OBSERVATIONS ON LIVING CELLS, MADE WITH THE MICROSCOPE-CENTRIFUGE

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(With Four Text-figures.)

THE microscope-centrifuge, recently described by Harvey and Loomis (1930), was devised in collaboration with Mr Alfred L. Loomis and constructed in his private laboratory at Tuxedo Park, N.Y. It is a device by which living cells can be observed while they are being centrifuged at speeds of 1000 to 4000 R.P.M. at a distance of 11 cm. from the centrifuge axis. The instrument is really a special head, containing part of a microscope, built to fit a $\frac{1}{2}$ in. shaft, the size of the type SB International Equipment Company electric centrifuge. The scheme is as follows.

A microscope-objective (*Obj*) is firmly set into one end of a bar of metal which revolves on the centrifuge axis (Fig. 1). The objective is purposely reversed from the ordinary position on a microscope so that the light (*L*), the focusing screw (*F*), and the slide containing living cells (*S*), will be easily accessible on the top of the centrifuge. After passing the objective, the light is carried by two total reflecting prisms, *A* and *B*, to the axis of the centrifuge and then vertically upward. The eyepiece (*Oc*) is stationary and mounted permanently in the protecting cover above the axis of the centrifuge. The counter weight (*W*) is used for balancing.

A slide to hold cells (Fig. 2) can easily be made from a hollow depression microscope slide by cementing with picene a cover-slip (*C*) over one-half of the depression. There is thus formed a niche (*O*) between cover-slip and surface of slide in which cells are thrown by the centrifugal force. Other bits of cover-slip (*G*) can be cemented around the edge of the depression to prevent evaporation by air currents. If only a few fertilised eggs are placed in the slide there is sufficient oxygen to allow perfectly normal cleavage, and such eggs could be kept under continual centrifuging and observation during development. The slide, cut to proper size, with cover-glass down, is laid on a flat strip of metal (Fig. 1, *D*) with holes at *H* and *H'* for passage of light, and held down by the clamp *G*. Its position in the direction of the centrifugal force can be adjusted by the screw *J*, so that distance *D* brings cells under the objective. The slide can be focused by moving the whole bar (*D*) up and down on the focus screw *F* (Fig. 1) and additional focusing during centrifuging is obtained by changing the vertical position of the ocular. In another design of the microscope-centrifuge head the objective fits accurately in a tube, but can be moved up and down by a rack and pinion. When the slide is in focus a set screw locks the objective in position. This device for focusing does away with the bar *D* and focusing screw *J*, and has worked perfectly at the highest centrifugal speeds.

If the cells are illuminated continuously the image in the eyepiece, while the centrifuge is running, would be whirling in a small circle. However, if we illuminate the cells for a few micro-seconds every time they reach a position in the revolution under the light, the appearance will be that of a succession of images, a moving picture. The cells will appear stationary, the granules within will be seen to move under the centrifugal force.

The illumination is a 2000 to 3000 volt condenser discharge in mercury vapour at atmospheric pressure or above (Fig. 3). The lamps can easily be made by sealing a tungsten wire into one end of a pyrex capillary tube which is partly filled with mercury. A portion of the tube near the other end of the capillary is enlarged to a small bulb and an iron wire pushed down the capillary to the proper distance from the tungsten to form the other electrode for the condenser discharge. This iron electrode is then sealed in with de Khotinsky cement, leaving the bulb filled with air. A heating coil around the capillary, not shown on

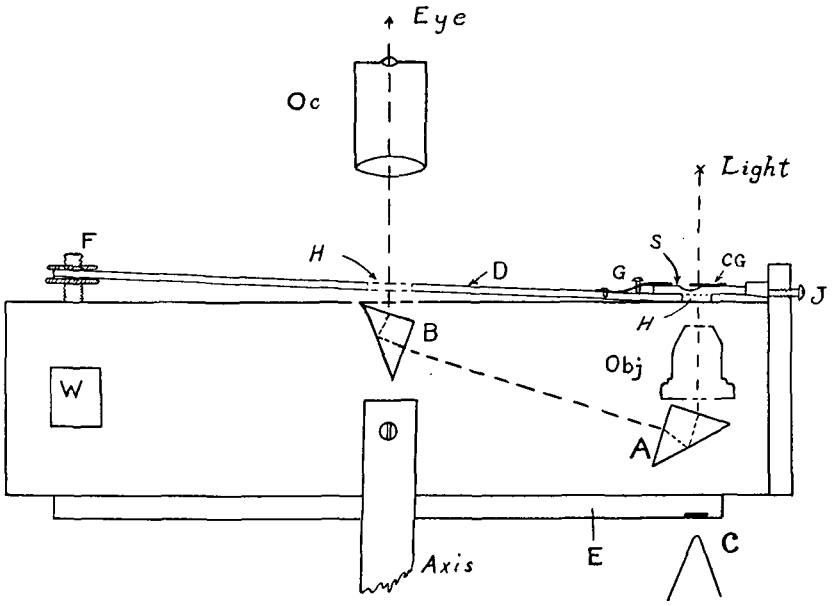

Fig. 1. Optical system of microscope-centrifuge. *Oc*, ocular; *CG*, cover-glass; *S*, slide; *G*, spring clip for holding slide; *J*, screw for adjusting slide; *Obj*, objective; *H*, *H'*, holes in focusing bar; *C*, contact point; *A*, *B*, right angle prisms; *D*, focusing bar; *F*, focusing screw; *W*, counterweight.

the figure, protected from air currents by an enclosing pyrex tube, boils the mercury in the capillary, forming mercury vapour through which the discharge takes place. A reservoir condenser is kept continually charged to 2000 to 3000 volts from a transformer and rectifying vacuum tube. The discharging condenser, of lower capacity, is in parallel with the reservoir condenser through a high resistance, made of a mixture of xylol and methyl alcohol. With each revolution of the centrifuge the contact point (*C*) on the bakelite disk (*E*) discharges the 2000 to 3000 volts through the mercury lamp, and during the remainder of the revolution the condenser is again charged through the high resistance and is ready for another discharge.

In actual practice the microscope-centrifuge has far exceeded expectations. The image is perfectly clear and steady with the highest centrifugal speed and highest dry objectives (× 62) and, because of the rather long tube length, the magnification obtainable with high power oculars is great. The adjustment of the special slide

has given no difficulty. The cells cannot help but be thrown in the proper place for observation once the objective has been focused. Old material can be removed from the slide and new cells inserted in half a minute. In fact a slide of this sort makes a far better container for centrifuging than a capillary tube, since the slide can be removed and the centrifuged cells immediately examined *in situ*. A bar of metal 16 inches long with wide grooves at each end and spring clips to hold two of the slides in place in the grooves makes a very convenient centrifuge head for obtaining forces many thousands of times gravity.

In order to test the possibilities of the microscope-centrifuge a number of observations were made on living cells, chiefly unfertilised marine eggs and protozoa. The radius is 11 cm.

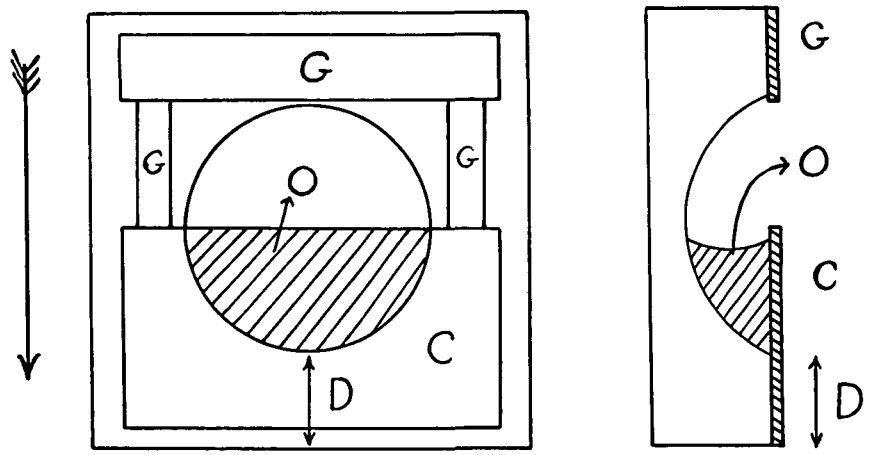

Fig. 2. Side and top view of slide for centrifuging cells. *C*, cover-slip; *G*, fragments of cover-slip to make slide level; *O*, fluid containing cells; *D*, distance to field of view under objective. The arrow indicates direction of centrifugal force. The curvature of the depression in slide is exaggerated in the side view.

Amoeba dubia. At low speeds (1000 R.P.M.) one can observe the organisms moving quite normally and adhering to the slide. The crystals are thrown down so readily that their velocity of fall can hardly be determined; in fact they look like falling stones. They are redistributed very rapidly (1 minute) after centrifuging by the streaming movements of the *Amoeba*. As the centrifugal force is increased the *amoebae* suddenly let go of the substratum and are thrown to the wedge-shaped side of the observation chamber, where they remain, tending to round up. Pseudopodia are not pushed out so readily at high speeds but I have seen them appear, slowly flowing against the centrifugal force at speeds of over 4000 R.P.M.

Paramoecium. The organisms are thrown down, forming a tightly packed mass, with the long axis at right angles to the centrifugal force. The under individuals cannot move because they are held by others on top, but the upper (toward axis of centrifuge) individuals can swim by ciliary movement back and forth at right angles to the force even at highest speeds (over 4000 R.P.M.). Crystals are rapidly thrown

down, as is also the nucleus, but the contractile vacuoles remain in position and can be observed to empty at the highest speeds. The rays of the contractile vacuole are most marked in the direction of the force.

Stentor. These organisms are thrown down in such a position that the ciliated end is toward the axis of the centrifuge. The green bodies rapidly collect in the centrifugal end at low speeds, forming a mass of green material from which (at higher speeds) the ciliated end pinches off and, after centrifuging, can be observed to swim around, a clear half *Stentor* with only a few green bodies. The cilia, like those of *Paramoecia*, keep beating even at the highest speeds.

Arbacia eggs. As is well known, the granules of the sea urchin, *Arbacia*, are much more difficult to move than those of the mollusc, *Cumingia*, so that the time required to effect marked stratification is much greater than the time for centrifugal force to pack the eggs into a fixed position. This is an important point in the study

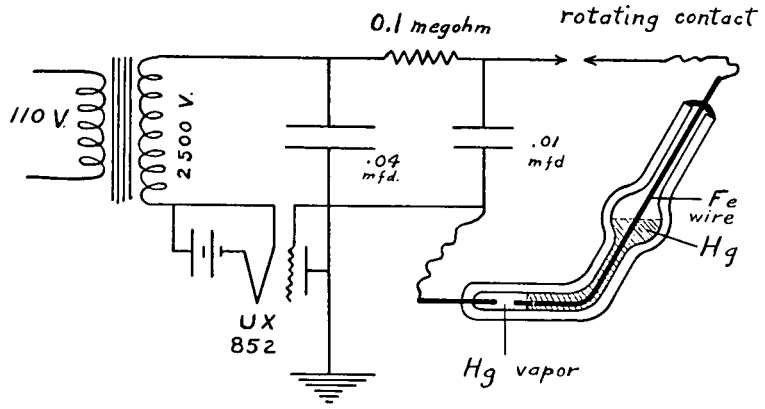

Fig. 3. Lighting system of microscope-centrifuge. The heating coil of nichrome wire around the mercury vapour lamp is not shown.

of viscosity, for it means that, in determination of the time of granule movements, the error involved for the centrifuge to come up to its proper speed and the error involved in the rolling over of eggs (which can be readily observed with the microscope-centrifuge) and consequent change in the direction of centrifugal force until they are in a fixed position, will be very small. On the other hand, although the eggs are surrounded by jelly, it is not very firm, and they are thrown out of the jelly and distorted by squeezing. The distortion makes it difficult to judge through what distance the granules as a whole have moved. It is not possible in these eggs to keep the eye on individual granules while the mass of granules is moving. The first indication of the granule movement as a whole is a clear crescent-shaped (following the round contour of the eggs) area at the centripetal pole, which gradually widens. The oil cap forms last. Fig. 4, fifth row, shows three stages in the stratification of *Arbacia* eggs photographed through the centrifuge-microscope while revolving 2100 R.P.M. It will be noted that the eggs show a slight tendency to elongate in the axis of the centrifugal force.

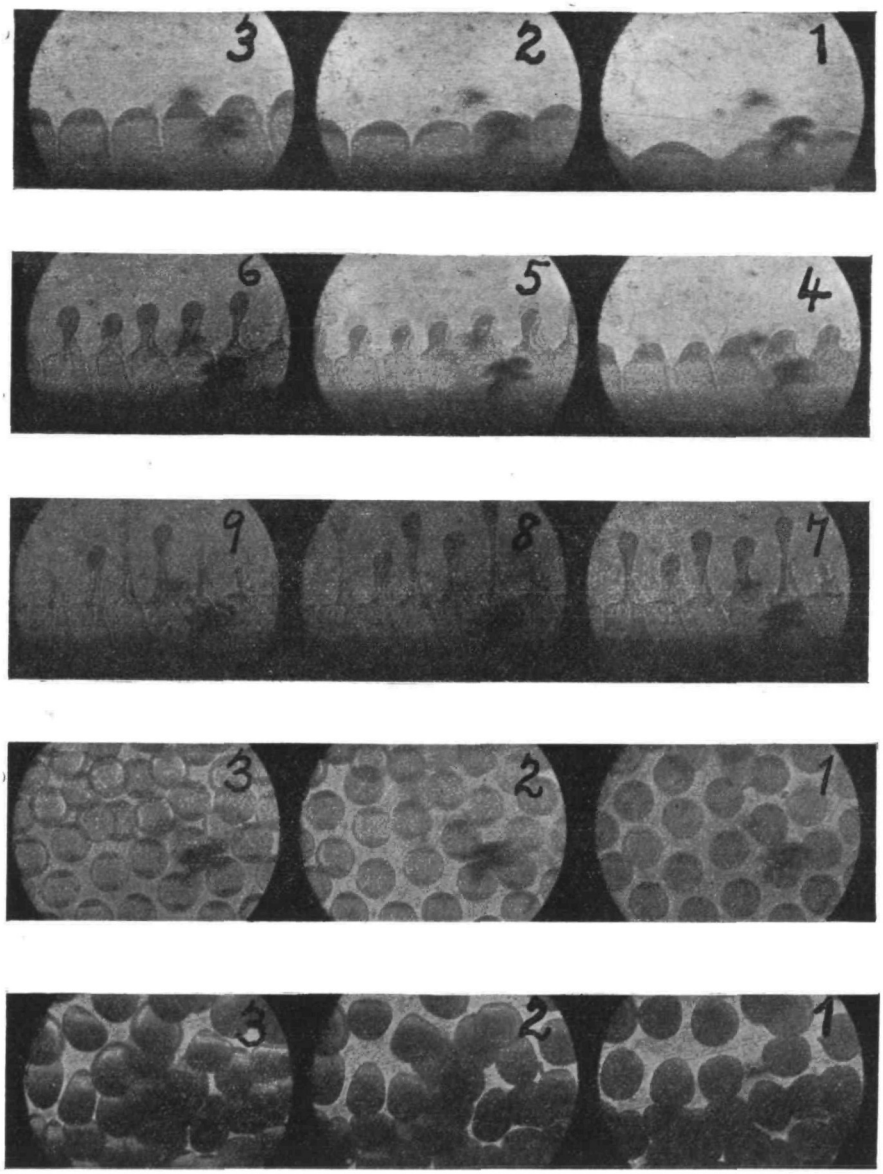

Fig. 4. Photographs of unfertilised eggs taken with a Leica camera through the microscope-centrifuge (radius 11 cm.), showing successive changes (examine from right to left) in stratification of granules and in shape of eggs. Magnification, 87 diameters. The direction of the centrifugal force is downward.

First three rows, *Chaetopterus* exposed for 5 seconds, at 35, 55, 95, 165, 205 and 265 (double exposure), 345, 375, 450, and 480 seconds after starting centrifuge. Speed of the microscope-centrifuge was 55 R.P.S. in the first row and 66 R.P.S. in the second and third rows. At the highest speed about 330 flashes of light occurred during the exposure.

Fourth row, *Cumingia* exposed 10 seconds at 35, 95 and 155 seconds after starting centrifuge. Speed 38 to 55 R.P.S. The eggs are separated by jelly.

Fifth row, *Arbacia* exposed 10 seconds at 45, 150 and 285 seconds after starting centrifuge. Speed 35 R.P.S. The eggs are partially separated by jelly.

Cumingia eggs. The eggs of this clam, as contrasted with *Arbacia*, always remain firmly embedded in jelly and retain an almost spherical form even at highest centrifugal speeds. The granules move so rapidly that one can actually observe the path of the granules change as the whole egg rotates in taking its final position on the slide, while the centrifuge is picking up speed. One can also see the stream of granules following the curvature of the egg surface as the oil layer forms, giving an appearance of rotary movements within the egg. There is some difference in time for stratification in different eggs. The oil layer is always completed before the yolk layer and in such a relation that if it takes 30 seconds to bring the oil into a layer occupying one-fifth the diameter of the egg it will take 45 seconds to stratify the yolk to a layer one-fifth the diameter of the egg, *i.e.* the oil is stratified in two-thirds the time necessary to stratify the yolk. As is well known, the oil and the yolk finally occupy about the same volume and present the appearance shown in Fig. 4, where three stages in stratification are shown (fourth line) photographed through the microscope-centrifuge.

Individual granules cannot be observed but it is easy to determine the time (45 seconds) with a stop-watch when the granules have been moved four-fifths the diameter of the egg, a distance of 0·0052 cm. This gives a velocity of fall of 0·000115 cm. per second, at 1100 times gravity.

The Stoke's equation for velocity of fall of spherical particles is $V = \frac{2cg(\sigma - \rho)a^2}{9\eta}$, where V is velocity of movement in cm. per second; c is centrifugal force in gravitational units; g the gravity constant, 980; σ, specific gravity of granules; ρ, specific gravity of the medium; a, radius of the particle in cm.; η, the viscosity in poises. Using Heilbrunn's values (1926) for the radius of a granule, specific gravity of the granule and specific gravity of the medium, the viscosity of *Cumingia* eggs work out as 0·068. Another determination at 612 times gravity gave 0·06 as the viscosity at 25°. These values agree well with Heilbrunn's determination from movement of granules across one-half the egg diameter at a much higher centrifugal force ($4968 \times g$) of 0·043 at room temperature. I do not believe the centrifugal method of determining viscosity can give more than order of magnitude. The greatest difficulty is in determining the size of granules whose diameter is of the order of wave lengths of light. Some measurements of the size of *Cumingia* yolk granules gave an average diameter of $0{\cdot}5\,\mu$ or a radius of $2{\cdot}5 \times 10^{-5}$ cm. instead of $1{\cdot}5 \times 10^{-5}$ cm., the value which Heilbrunn used. Remembering that this radius must be squared when substituted in the Stokes' equation for viscosity by fall of particles, we find that if the particles really average that much larger the viscosity will be about three times as great.

Chaetopterus eggs. The eggs of this annelid are not surrounded by jelly and behave in a most interesting manner for observation under the microscope-centrifuge. The eggs pack tightly together side by side and later, as observed by Lillie (1908), pull apart into fragments. The entire process of fragmentation can be observed. At one stage the protoplasm is drawn out into long filaments with the oil globules forming a spherule at one end. Fig. 4, the first three rows, shows ten stages in the formation of the oil spherule photographed through the microscope-

centrifuge while revolving about 4000 R.P.M., 5 seconds' exposure. It will be noted that No. 5 is a double exposure (by mistake) and shows the exact change in position which takes place in 1 minute. In the pulling off of this oil spherule the appearance is that of a drop of molten glass slowly falling from a heated rod. When the oil finally breaks away it *slowly* floats centripetally to the surface of the sea water. Sometimes a clear fragment containing no yolk or oil globules will separate. These clear fragments are denser than sea water and sink; in fact a clear fragment half filled with oil globules is heavier than sea water.

The pulling away of an egg spherule containing oil occupies about 7 minutes at 1916 times gravity, and it will be noted that when an oil spherule breaks away the stalk does not round up immediately but remains for some time and only slowly rounds off. The stalk does not behave as if it were elastic like rubber which would immediately recover when stretched, nor is it stiff, for one can observe these stalks waving back and forth in currents in the sea water during centrifuging. They appear like a very slow flowing plastic material such as tar. I have calculated the force necessary to pull this stalk apart and discussed its significance elsewhere (Harvey (1931)).

As the stalk appears to be highly viscous, it seemed worth while to obtain some idea of the velocity of granules moving through *Chaetopterus* eggs, and particularly to compare rate of movement of granules determined in two ways with the microscope-centrifuge: (1) by watching the rate of movement of the *boundary* of the yolk granules *as a whole* a certain distance across the egg; (2) by timing the movement of *individual* granules as they pass along a micrometer scale in the ocular of the microscope-centrifuge. Such granules can be easily observed when most of the yolk has been thrown down and the eggs are in the stage represented by pictures 4, 5 and 6 of Fig. 4.

It should be stated that these granules do not always have a uniform velocity, but move and stop, or move slowly, then faster, then slowly, then faster, etc. Whether they move through a network, occasionally catching on a mesh, or whether they roll down the egg surface, to which they occasionally stick, cannot be stated. I should incline to the latter view. At any rate, I recorded the time of movement of forty individual granules whose velocity was most uniform and obtained an average of 0·00044 cm. per second at 1390 times gravity.

Using the other method of determining the rate of movement of these granules, namely, watching the time of stratification of the yolk as a whole a certain distance from the egg boundary, I obtained rates at 23° C. of 0·000082 cm. per second for $602 \times g$ and 0·00014 cm. per second for $1014 \times g$. These values are less than one-half as large as by the previous method, making allowance for the difference in centrifugal force used. Part of the difference may be due to the fact that, where individual granules are watched, the centrifuge has been running some time and the temperature rises to 29° C., whereas in the other method the temperature was 23° C. Again, since the eggs elongate so much, it is difficult to evaluate the distance through which the yolk as a whole has moved.

The yolk granules in *Chaetopterus* vary considerably in size, from 1 to 2 μ in diameter. As the largest ones would be most likely to be observed, we might take

1×10^{-4} cm. as the radius of a granule. Not knowing the specific gravity of a granule or the medium, we might assume some likely value for $\sigma - \rho$, namely 0·1, the average of Heilbrunn's determination for *Arbacia* and *Cumingia* eggs. Using these constants and neglecting the Cunningham correction, the viscosity works out as 0·68 by the first method ($c = 1390 \times g$) and 1·62 ($c = 602 \times g$) and 1·54 ($c = 1014 \times g$) by the second. It should be recalled that the viscosity of water is 0·01, olive oil 0·99 and glycerine 10·69.

However, I do not propose these values as accurate determinations of the viscosity of *Chaetopterus* eggs. Many determinations might average to give a fairly respectable value, but even then, if the granules are one-half the diameter measured (an extraordinarily difficult measurement) the viscosity must be divided by 4. I only publish the figures for rate of movement to show what may be done with the microscope-centrifuge, an instrument that will have many uses in the field of protoplasmic studies and experimental embryology.

SUMMARY.

The microscope-centrifuge is described in detail, together with some observations on living *Amoeba*, *Paramoecium* and *Stentor*, and granule movement in unfertilised eggs of *Arbacia*, *Cumingia* and *Chaetopterus*.

REFERENCES.

HARVEY, E. N. (1931). *Biol. Bull.* **60**, 67.
HARVEY, E. N. and LOOMIS, A. L. (1930). *Science*, **72**, 42.
HEILBRUNN, L. V. (1926). *Journ. Exp. Zool.* **44**, 255.
LILLIE, F. R. (1908). *Biol. Bull.* **16**, 54.