to fragmentation, had been stratified by centrifugation. The cuts were made so as to sever the "light" end of the egg, with nucleus, from the "heavy" anucleate end. The differential distribution of various cytoplasmic materials was found to make no difference to cleavage rate, the centrifuged fragments cleaving at exactly the same rates as comparable non-centrifuged fragments. However, the delay of haploid over diploid fragments was once more confirmed (in this case diploid fragments were found to cleave slightly faster than whole egg controls), and WHITAKER concluded that the nuclear/cytoplasmic ratio is a determining factor in cleavage rate. However, the differ-

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ences of rate observed by WHITAKER were in fact very small indeed (of the order of 6 min in 50), and later evidence, which we shall now consider, indicates that his conclusions were not justified.

MOORE[2] has made a very elegant experimental analysis which has exposed the roots of the problem. Advantage was taken of the fact, already mentioned, that the eggs of different species of sea urchins cleave at different rates. The eggs of *Strongylocentrotus franciscanus* at 20° C take approximately 95 min to pass from fertilization to the first cleavage, and succeeding divisions take about 47 min each. The eggs of *Dendraster excentricus* divide nearly twice as rapidly, the first cleavage occurring after 57 min, and the interval between subsequent divisions being approximately 28 min. When MOORE crossfertilized these two species he found that the cleavage rate of the hybrids was precisely that characteristic of the egg and independent of the sperm. This experiment fails to differentiate between the contribution of the egg cytoplasm and egg nucleus. To answer this question, MOORE cut unfertilized eggs into pieces and then made cross-fertilizations. All such fragments, with or without the egg nucleus, were found to cleave at the rate characteristic of the original eggs. These experiments show not only that it is the egg cytoplasm which determines cleavage rate, but also that this rate is, at least within certain limits, independent of egg size. MOORE interprets WHITAKER's results as being ascribable to slight inequalities in the distribution of some critical cytoplasmic material between haploid and diploid egg fragments.

The question which next presents itself is whether by means of experiment, a factor determining cleavage rate can actually be located within the egg cytoplasm. Such a substance had already been envisaged by LOEB AND CHAMBERLAIN[6] in their attempt to provide a physico-chemical explanation for inter-egg variability of cleavage rate within a single species. The centrifuge experiments of E. B. HARVEY[8, 9] provide relevant information. Under the action of centrifugal force *Arbacia punctulata* eggs stratify into five layers: oil, clear layer, granular layer (the granules being identified as mitochondria), yolk layer and pigment. The oil cap is at the centripetal, the pigment at the centrifugal end of the egg, while the nucleus lies in the clear layer. With stronger centrifuging the stratified eggs may be pulled apart into "lighter" and "heavier" half-eggs, the lighter half containing oil, nucleus, clear layer and part of the granular layer, the heavier half containing the rest of the granular layer together with yolk and pigment.

The light half-eggs, when fertilized, cleave at approximately the same rate as whole eggs, though occasionally the rate is perceptibly higher. The heavy half-eggs, when fertilized, undergo cycles of nuclear division which are somewhat delayed as compared with whole eggs. These nuclear divisions are not at first accompanied by cytoplasmic cleavage; however later the cytoplasm splits up between the nuclei. HARVEY recentrifuged the light half-eggs, which then separate into quarter-eggs, the centripetal quarter-egg containing oil, nucleus and part of the clear layer, while the centrifugal quarter-egg contains the rest of the clear plus the granular layer. When these quarter-eggs are fertilized the nucleated quarter cleaves exceedingly slowly: the granular quarter, on the other hand, cleaves at nearly the same rate as whole eggs. HARVEY[9] found the same rules to hold for centrifuged eggs of *Arbacia lixula* and *Sphaerechinus granularis*. The situation is, however, somewhat different in two other species *Paracentrotus lividus* and *Psammechinus microtuberculatus*, which do not stratify in the same order as do *Arbacia* and *Sphaerechinus*.

MOORE[10] applied HARVEY's centrifuge technique to the eggs of *Dendraster excen-*

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*tricus*. The eggs were stratified and pulled into dumb-bell shape but not completely split into fragments. They were then activated parthenogetically. In these stratified eggs the anucleate centrifugal end always cleaved in advance of the nucleate centripetal end, while in some egg batches so treated, no cleavage of the centripetal end took place at all. Topographically speaking, MOORE's results are the antithesis of HARVEY's results with *Arbacia* and *Sphaerechinus*, but this may well be due to differences in the order of stratification of the various cytoplasmic components. On the basis of HARVEY's results and his own, MOORE concludes that under the action of centrifugal force a "cleavage-substance" can be differentially distributed through the egg cytoplasm and that the rates of cleavage of the various egg regions or fragments are then determined by the relative concentrations of this substance.

However, one point which emerges from the work of HARVEY AND MOORE has been, in my opinion, somewhat overlooked by the latter author. If the word "cleavage-substance" is used to define some stratifiable material of the egg cytoplasm whose concentration is the limiting factor determining normal cleavage rate, then it should be possible not only to decrease its concentration, thereby decreasing the rate, but also to increase its concentration, in which case a faster rate should result. Cleavage rate can undoubtedly be reduced by stratification: but at the same time there is no experimental evidence for a clear cut and striking increase in cleavage rate in any way comparable to the interspecific differences which exist in nature. In the absence of this evidence the reduction of cleavage rate observed in egg fragments and regions produced by centrifugation is better envisaged as the imposition of a new limiting factor rather than the differential distribution of a factor limiting the normal cleavage rate in the entire egg. I therefore suggest that the use of the word "cleavage substance" be discontinued or at least reserved until such time as a normal limiting factor is discovered.

There remains another line of work which bears directly on cleavage rate determination and which we must consider before turning to the experimental results reported in this paper. HÖRSTADIUS[11] has shown that the winter and summer eggs of *Paracentrotus lividus* cleave at different rates when exposed to the same temperature. At 13° C the winter eggs cleave faster than the summer eggs. At 26° C the summer eggs cleave faster than the winter eggs. This phenomenon, which may be best described as acclimatization to the normal environmental temperature, has also been studied by FOX[12, 13] from a somewhat different point of view. FOX determined the cleavages rates of closely related species of *Psammechinus* and the same species of *Paracentrotus* from different localities. He found that at a given temperature (ca. 20° C) the material deriving from colder waters cleaved more rapidly than that deriving from warmer waters.

It will thus be clear that although there are specific differences in cleavage rate, these rates are not rigidly controlled and may undergo adaptation. HÖRSTADIUS has suggested that this adaptation involves alterations in the dispersion of the protoplasmic colloids.

## II. CLEAVAGE RATES OF THE NEAPOLITAN SEA URCHIN SPECIES

*Psammechinus microtuberculatus* (Blainv.), *Sphaerechinus granularis* (Lam.), *Arbacia lixula* (L.) and *Paracentrotus lividus* (Lam.) are four species of sea urchins which occur commonly in the Gulf of Naples. For the purposes of the experiments to be described in this paper, these species were used during the months of March and April 1947.

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TABLE I

| Species | Individual | Time in minutes between: fertilization and first cleavage | first and second cleavage | second and third cleavage |
|---|---|---|---|---|
| *Psammechinus microtuberculatus* (Blainv.) | A | 58 | 38 | 34 |
| | | 58 | 37 | 35 |
| | B | 63 | 37 | 33 |
| | | 62 | 37 | 34 |
| | C | 63 | 37 | 36 |
| | | 62 | 39 | 37 |
| | Averages | 61.0 | 37.5 | 34.8 |
| *Paracentrotus lividus* (Lam.) | A | 74 | 43 | 43 |
| | | 74 | 43 | 44 |
| | B | 79 | 43 | 45 |
| | | 79 | 43 | 45 |
| | C | 76 | 42 | 45 |
| | | 77 | 43 | — |
| | D | 74 | 43 | 44 |
| | | 75 | 42 | 44 |
| | Averages | 76.0 | 42.7 | 44.3 |
| *Arbacia lixula* (L.) | A | 97 | 54 | 52 |
| | | 100 | 53 | 55 |
| | B | 95 | 52 | 57 |
| | | 97 | 52 | 58 |
| | C | 102 | 53 | 55 |
| | | 105 | 57 | 54 |
| | D | 99 | 56 | 55 |
| | | 100 | 56 | 55 |
| | Averages | 99.4 | 54.1 | 55.1 |
| *Sphaerechinus granularis* (Lam.) | A | 103 | 57 | 56 |
| | | 101 | 57 | 58 |
| | B | 99 | 59 | 54 |
| | | 100 | 58 | 52 |
| | C | 99 | 58 | 57 |
| | | 101 | 57 | 55 |
| | Averages | 100.5 | 57.7 | 55.3 |

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Eggs and sperm were collected by clipping away the oral half of adult specimens and inverting the aboral half over bowls containing filtered sea water. This method of egg collection is preferable to the excision of whole ovaries, as the egg batches so obtained contain hardly any immature ocytes.

The egg batches were repeatedly rinsed with large quantities of clean sea water, and trial inseminations were made about one hour after shedding. Occasionally, for reasons which are not apparent, seemingly mature eggs cannot be fertilized. Such egg batches were rejected. When trials showed that effectively 100% fertilization followed insemination, small quantities of eggs from the same parent batch were transferred to 100 ml Erlenmeyer flasks containing about 40–50 ml of clean sea water: the flasks were then placed in a water bath maintaining a temperature of 18° ± 0.2° C. The temperature of the circulating sea water of the laboratory was at this period about 13° C. After equilibrating for half an hour, small quantities of sperm were pipetted into the flasks, and the contents momentarily agitated to ensure even distribution of sperm and eggs.

The rate of development of the fertilized eggs was followed at regular interval by pipetting out a small sample and examining it under the microscope. In the conditions of the experiment outlined above, the rate of development is remarkably regular; no attempt was therefore made to treat the material statistically. The time of the first cleavage was taken as being that where 50% of the eggs in any one sample showed a well-marked cytoplasmic furrow, the remaining 50% being uncleaved. A similar criterion was used for the second and third cleavage stages. In Table I the time intervals between fertilization and the first, second and third cleavages are tabulated to the nearest minute. Any one entry would require the figures ± 2 to 3 min in order to include practically all of the slowest and fastest cleaving eggs from the batch in question. The figures show that *Psammechinus* is the fastest cleaving species, *Arbacia* and *Sphaerechinus* cleave most slowly and at about the same rate as one another, while *Paracentrotus* falls between the two extremes.

In a short series of subsidiary experiments, *Psammechinus* at 13° C was found to cleave at the same rate as *Arbacia* and *Sphaerechinus* at 18° C. However, the converse experiment was not possible: 28° C proved to be the upper limit of temperature for *Sphaerechinus* egg development, and at no temperature below this limit does *Sphaerechinus* cleave as rapidly as does *Psammechinus* at 18° C.

### III. OXYGEN CONSUMPTION DURING CLEAVAGE

GRAY[14] has stated that the rate of cell division during segmentation of the egg of *Echinus miliaris* bears no obvious relationship to the rate of metabolism during this process. Thus, though the early cleavage divisions occur after equal intervals of time, the cleavage rate being therefore constant, the rate of oxygen consumption slowly rises as more and more reserve material is incorporated into the respiring protoplasm.

However, despite the absence of a direct correlation between cleavage and metabolic rate, we are bound to admit that the two processes are very intimately related to some common denominator. This conclusion derives from the experiments of EPHRUSSI[15] and TYLER[17] who studied the oxygen consumption and developmental rates of various echinoderm eggs at different temperatures. These authors found that the total quantity of oxygen consumed in reaching a given developmental stage is the same at different temperatures. In other words, the temperature coefficients of cleavage and respiration

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are identical, and the two processes cannot be dissociated by varying the culture temperature, provided that this remains within the physiological range.

The following experiment was designed in order to determine whether the relationship between cleavage and metabolic rate within a species established by EPHRUSSI AND TYLER can be extended to cover related species whose cleavage rates differ.

Eggs from a number of females of one of the four sea urchin species were collected. Samples were tested for maturity by trial inseminations, and those eggs batches which passed the test were pooled together, washed in filtered sea water and fertilized in bulk. The fertilized eggs were then thoroughly washed four or five times with fresh sea water and finally concentrated in about 40 ml volume. The concentration was checked roughly by centrifuging a small sample.

The final egg suspension was thoroughly mixed and pipetted, 3 ml at a time, into five WARBURG respirometer vessels. Aliquot volumes of the same suspension were pipetted into three centrifuge tubes and three KJELDAHL flasks.

The central chambers of the WARBURG respirometer vessels were fitted with filter-paper rolls wetted with 6 drops of 5 N potassium hydroxide, and the vessels attached to their respective manometers. They were then placed on rocking racks in a thermostatically-controlled water bath at 18° C and rocked gently during the course of the observations. The first manometer readings were taken two hours after the time of fertilization and continued at ten minute intervals for a further two and a half hours. At the end of each run the eggs were checked for regularity of development. Out of over twenty separate determinations of oxygen consumption, two were rejected on the score of developmental irregularities.

The manometric readings were plotted graphically, after conversion to volumes at N.T.P., as total oxygen consumption against time. The curves so obtained (cf. GRAY[17]) are effectively linear over the time range 2–4 ½ hours after fertilization and were treated as such.

Aliquot volumes of egg suspension pipetted into centrifuge tubes were to serve for dry weight determinations. Since the eggs of sea urchins are surrounded by a mucilaginous jelly-coat which otherwise makes a considerable "dead weight" contribution to dry weight, the jelly-coats were removed by suspending the eggs for fifteen minutes in 0.52 M sodium chloride solution. This treatment, coupled with mechanical agitation, is generally adequate. The stages in the removal of the jelly-coat can be readily observed under the microscope if a small quantity of Indian ink be added to the fluid in which the eggs are lying. I am indebted to Dr. MONROY, of the Stazione Zoologica, for details of this useful technique (cf. MONREY AND RUFFO[18]).

The eggs, freed from their jelly-coats, were again suspended in sea water, and then strongly centrifuged. The supernatant water was removed at the pump and all but the last traces absorbed at the sides of the centrifuge tubes by filter paper. The eggs and tubes were then dried to a constant weight in an oven at 110° C. Owing to the impossibility of removing all sea water before drying the material, the dry weight determinations are slight overestimates of the true dry weight of eggs. However, the salt error involved has been calculated to represent less than 5% of these determinations.

The aliquot volumes of egg suspension in KJELDAHL flasks were also treated so as to remove the jelly-coats from the eggs. Total nitrogen determinations were subsequently made by a micro-KJELDAHL method.

The results of this experiment are given in detail in Table II. It will be seen that

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TABLE II

| Species | Oxygen consumption per hour (μl) | Aliquot dry weight determinations (mg) | Oxygen consumption per hour per 100 mg dry weight (μl) | Aliquot total nitrogen determinations (mg) | Oxygen consumption per hour per 1 mg total nitrogen (μl) |
|---|---|---|---|---|---|
| *Psammechinus microtuberculatus* (Blainv.) | 2.50<br>2.66<br>2.54<br>2.79<br>2.37 | 44<br>46<br>50<br>52 | | 2.59<br>2.95<br>2.98 | |
| Averages | 2.57 | 48 | 5.35 | 2.84 | 0.90 |
| *Paracentrotus lividus* (Lam.) | 3.13<br>3.18<br>3.51<br>3.45<br>3.16 | 66<br>71<br>75 | | 4.58<br>4.88 | |
| Averages | 3.29 | 71 | 4.63 | 4.73 | 0.70 |
| *Arbacia lixula* (L.) | 3.55<br>3.34<br>3.80<br>3.37 | 94<br>94<br>96<br>96 | | 6.13<br>6.55<br>6.61 | |
| Averages | 3.51 | 95 | 3.70 | 6.43 | 0.55 |
| *Sphaerechinus granularis* (Lam.) | 4.11<br>4.69<br>4.49<br>4.43 | 94<br>101<br>102 | | 6.40<br>6.52 | |
| Averages | 4.43 | 99 | 4.47 | 6.46 | 0.69 |

TABLE III

| Species | Average time interval between successive cleavages (min) | Oxygen consumption per hour per 100 mg dry weight (μl) | Oxygen consumption between successive cleavages per 100 mg dry weight (μl) |
|---|---|---|---|
| *Psammechinus* (18° C) | 36.1 | 5.35 | 3.22 |
| *Psammechinus* (13° C) | 60.0 | 3.18 | 3.18 |
| *Paracentrotus* (18° C) | 43.2 | 4.63 | 3.34 |
| *Arbacia* (18° C) | 54.6 | 3.70 | 3.37 |
| *Sphaerechinus* (18° C) | 56.5 | 4.45 | 4.19 |

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*Psammechinus* respires most rapidly of the four species, *Arbacia* most slowly, while *Paracentrotus* and *Sphaerechinus* lie between the two extremes and have equal rates of respiration.

In Table III the cleavage and respiration rates of the four species are set beside one another together with the computed quantities of oxygen consumed between two successive cleavages. The data from a single respiration determination for *Psammechinus* at 13° C is also included. (At this temperature *Psammechinus* cleaves at the same rate as do *Arbacia* and *Sphaerechinus* at 18° C). While *Psammechinus*, *Paracentrotus* and *Arbacia* absorb comparable amounts of oxygen between successive cleavages per unit of dry weight, *Sphaerechinus* is out of line in having a considerably higher oxygen requirement.

The exceptional behaviour of *Sphaerechinus* bears out GRAY's contention that there is no direct and simple correlation between metabolic and cleavage rates. It may be that this species possesses proportionately less non-respiring reserve materials. Nevertheless, the identity in oxygen requirement of the other three species when performing a comparable act of development should not be overlooked since it may indicate that, other things being equal, the metabolic cost of cleavage is proportional to its rate.

## IV. THE RIBOSE NUCLEIC ACID CONTENT OF SEA URCHIN EGGS

On the basis of HARVEY's experiments with half- and quarter-eggs produced by centrifugal force, MOORE has argued that the mitochondrial granules may represent the "segmentation stuff" of LOEB AND CHAMBERLAIN, whose concentration in the cytoplasm determines cleavage rate. In the introduction to this present paper the logic of MOORE's argument has been questioned: however, since ribose nucleic acid has been shown by BRACHET and by CASPERSSON to be a characteristic component of rapidly dividing tissues, (see BRACHET[19]) and since CLAUDE[20] has put forward the view that the granules rich in ribose nucleic acid are, in fact, the mitochondria of cytologists, I thought it of some interest to determine the relative concentrations of ribose nucleic acid in the virgin eggs of the four neapolitan sea urchin species.

The determinations were made according to the method of BRACHET[22]. Mature virgin eggs were obtained from a number of females. The eggs were thoroughly washed in clean sea-water, and the jelly -coats removed by the method already described. The eggs were then washed once more, concentrated into a small volume of sea water and fixed in a 10% solution of trichloroacetic acid. After prolonged extraction in three changes of this solution the eggs were pipetted on to weighed fragments of coverslips and dried out to constant weight at 110° C. Neutral fats were then removed by extraction in hot ether, and the resultant material dried and weighed.

The weighed samples were then hydrolysed and steam distilled in a sulphuric acid-zinc sulphate-potassium sulphate mixture, as described by BRACHET: the furfural content of the resulting distillate was subsequently determined by means of a PULFRICH photometer, use being made of the coloured product formed between furfural, aniline and acetic acid. Among the various sources of error involved in this technique, the following are noteworthy:

a. Jelly-coats must be completely removed before fixation of the eggs, not only because they contribute to the figures of dry weight, but also because they generate furfural on hydrolysis.

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b. Prolonged extraction in trichloro-acetic acid is necessary to remove soluble sugars and glycogen which would otherwise also yield furfural.

c. Failure to extract neutral fat leads to turbidity in the distillate and hence to inaccuracies in the colorimeter readings.

The results of the determinations are given in detail in Table IV. (For the calculation of ribose nucleic acid content, 1303 parts by weight of this substance are taken as yielding 384 parts by weight of furfural).

From these figures it is evident that the content of ribose nucleic acid cannot be the sole or even the overriding determinant of the cleavage interval. This does not exclude the possibility that it plays a role in the whole physiological system on which cleavage depends, but for the solution of this question, interspecific comparisons are not an adequate method.

TABLE IV

| Species | Dry weight of ether extracted virgin eggs (mg) | Yield of furfural ($\gamma$) | Estimated ribose nucleic acid (mg per g) |
|---|---|---|---|
| *Psammechinus microtuberculatus* (Blainv.) | 51.4 | 19.6 | 1.30 |
| | 41.5 | 19.2 | 1.57 |
| | 50.1 | 21.2 | 1.44 |
| | 39.1 | 16.6 | 1.44 |
| | 37.2 | 15.1 | 1.37 |
| | | | Average 1.42 |
| *Paracentrotus lividus* (Lam.) | 39.6 | 11.0 | 0.94 |
| | 39.1 | 9.0 | 0.78 |
| | 38.4 | 8.5 | 0.76 |
| | 39.7 | 11.1 | 0.95 |
| | 37.7 | 9.6 | 0.86 |
| | | | Average 0.86 |
| *Arbacia lixula* (L.) | 39.4 | 11.2 | 0.97 |
| | 40.4 | 12.4 | 1.04 |
| | 36.5 | 10.9 | 1.01 |
| | 41.8 | 12.1 | 0.98 |
| | | | Average 1.00 |
| *Sphaerechinus granularis* (Lam.) | 41.0 | 16.4 | 1.36 |
| | 39.8 | 14.0 | 1.20 |
| | 51.9 | 21.2 | 1.39 |
| | 32.6 | 13.1 | 1.37 |
| | 26.8 | 10.9 | 1.38 |
| | 27.8 | 11.8 | 1.44 |
| | | | Average 1.36 |

## V. THE ORDER OF STRATIFICATION OF THE CYTOPLASMIC COMPONENTS

In the preceding parts of this paper it has been shown that differences in cleavage rate of sea urchin eggs cannot be directly related either to differences in total

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metabolic rate or to differences in the initial ribose nucleic acid content prior to cleavage.

There remains, however, another property of these sea urchin eggs which differs in a graded way between the species, and the seriation is in accordance with the seriation of the cleavage rates.

HARVEY[9], by means of the centrifuge technique, was able to show that the cytoplasmic components of the eggs stratify in different order in different species. I have repeated her experiment and have obtained similar results. The order of stratification is shown diagrammatically in Fig. 1. Working from the centripetal to the centrifugal ends of the eggs, the fast-cleaving *Psammechinus* egg is stratified into oil, yolk (with nucleus), clear zone and mitochondria; the *Paracentrotus* egg into oil, clear zone (with nucleus), yolk and mitochondria: the slow cleaving *Arbacia* and *Sphaerechinus* eggs into

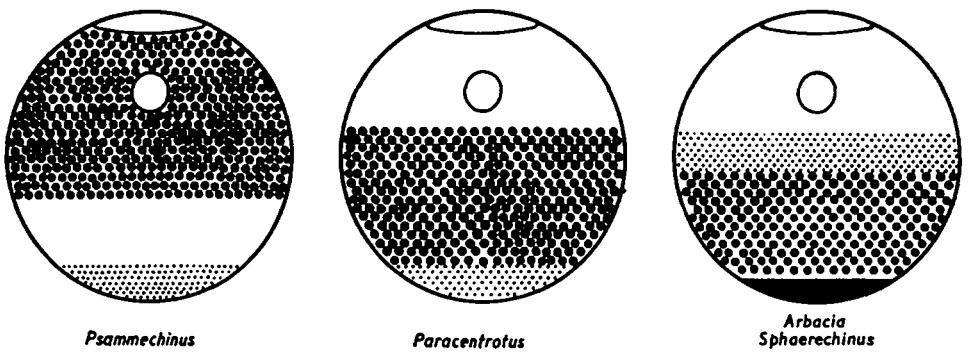


Fig. 1. Diagrammatic representation of the stratified cytoplasmic components of sea urchins' eggs: centripetal pole, with oil cap, is uppermost; coarse granules are yolk, fine granules mitochondria; the black area represents pigment in *Arbacia* and "heavy" clear cytoplasm in *Sphaerechinus*.

oil, clear zone (with nucleus), mitochondria and yolk. Thus the relative density of the yolk granules is least in *Psammechinus*, greatest in *Arbacia* and *Sphaerechinus* with *Paracentrotus* occupying the intermediate position.

It would be entirely premature to discuss this possible correlation, which may be purely fortuitous, before appropriate experiments have been undertaken to test its validity. A direct correlation between relative yolk density and cleavage rate is improbable, considering what is known of the cleavage rates of non-yolky half-and quarter-eggs. However the correlation may well be an indirect one, depending on the density or some linked property of another of the stratifiable cytoplasmic components.

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## SUMMARY

1. There are striking differences in the cleavage rates of the eggs of four neapolitan sea urchin species.

2. These cleavage rates are not directly correlated with differences in the rate of oxygen consumption of the eggs during the early cleavage stages, nor

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3. are they correlated with differing ribose nucleic acid concentrations in the virgin eggs.
4. There is a hint that cleavage rate differences may be correlated with variations in the order of stratification of the cytoplasmic constituents when eggs are subjected to centrifugal force.

## RÉSUMÉ

1. Il existe des différences frappantes dans les vitesses de division des œufs de quatre espèces napolitaines d'oursins.
2. Ces différences ne correspondent ni à des différences dans les intensités de consommation d'oxygène au cours des premiers stades de division,
3. ni à des différences dans les concentrations des œufs vierges en acide ribonucléique.
4. L'action de la force centrifuge semble montrer que ces différences de vitesse de division dépendent de variations dans la stratification des constituants cytoplasmiques.

## ZUSAMMENFASSUNG

1. In den Spaltungsgeschwindigkeiten der Eier von vier napolitanischen Seeigelarten treten starke Unterschiede auf.
2. Diese Spaltungsgeschwindigkeiten hängen nicht direkt mit Unterschieden in der Rate des Sauerstoffverbrauchs in den frühen Spaltstadien zusammen, noch
3. sind sie von verschiedenen Ribosenukleinsäurekonzentrationen in den unbefruchteten Eiern abhängig.
4. Es sind Anzeichen vorhanden, dass die Spaltungsgeschwindigkeitsunterschiede von Variationen in der Streckungsordnung der Zytoplasmabestandteile, wenn die Eier der Zentrifugalkraft unterworfen werden, abhänging sein könnten.

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