

The Air Turbine Ultracentrifuge, together with some Results upon Ultracentrifuging the Eggs of *Fucus serratus*.

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With Plate II and 8 Figures in the Text.

INTRODUCTION.

GENERALLY, the force of gravity has little or no effect upon the distribution of the various materials within cells, although they may differ considerably in their relative specific gravity. However, by means of the centrifuge forces can be obtained many times greater than gravity, which otherwise exist only on the very largest planets. Because of this fact, the centrifuge has proved to be a very valuable instrument in the study of many problems of biology. For instance, in experimental cytology and embryology, the subjects with which we are here concerned, it has been used extensively to bring about a redistribution of the various materials within the animal egg, such as the yolk, the pigment, the protoplasm and the fat. From such experiments the role of these various substances as possible organ-forming materials and as affecting cellular differentiation has been studied. Other problems such as fragmentation of the egg, viscosity of the protoplasm, membrane strengths, influence of gravity upon development, differential injury to eggs, molecular weight determinations, polarity, and the cytoplasmic components and inclusions have all been profitably investigated by means of the centrifuge.

The types of devices that have been used to generate centrifugal force in biological experiments are many and varied. For instance, we find a gradual evolution from a simple wagon-like wheel used by Knight in 1815 to study the effect of centrifugal force upon developing plants, to various types of hand centrifuges with high gear ratios, to the fly-wheel of an engine as used by Morgan (1902), to electric and water driven centrifuges, to the Sharples supercentrifuge which develops forces upward to 62,000 times gravity, to the Svedberg oil turbine centrifuge capable of developing forces upward to 400,000 times gravity, and finally to the recently

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developed air turbine ultracentrifuge, constructed of air spun rotors without bearings which give rotations of several thousand times a second and develop forces of the order of 400,000 to 7,000,000 times gravity. Forces developed by such centrifuges are limited mainly by the tensile strength of the metal of which the rotors are made. It is this latter type of ultracentrifuge that has been used in the experiments to be described below.

THE ULTRACENTRIFUGE.

Since the air turbine ultracentrifuge has only recently been applied to studies in biological work and since it promises to have a rather wide application in biological experiments, it seemed desirable to give illustrations and a brief description of it here. I shall not attempt to give a detailed explanation of the various parts of the ultracentrifuge, but only to point out its important characteristics so that the reader may have a fair understanding of its construction and operation. Those desiring to construct the apparatus will find complete specifications in the papers by Beams (1930), Beams and Weed (1931), Beams, Weed and Pickels (1933), and Beams and Pickels (1935). The cost of constructing the ultracentrifuge is very low in comparison with that of other types of high speed centrifuges.

The first investigators to use the air turbine for centrifuge work seem

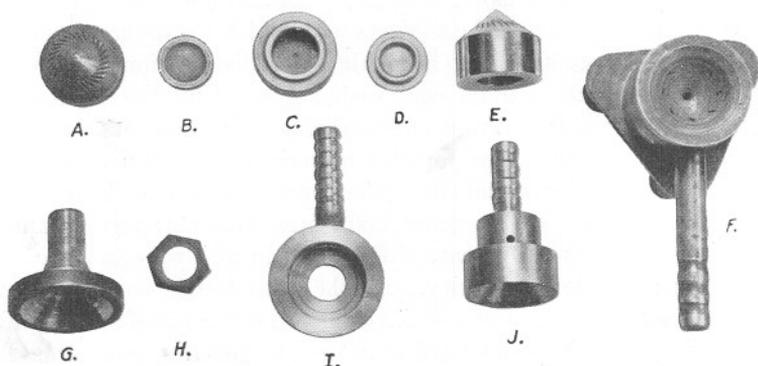


FIG. 1.—The ultracentrifuge. A, bottom of rotor cone showing flutings; B, top of cap which screws into rotor from above; C, top of rotor showing centrifuge cavity; D, bottom of cap as in B; E, side view of rotor; F, assembled stator showing stator cup and eight diagonal holes; G-I, unassembled parts of stator; J, stator with air entering from below instead of from side, as in F.

to have been Henriot and Huguenard (1927). However, it has been greatly modified and developed into a practical laboratory apparatus by Professor J. W. Beams and his associates of the University of Virginia.

The ultracentrifuge which I have used is composed of two principal

parts: the rotor and the stator (Figs. 1, 2, and 3). The rotor is a small, one and one-eighth inch diameter, all metal (steel, brass, duraluminum, monel metal) structure, shaped like a schoolboy's top, but with a cavity in the centre which holds approximately 4 c.c. of fluid (Fig. 1, A-E). However, rotors may be constructed which hold many times this amount. A small screw cap seals the centrifuge cavity from above (Fig. 1, B and D). The lower portion, or cone-shaped part of the rotor, which is grooved with a series of flutings is mounted in a cup, the stator (Fig. 1, F), also of conical shape, but of a slightly different angle so that the rotor cone touches the

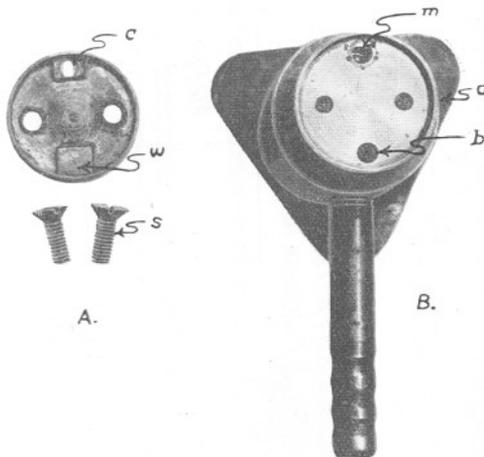


FIG. 2.—The microscope ultracentrifuge. A, detachable top showing centrifuge chamber (c) above and balance weight (w) below; machine screws (s) fasten detachable top into position on rotor in B; B, upper view of rotor (a) resting in stator and showing tube leading to mirror (m) with counter balance tube (b) below.

stator cup when not running at its top only, which is also its largest diameter (Figs. 2, B; 3, A). To start the ultracentrifuge the rotor is placed in the cup of the stator and air under pressure (2 to 150 lb. per sq. inch) is released and passes through the tube labelled "air pressure" into the stator chamber and then through the eight diagonal holes into the stator cup where it impinges on the flutings of the rotor and starts it rotating (Fig. 3, A). The air that is used to cause rotation then escapes between the surface of the rotor cone and of the stator cup, floating the rotor on a cushion of air just above the stator cup, entirely free of any mechanical contact. At the same time that air under pressure is escaping between the surfaces of the stator and the rotor, air is entering from the atmosphere through a small hole at the vertex of the stator because of the reduced pressure at this point (Fig. 3, A, labelled "stabilising air flow"). This, according to Beams and Pickels (1935), greatly improves

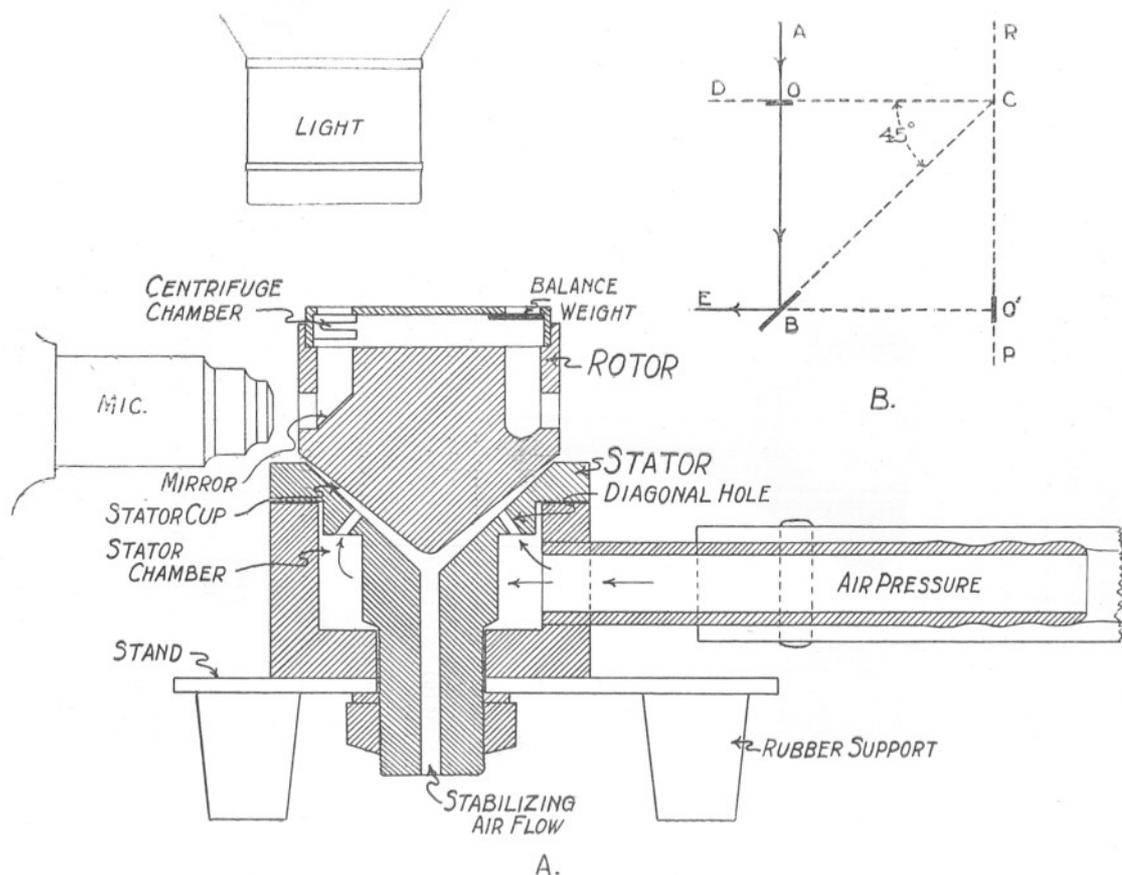


FIG. 3.—A, section through microscope ultracentrifuge. Labels are self-explanatory. B, diagram showing the optical system of the rotor in A (modified from Pickels). The centrifuge chamber has a diameter large in comparison with its thickness. "B, is a small plane mirror mounted on the rotor. Light from a straight filament lamp is focused upon O so that the image of the filament lies along CD and consequently transverse to the direction of the motion of the object thus illuminated. As O then revolves about RP, it becomes visible to the naked eye or in a microscope only as it passes through the indicated position. Consequently, as viewed from E the virtual image O will appear stationary and under apparently continuous illumination when the speed is high enough to prevent flicker" (Pickels, 1936).

stability and helps to adjust automatically the air cushion for different air pressures, speeds, and weights of rotors. This is an important improvement over the first models in which the rotors were supported by a separate

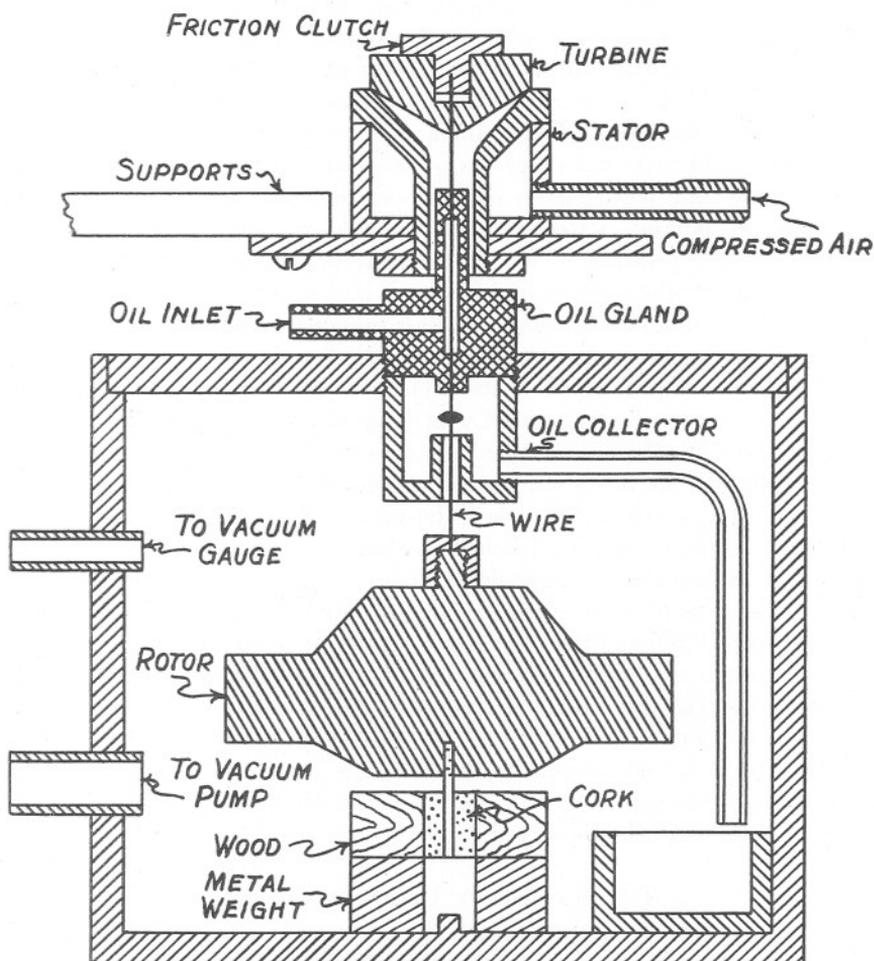


FIG. 4.—Air turbine vacuum ultracentrifuge (redrawn from Beams and Pickels, 1935). In this design the driving mechanism is simply an air turbine rotor as above described (Fig. 1). From the vertex of the driving rotor or turbine a small steel wire extends downward through an oil gland into a vacuum chamber and is attached to a much larger rotor which spins in the vacuum chamber where the material to be centrifuged is placed. Since the rotor which spins in the vacuum chamber is of much greater diameter than the turbine rotor, enormous centrifugal forces may be obtained.

column of air from that which was used to cause rotation. The rotor is prevented from being blown out of the stator cup by the principle of Bernoulli. The forces involved in this principle are so great at high

pressures that the entire ultracentrifuge may be inverted without the rotor falling out of the stator; because of this fact, too, it is not essential that the rotor be exactly balanced. Another important feature of this type of ultracentrifuge, especially in biological work, is that the temperature does not vary in the rotor chamber over a few degrees from that of the atmosphere even though the rotor may be operating at very high speeds.

The speed of the rotor may be accurately and easily determined by the stroboscopic method and the amount of centrifugal force developed calculated. The size and shape of the rotors are subject to wide variation and Beams and Weed (1931) have described rotors in which materials may be introduced, separated, and the lighter and heavier materials collected while rotating. They have also designed rotors to observe the sedimentation velocity of particles at very high speeds. To stop the rotor when it is running at high speeds the air pressure is reduced to 3 to 5 lb., which is sufficient to sustain an air column between the rotor and the stator. When the rotor has reduced its speed (as determined by pitch), the fingers are placed about it and pressed gently against the sides until it stops. The air pressure should never be completely cut off while the centrifuge is operating as the rotor will drop down on to the stator and then jump off. Caution is urged that, when the ultracentrifuge is to be operated at high speeds, it be placed behind a suitable barricade to protect the operator in case the rotor should explode.

Another type of ultracentrifuge has recently been described by Beams and Pickels (1935) and they have very kindly permitted me to reproduce it in Figure 4. In brief, the advantage of this model over the one above described is that the rotor which contains the material to be centrifuged spins in a vacuum and may be kept thermally insulated, which is an important factor in preventing troublesome convection currents from occurring in the centrifuged liquid, especially when the rate of molecular sedimentation is being observed.*

THE MICROSCOPE ULTRACENTRIFUGE.

Recently Harvey and Loomis (1930) have described a unique and ingenious type of microscope centrifuge in which the materials may be continuously observed while centrifuging. Harvey (1934) has adapted this principle to the air turbine ultracentrifuge mentioned above. In such a microscope ultracentrifuge he was able to take clear photographs of eggs being centrifuged at 84,000 times gravity. Figures 2 and 3 show rotors adapted as microscope ultracentrifuges which were constructed for me by Dr. E. G. Pickels in Dr. J. W. Beams' laboratory. The design of the optical system differs considerably from that of Dr. Harvey (Fig. 3, B). The

* See also Biscoe, J., Pickels, E. G., and Wycoff, W. G., *Jour. Exp. Med.*, 1936, Vol. 64, p. 39.

chamber which holds the material to be centrifuged is made of a specially treated glass (Beams, Weed, and Pickels, 1933), sealed at one end and cemented into position on the detachable top of the rotor (Figs. 2, A; 3, A). The top (Fig. 2, A) is placed over the body of the rotor, as in Figure 3, A, and fastened in position by machine screws so that the chamber containing the material to be centrifuged is directly over the mirror. The mirror is stellite or polished steel and is placed at a 45-degree angle to the chamber (Fig. 3, A, B). The light source is placed directly over the cell containing the material to be observed. When the rotor is turning fast enough to prevent flickering, one obtains through the microscope an almost perfect image of the material being centrifuged (Fig. 3, A).

As pointed out by Harvey, one of the important problems in high speed centrifuging is to prevent crushing of the material that is centrifuged. This may be obviated by supporting this material in an isotonic medium of graded density so that the material comes to lie in a medium of the same density. Isotonic sucrose, neutralised gum arabic or soluble starch solutions may be used for this purpose. Where large pieces of organs, such as the liver, are centrifuged, the cells next to the sides of the ultracentrifuge, i.e. on the centrifugal side of the liver mass, may be somewhat crushed while serving as a buffer for the rest of the cells which are not affected by the crushing action.

MATERIAL AND TECHNIQUE.

Several species of *Fucus* may be easily collected in abundance upon the rocks a few feet below the high-tide limits along the shores of Plymouth Sound. *Fucus vesiculosus*, *Fucus platycarpus*, *Fucus serratus*, and *Ascophyllum nodosum* were collected during the months of April, May, and June of 1935 at a point just below the Plymouth laboratory. Observations were made upon all the above-named species of *Fucus* for comparison, but only those upon *Fucus serratus* are recorded here.

The sexes of *Fucus serratus* can easily be distinguished by cutting and examining the conceptacles. The plants were collected a short time after they had been exposed by the ebb-tide and taken into the laboratory, and the males and females placed in separate dishes. After 6 to 10 hours in the laboratory the fruiting tips were observed to extrude the gametes. Those of the female were extruded in capsules of eight forming an olive-green mound-like mass upon the conceptacle. Those of the male were extruded in capsules containing many sperm (antherozoids) and were of an orange colour. A considerable amount of mucilaginous material is secreted, too, if the plants are kept moist. After tips of the fruiting plants were submerged in sea water, the mucilaginous material and the gametes were washed free; they then settle readily to the bottom of the dish.

If the female gametes are examined immediately they will be found in groups of eight surrounded by a gelatinous capsule consisting of a definite membrane or membranes, which dissolve after a few minutes in sea water setting the individual eggs free. If the capsules containing the eggs are extruded under unfavourable conditions, such as rapid drying or at too high a temperature, one frequently finds two or more of the eggs fused together giving rise to a capsule of 1 large egg and 6 of normal size (Fig. 5, A). In extreme cases as many as 6 eggs may fuse, giving rise to 1 giant egg and 2 of normal size within the capsule (Fig. 5, B).

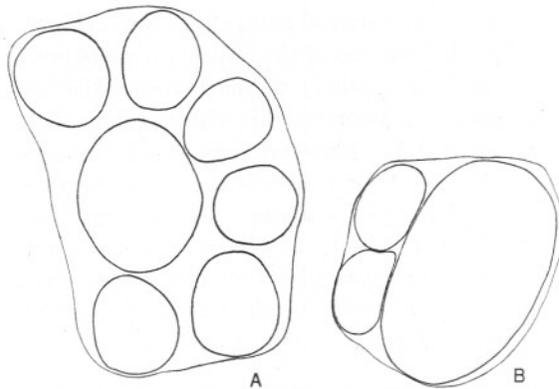


FIG. 5.—Fusion of *Fucus* eggs inside capsule. A, two eggs fused giving rise to one large egg and six of normal size; B, six eggs fused giving rise to one giant egg and two of normal size.

In addition to observing the eggs alive, they were fixed in Bouin's, Flemming's strong, Flemming's weak, and Champy's solutions. The best results were obtained with Flemming's strong solution made up in sea water. The sections were bleached and stained in Heidenhain's haematoxylin.

THE UNFERTILIZED EGG.

Notwithstanding the fact that the centrifuge has proved to be a valuable instrument in the study of many problems of experimental cytology and embryology of animal eggs (see Morgan, 1928, and Wilson, 1925, for reviews of the literature), it has been almost entirely neglected or no results have been obtained in similar types of studies upon plants. Shimamura (1929) centrifuged the egg of *Pinus thunbergii* and reports that "there is no stratification or separation of the contents in the egg cytoplasm." More recently, however, Whitaker (1931) succeeded in stratifying the cytoplasmic materials in the unfertilized eggs of *Fucus vesiculosus*. Various somatic cells of plants have been centrifuged and for a review of the

literature on this aspect of the subject the reader is referred to a recent paper by Beams and King (1935).

For a detailed description of the normal cytology of fertilization and of the early cleavages in *Fucus* the reader is referred to the excellent works of Farmer (1896), Farmer and Williams (1898), Strasburger (1897), Yamanouchi (1909), Oltmann (1922), and Walker (1930).

The normal unfertilized eggs of *Fucus serratus* vary considerably in size (60–90 μ) and possess an alveolar or foam-like type of cytoplasm in which is embedded a well-defined nucleus (Pl. II, Fig. 1). Irregularly distributed

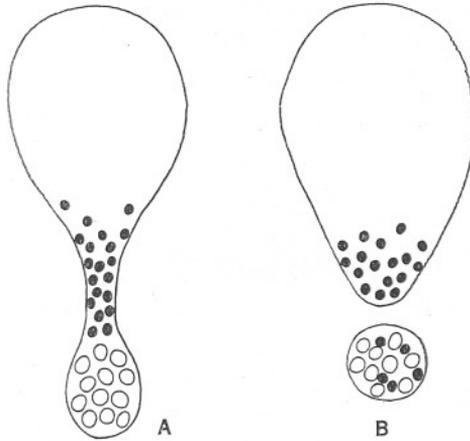


FIG. 6.—Ultracentrifuged unfertilized eggs. A, egg greatly elongated in the direction of the centrifugal force; fat globules and some of the chromatophores fragmenting from the egg. B, later stage of A.

throughout the cytoplasm are many chromatophores, fat-bodies and certain other granules of undetermined character. Farmer and Williams have described the chromatophores as located at the surfaces of the foam cavities with their long axes directed away from the nucleus. A cell membrane encloses the cytoplasm and the eggs have been described by some investigators as undergoing amoeboid movement. When the unfertilized egg is centrifuged its components become stratified into three distinct layers from the centrifugal to the centripetal pole as follows (Pl. II, Fig. 2): (1) A layer of very dense alveolar-like clear cytoplasm constituting over two-thirds of the total volume of the egg; (2) a layer of green chromatophores; and (3) a layer of fat in the form of globules or vacuoles at the centripetal pole. The nucleus takes up a position between the chromatophores and fatty globules. Sometimes the eggs become greatly elongated in the direction of the centrifugal force and the fatty layer and part

of the chromatophores may be completely removed from the egg (Figs. 6, A, B; Pl. II, Fig. 3). The eggs are very dense as compared to sea-urchin eggs and it was difficult to find a medium of the same density in which they could be suspended while centrifuging without causing a marked osmotic effect. I employed gum arabic and a sucrose solution made up in sea-water, but even then I was not successful in getting a solution of the same specific gravity as the eggs.

THE FERTILIZED EGG.

In about 10 to 15 minutes after fertilization of the eggs of *Fucus* a distinct cell wall is formed around them which prevents a marked distortion of the eggs during centrifuging. When a fertilized egg with a cell

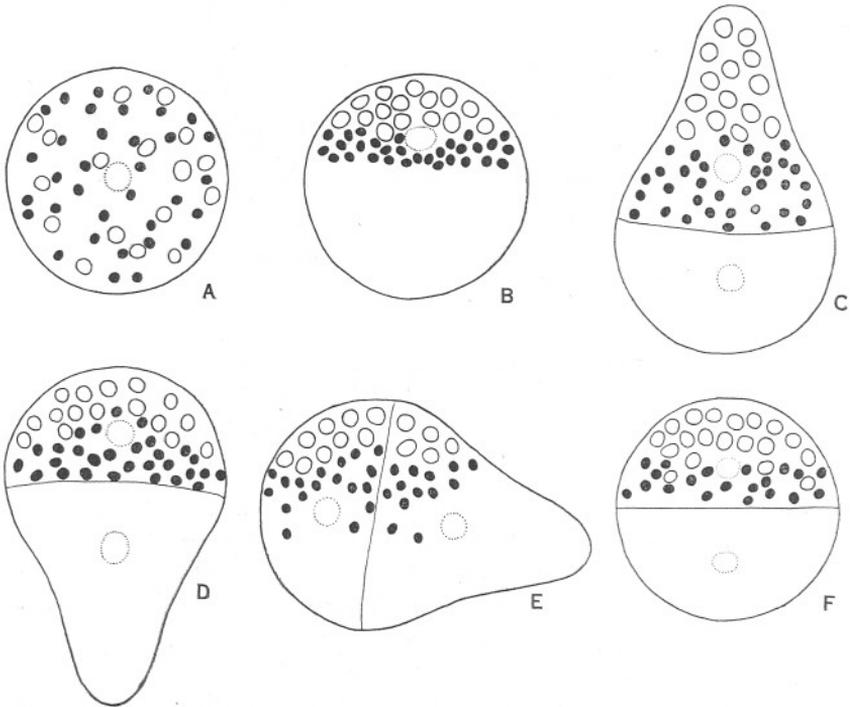


FIG. 7.—A, normal egg; B, stratified egg; C-E, eggs showing appearance of rhizoid protuberances, first cleavage planes and their relations to the planes of the stratified materials. F, egg cleaved without forming rhizoid rudiment.

wall is centrifuged in sea-water at 150,000 times gravity a sharp stratification of its protoplasmic components takes place (Pl. II, Fig. 4). Here, as in the unfertilized egg, three distinct zones are present in the order of their decreasing specific gravity as follows: (1) a layer of dense alveolar

cytoplasm; (2) a layer of chromatophores and bodies which I have tentatively interpreted as mitochondria; and (3) a layer of fat globules which are readily blackened by osmic acid. The nucleus lies just centripetal to the layer of chromatophores. The egg illustrated in Plate II, Figure 4, was not fixed until one half-hour after it had been centrifuged and shows the initial stages of the migration of the nucleus back to the centre of the egg, its usual position. Conklin (1917) has attributed this phenomenon to the activity of the "spongioplasm." The writer is inclined to believe that the factors involved in the redistribution of the

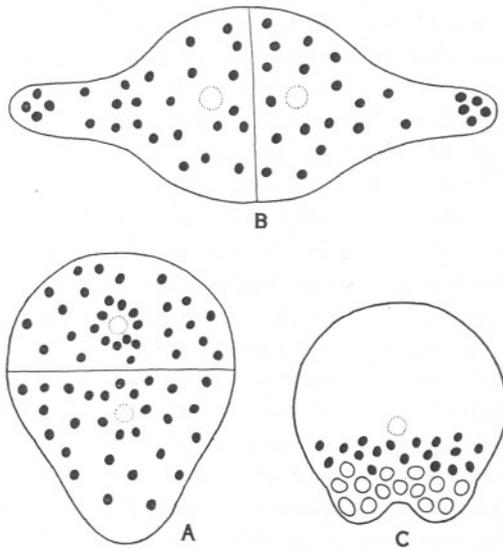


FIG. 8.—A, normal egg; B, bipolar uncentrifuged egg; C, ultracentrifuged egg with two rhizoid protuberances.

stratified materials are more complex than assumed by Conklin. This point will be further discussed in a forthcoming paper dealing with the effects of centrifuging the eggs of *Ascaris suum*. Small granular bodies are found in the fixed preparations that are readily stratified by ultracentrifuging into a position between the chromatophores and fat globules. I am inclined to interpret them as mitochondria; however, they are obviously in need of further investigation. In Plate II, Figure 5, is depicted a cell fixed 6 hours after centrifuging. It will be observed that the nucleus has returned almost to its usual position within the cell, carrying with it numerous chromatophores and fat globules. However, less frequently, following strong centrifuging cells may be found where only the nucleus migrates to the centre of the egg where cleavage takes place and all or most of the chromatophores and fat globules remain stratified (Fig. 7, C-F;

Pl. II, Figs. 6-7). In no case was I ever successful in fragmenting the fertilized eggs of *Fucus* as has been done with the eggs of certain sea-urchins.

As previously pointed out the unfertilized and the fertilized eggs of *Fucus* for the first 6 to 8 hours after fertilization are unlike many animal eggs, for example, the frog's egg, in that they show no indication of polarity. I have attempted to show diagrammatically (Fig. 7) how the various materials may be stratified in relation to the appearance of the rhizoid protuberance and of the first cleavage plane. Figures 7, A and B, show the typical appearance of the fertilized egg before and after centrifuging, respectively. It will be observed by an examination of Figure 7 that the appearance of the rhizoid protuberance and of the first cleavage plane seems to be irrespective of the plane of the stratified materials. For instance, in Figure 7, C, the rhizoid protuberance appeared at the centripetal pole of the stratified egg and the first cleavage plane occurred parallel to the stratified material separating all of the chromatophores and fat globules into the daughter cell possessing the rhizoid protuberance. However, in Figure 7, D, the rhizoid protuberance has appeared at the centrifugal pole of the egg, and the first cleavage plane, as in the above case, occurred at right angles to the stratified materials dividing the egg so that the daughter cell possessing the rhizoid rudiment is composed chiefly of alveolar cytoplasm and is free of chromatophores and fat globules. In Figure 7, E, the rhizoid protuberance has occurred laterally and the first cleavage plane at right angles to the stratified materials. In still other cases eggs are found which do not give rise to rhizoid rudiments, yet cleavage may take place as indicated (Fig. 7, F).

In some of the control eggs, as well as in the centrifuged material, I have observed certain rather interesting abnormalities. In Figures 8 B and C are shown cells with a dual polarity. Whitaker (1931) has likewise described similar abnormalities in *Fucus vesiculosus*. Although the complete history of these two cells is unknown it seems probable that their dual polarity is due to a fusion of 2 eggs, each of which retained its original polarity. Eggs with as many as 4 nuclei have been found but it is not known whether this is due to a fusion of 4 eggs, polyspermy, or to a division of the nucleus without division of the cytoplasm (Pl. II, Fig. 8).

DISCUSSION.

It has been demonstrated in this paper that the visible inclusions in the eggs of *Fucus serratus* may be shifted and stratified in the order of their relative specific gravity without affecting the normal development or apparently modifying the original polarity, i.e. the original axis of the egg. Neither do the stratified materials seem to play a role as organ-

forming substances. In this respect the *Fucus serratus* egg differs from the somatic tissue of another alga, *Griffithsia bornetiana*, recently investigated by Schechter (1934). Schechter found that if tufts of the alga were centrifuged at 150 times gravity for 24 hours or longer, normal shoots appear from the cells at a point where the heavier substances, in this case the chromatophores, have concentrated. In this way reversal of polarity may be produced anywhere along the plant axis. He suggests, however, that the centrifuged substances (inclusions) are not directly determinative of the polarity, but act as stimuli.

The question then follows in what structure or structures of the cells does the polarity reside. This is an old question among students of embryology and one that has never been satisfactorily answered. Lillie (1909), from his work on centrifuged *Chaetopterus* eggs, concludes that "polarity is a property of the ground substance of the protoplasm" which cannot be disturbed by ordinary centrifuging because it is assumed to be of molecular organization. Conklin (1917, 1924) holds that polarity inheres in the "viscid spongioplasm" which forms a peripheral layer around the egg and a continuous framework throughout the cell that is connected to the nucleus. It is this structure which, he holds, is responsible for the return of the nucleus and other inclusions or components to their usual position within the cell after artificial displacement by centrifuging. The yolk, oil, water, and pigment granules are thought to lie in the meshes of the "spongioplasm." As regards the effect of centrifuging upon the "spongioplasm" Conklin states: ". . . at the same time the strands of the framework may be stretched or bent, but unless the centrifuging is strong enough to kill the egg, this substance is not stratified with the other cell contents."

If one assumes that the "ground substance" of Lillie and the "spongioplasm" of Conklin are molecular, a condition that now applies to protoplasm generally, it might be possible, in view of the recent work of Svedberg (1928), to produce a stratification within the protoplasm of its colloidal components. In this connexion Taylor (1931) has recently raised the question, "But to what extent the living ground substance would endure the rigours of such enormous forces (10,000 to 100,000 times gravity) and remain living is extremely problematical."

It is clear from this work on *Fucus* that centrifugal force of 150,000 times gravity for 30 minutes does not kill the eggs. But to what extent the "spongioplasm" has been affected by such enormous forces one is unable to state. However, Beams and King (1936) have recently subjected the eggs of *Ascaris suum* to a centrifugal force in excess of 400,000 times gravity for one hour without killing them. This is a force equal to the maximum employed by Svedberg to produce sedimentation of colloids. *Ascaris* eggs have also been subjected to 150,000 times gravity for 10

days; they not only remain living but undergo cleavage in the ultra-centrifuge. It has not yet been determined whether or not a stratification of the colloidal cytoplasmic components has taken place, but if such does take place, it is of particular interest, for then the normal spatial relationship of the separate elements cannot be of vital importance for the maintenance of life. However, if, as we are inclined to believe, little or no separation or stratification of the components has taken place in this material, they must be held together in a firmer way than those in the colloidal systems examined by Svedberg. In other words, the conditions present in this living colloidal system (protoplasm) seem to be different from those in non-living ones.

It would seem that the killing of cells by the present methods of centrifuging is usually due to mechanical distortion or disruption (prevented in the fertilized *Fucus* egg by a very resistant cell wall and in *Ascaris* eggs by a shell) rather than to a disturbance of the spatial relationship of their molecular parts.

In *Fucus* it has been demonstrated by Farmer and Williams (1898), Hurd (1920), Whitaker (1931), and others that light may alter the polarity of the developing spore. They found, and I have also observed, that the rhizoid protuberance always appears on the side of the egg opposite the light (i.e. the shaded side). Hurd further observed that the shorter rays at the blue end of the spectrum were the most effective in determining the polarity. She suggests that this effect may be due to a more rapid oxidation of the egg on the side exposed to the light, setting up a metabolic gradient which is responsible for the polarity. Hurd also found that when eggs were placed in groups very close to one another, each develops a polarity in such a way that its apical point faces toward the group. To this phenomenon, which is even more influential in determining polarity than light, she has given the name of "group orientation effect." Whitaker has shown that even unfertilized eggs of another species may control the polarity in the "group effect." The direct effect, he concludes, cannot be due to any agency depending upon nuclear or cell division.

Lund (1923) has found by passing an electric current through sea water containing eggs of *Fucus inflatus*, that they will all become polarized with the rhizoid protuberance toward the positive pole. This is interesting in view of the findings of McClendon (1910) that the chromatin in the nucleus of the onion root tip moves to the positive pole when exposed to an electric field. However, since Lund did not examine the eggs cytologically it is unknown whether or not the electric current directly affected the mitotic spindle.

It is evident that in many of the eggs like those in Figure 7, all of the chromatophores are segregated into one of the daughter cells at the first cleavage. However, as the spore grows, new chromatophores arise in

those cells derived from the chromatophore-free blastomere. Although I do not want to urge the view too strongly, it seems probable that the new chromatophores may arise *de novo* in the cell, perhaps by the activity of certain of the cytoplasmic components.

A few preparations were made by the Kolatchev method in an effort to demonstrate the osmiophilic platelets, but they were unsuccessful. The whole problem of the nature of the mitochondria and the osmiophilic platelets and their relationships to the other cell inclusions of the *Fucus* egg is badly in need of further investigation.

It has been the author's experience that many of the methods designed to be used on the tissues of fresh-water animals and plants do not always work so successfully upon the tissues of marine animals. It is also a fact of experience that many of the well-known methods fix tissues and cells of marine animals better if they are dissolved in sea water instead of the usual method of using distilled water. In general, a distinct need is felt for a modification of many of our cytological fixatives in order that they will preserve more successfully the cells of marine animals.

SUMMARY.

1. An air turbine ultracentrifuge suitable for biological work and capable of developing a centrifugal force from 10,000 to 500,000 times gravity has been described. Advantages of the ultracentrifuge are: (1) The temperature does not vary in the centrifuge chamber over 2 or 3 degrees from that of the atmosphere; this is not sufficient to be an important factor in general biological work. (2) The cost of constructing the apparatus is very low in comparison with that of other high speed centrifuges.

2. A modification of the air turbine ultracentrifuge in which the turbine rotor drives a second rotor, in this case the centrifuge rotor which spins in a vacuum, has been described. Here the centrifuge chamber is thermally insulated which is an important item in preventing troublesome convection currents from arising in the centrifuged material where such special problems as the rate of molecular sedimentation are being observed. Forces in excess of one million times gravity can easily be obtained by such an apparatus.

3. A microscope ultracentrifuge in which the biological materials may be observed while centrifuging at high speeds (10,000 to 200,000 times gravity) has been illustrated.

4. The stratification in *Fucus serratus* eggs of the visible inclusions in the order of their relative specific gravity by means of the ultracentrifuge has been described. After centrifuging fertilized *Fucus serratus* eggs at 150,000 times gravity for one half-hour they apparently develop normally.

5. The polarity in centrifuged *Fucus* eggs as determined by the appearance of the rhizoid protuberance and of the first cleavage plane is unaffected by a stratification of the visible inclusions.

6. The fusion of as many as six unfertilized eggs has been observed.

7. Normal and centrifuged bipolar eggs, i.e. with two rhizoid protuberances, have been found. This condition is probably due to the fusion of two eggs each of which has retained its original polarity.

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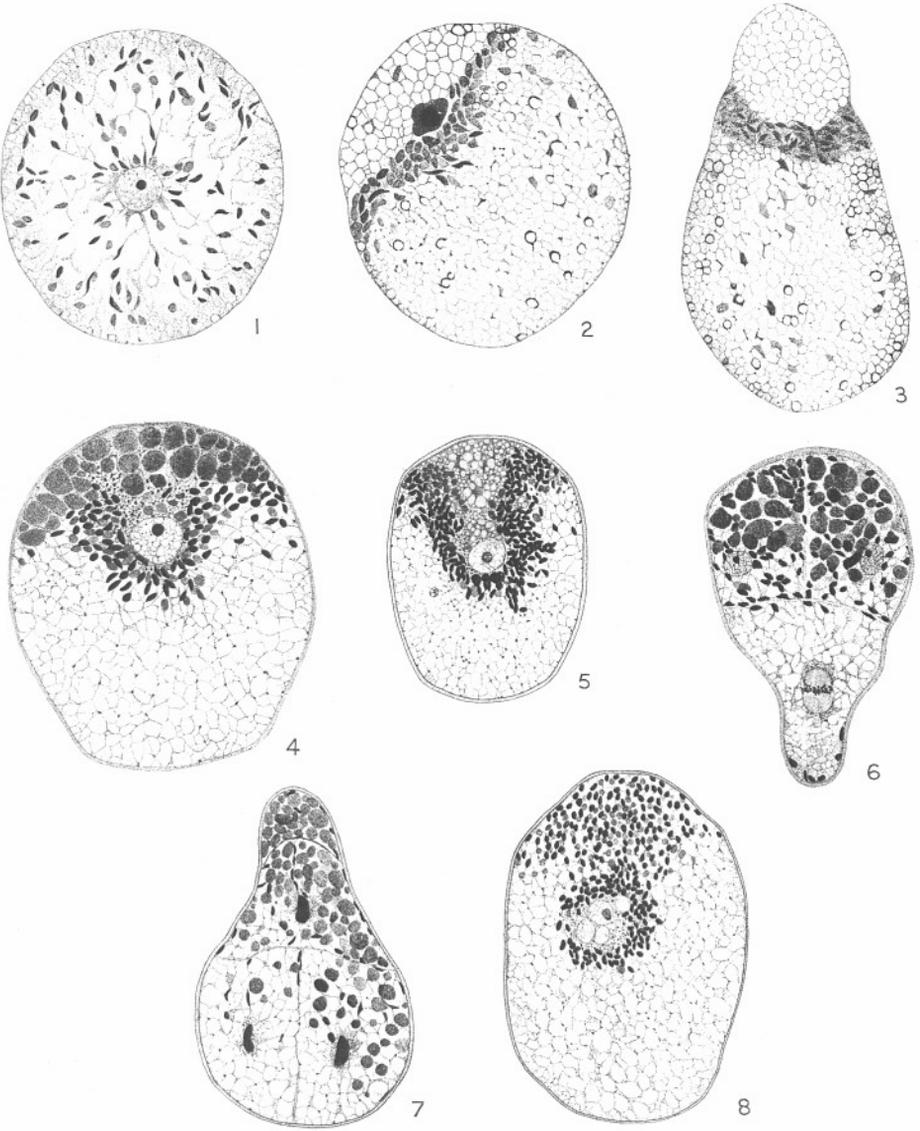
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EXPLANATION OF PLATE II.

All drawings were made from sections at an approximate magnification of 2,000 times. Centrifugal ends of the cells, except Figure 1, are directed toward the bottom of the plate.

- FIG. 1.—Normal unfertilized egg.
- FIG. 2.—Ultracentrifuged unfertilized egg showing stratification of the visible materials.
- FIG. 3.—As in 2, but elongated in the direction of the centrifugal force.
- FIG. 4.—Fertilized ultracentrifuged egg showing stratification of materials.
- FIG. 5.—As in 4. Nucleus and inclusions shown in the process of returning to their usual position within the egg.
- FIG. 6.—Spore derived from egg in which most of the inclusions had been segregated into the daughter cell opposite the one possessing the rhizoid protuberance at the first cleavage.
- FIG. 7.—Spore derived from egg in which most of the inclusions had been segregated into the daughter cell possessing the rhizoid protuberance at the first cleavage.
- FIG. 8.—Uncleaved ultracentrifuged egg with four nuclei.



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