# THE PROTOPLASMIC VISCOSITY OF *PARAMECIUM*

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(With Three Text-figures)

Since Heilbrunn's work in 1926 most workers have agreed that the viscosity of protoplasm is low; all the recent observations, with one exception, have given figures which are not markedly different from each other. The exception, the work of Fetter (1926) on *Paramecium*, gives a viscosity value which is much higher than that found in other material. The present paper constitutes a re-examination of the viscosity values given by Fetter.

## MATERIAL AND METHOD

The animals used were obtained from a stock culture of *Paramecium* sp. The experimental method was originally based on that of Fetter, but it required so much modification in detail, that in its final form little trace of the original method remained.

When the animals were fed with particles of different kinds (iron, indian ink, and starch were among those tried), it was found that there was a very great variation in their degree of movement in a centrifugal field. It seemed probable that this was due to differences in the orientation of the animals in the centrifuge tube. Various attempts were made to control this factor, the final method being as follows.

A few cubic centimetres of culture were put into a watch-glass and a small quantity of iron particles mixed with the water. The iron used was the so-called "dust iron" manufactured for use in high-frequency radio circuits. These particles are nearly spherical and between 3 and 4 $\mu$ in diameter. They were ingested very readily by the *Paramecium*; after 2–3 min. a majority of the animals had one or more vacuoles containing iron. An animal which had not ingested too much metal was selected under a dissecting microscope and was drawn into a capillary tube about 3–4 cm. long. The bore of the tube was very little greater that the cross-section of the animal; the capillary was sealed at both ends and cemented to a glass slide. The position of the *Paramecium* in the capillary was marked by a line drawn on the slide and the latter placed in a special carrier (Fig. 1). This carrier was rotated by an electric motor at a predetermined speed. At the end of the required time the motor was stopped and the slide examined under a microscope, and the position of a certain vacuole noted. The slide was then replaced in the carrier and

the procedure repeated. When the particles had been moved to one end of the animal, the slide was rotated through 180° and replaced in the carrier. The vacuoles could then be moved back towards the end at which they started. This could be repeated many times; actually the largest number of measurements carried out on a single animal was seventeen.

Since the *Paramecium* nearly filled the bore of the tube, their rate of movement along it during centrifuging was slow, so that the radius of rotation, and therefore the centrifugal force, remained constant during an experiment. The amount of movement of the animal could of course be determined by reference to the mark made on the slide before centrifuging. The carrier was graduated in centimetres from the axis, so that the radius of rotation could be read accurately.

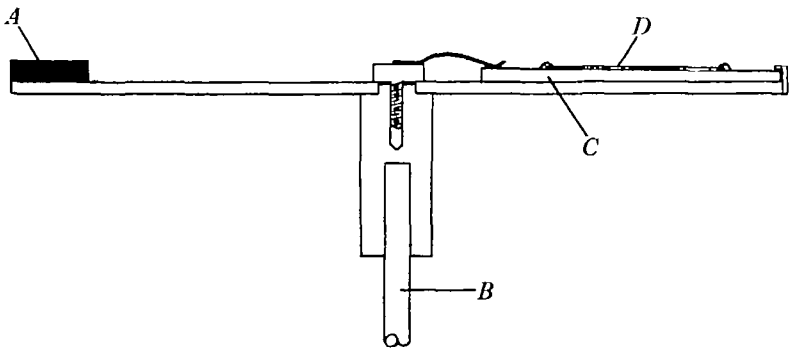


Fig. 1. Centrifuge head to carry slide. *A*, balance weight; *B*, centrifuge shaft; *C*, slide; *D*, capillary tube.

The method of speed control was simple and satisfactory. A black disk with a radial white bar was mounted on the end of the shaft carrying the slide holder, and was illuminated by a neon lamp connected to the 50-cycle A.C. supply. This formed a very satisfactory stroboscope, with which it was possible to maintain a constant speed at various levels from 10 to 50 rev./sec. All the measurable speeds are, of course, submultiples of the hundred flashes a second given by the neon lamp.

The motor speed was adjusted by a resistance before the slide was placed in the carrier; and the current was then switched off without altering the resistance. With the slide in place, the motor was restarted, and the chosen speed reached in a few revolutions.

Within certain limits, the above technique gave results which seemed to be reliable, but irregularities appeared when attempts were made to use very low forces. All attempts to find any constant factor in these irregularities failed; it was decided therefore that a microscope centrifuge would be the only means by which it would be possible to study the effect of low forces.

The choice of instrument lay between the type designed by Harvey (1933), or that described by Beams (1937). These differ greatly in their optical systems, each having its own particular merits; the type due to Beams is carried on the air-turbine centrifuge, while Harvey's instrument has an electric drive. The simplicity and

vibrationless running of the air-turbine centrifuge is exceptionally valuable for an optical instrument, but unfortunately the minimum speed obtainable is much too high for the present type of work. On the other hand, the constructional simplicity of Beams's design is a very useful feature. With these and other points in mind, an attempt was made to design a centrifuge which would combine some of the advantages of both types.

The design is shown in Fig. 2. The instrument incorporates the Pickels's (1936) optical system and revolves on a fixed horizontal axis. The advantage of the hori-

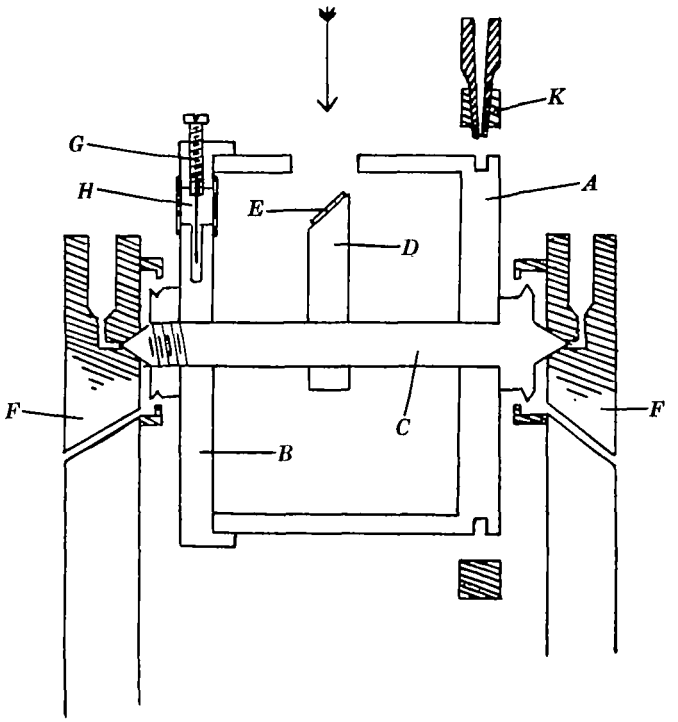


Fig. 2. Direct vision microscope centrifuge (vertical section). *A*, rotor case; *B*, rotor cap containing capillary; *C*, mounting rod; *D*, mirror carrier; *E*, mirror; *F*, supports and bearings; *G*, adjusting screw carrying capillary; *H*, cross-hole closed by cover-slips and filled with clove oil; *K*, ring carrying driving jets. Arrow shows optic axis of microscope.

zontal mounting lies in the fact that the lighting system can be mounted on the bench where adjustments can be made conveniently; also, the microscope axis can be vertical or inclined at any convenient angle.

The rotor is a hollow cylinder (*A*) closed at one end and fitted with a removable cap (*B*) at the other. A steel rod (*C*) runs through the axis and is threaded at one end, a nut on this thread holding the cap in place. The ends of this rod are conical and are case-hardened and polished; these form the bearing surfaces.

The mirror carrier is a brass bar (*D*) fitting tightly on to the rod; this bar is bevelled at its outer end to form a flat surface at 45° to the axis. On this surface a stainless steel mirror (*E*) is fastened.

The stationary frame (*F*), which carries conical brass bearings, is mounted on the condenser carrier of a microscope and can therefore be centred under the objective. The bearings are fed with oil as shown in Fig. 2; as the oil works through the bearing, it is thrown off the rod and caught in the stationary rings from which it drains into a reservoir.

The method of mounting the specimen is as follows: the cell or cells to be tudied is drawn into a glass capillary as already described. The capillary is then sealed and cemented to the end of a screw (*G*) that fits into a radial hole in the rotor cap. This hole is crossed by a large hole (*H*) parallel to the axis, which is closed on each side by a cover-glass. The chamber so formed is filled with cedar-wood oil. The capillary after insertion is adjusted radially to bring the specimen into the centre of the field.

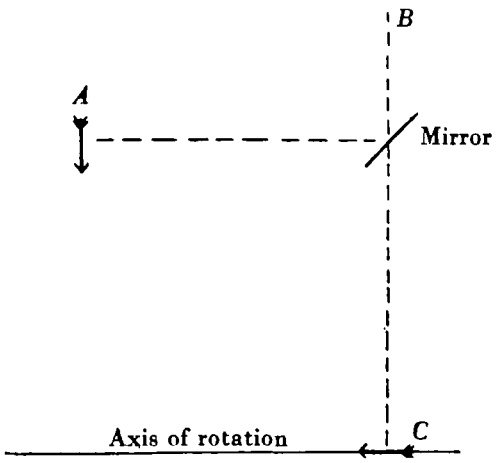


Fig. 3. Optical system. For explanation, see text.

Rotation is effected by compressed air. On the periphery of the rotor is a ring of shallow holes bored at a slight angle to the radius. Attached to the stationary frame is a ring (*K*) concentric with the rotor; this carries three air jets which are directed at right angles to the axis, and in line with the holes.

The light source is a pointolite lamp, whose electrode is focused on to a slit through a cylindrical lens. The slit is then focused on the object through a low-power microscope objective.

The theory of the optical system is shown in Fig. 3. The object is at *A* and is observed from *B*. The virtual image in the mirror is at *C*. The distances are adjusted so that this image lies on the axis of rotation. The result is, of course, that there is no apparent displacement of the object during the time it is being observed, but only a small rotation which is hardly perceptible.

Since high speeds were not required, the measurement and control of the speed were carried out by means of the disk and neon lamp as described before. The instrument was very simple to use, and, though not entirely satisfactory, it gave a clear solution to the problems for which it was constructed.

## OBSERVATIONS

The first point of importance which must be stated, is that the iron particles were found to remain in a vacuole which contained a large proportion of water. This vacuole moved as a whole; consequently the diameter of the moving body is that of the vacuole and not the diameter of the iron particles.

The forces used were much lower than those employed by other workers (50–100 g.), and actually might, with advantage, have been still lower, since the vacuoles moved rather too quickly for satisfactory timing. However, for various reasons, attempts to use lower forces were not successful; very marked irregularities appeared. For example, one application of the force might cause a movement of 20 $\mu$, while the next might produce no effect at all. The observations with the microscope centrifuge are thought to give a possible explanation of this phenomenon.

The calculation of the results was carried out as follows. The viscosity is obtained from Cunningham's (1910) modification of Stokes's formula,

$$V = \frac{2cg(\sigma-\rho)a^2}{9\eta},$$

where $c$ is the acceleration as a multiple of gravity, $\sigma$ the specific gravity of the particle, $\rho$ the specific gravity of the fluid, $a$ the radius of the particle, and $\eta$ the viscosity.

The average food vacuole is about 14 $\mu$ in diameter and might, for example, contain seven iron particles, 10% of its volume. If we assume that the rest of the vacuole is filled with water, its density as a whole works out at 1·7. Actually there is some food material in the vacuole, but, since the normal vacuole tends to move in a centripetal direction, its density cannot be more than 1·038 that of the animal. This will make no significant difference to the figure already given.

When a *Paramecium* with such a vacuole was subject to a centrifugal force of 67 g., it was found that the average velocity of movement of the vacuole was 0·005 cm./sec. Actually the average distance moved in nine consecutive runs was 160 $\mu$. The variation was about 10%. These figures give a viscosity of 0·5 c.g.s. units. It is considered that this figure is probably too high.

The effect of temperature changes on the viscosity was studied with numerous specimens, but no reliable result could be obtained. It was clear, however, that there was no trace of a maximum around 16° C. as stated by Heilbrunn (1928). The results tended to show a decrease from about 0·9 to 0·1 in passing from 10 to 38°, but were too irregular to give any indication of the rate of change between these temperatures.

The microscope centrifuge gave some useful results. The first point noticed was the effect of using a rather wide tube in which to centrifuge the animals. The *Paramecium* tended to turn obliquely across the tube, so that the vacuole, moving radially, came against the side and moved along it at a very greatly reduced speed. On repeating this with a low force, it was found that, when subjected to about 45 g. or less, the vacuole would cross the animal obliquely and then remain against

the pellicle without moving. The movement could be restarted by increasing the force.

It was also possible to see the interference caused to the movement by the nucleus. Further, the movement was sometimes checked for no apparent reason; it is suggested that the excretory canals may be responsible for this effect.

With the design of centrifuge used it was not possible to control the speed with sufficient accuracy to enable any viscosity measurement to be obtained. It is hoped to measure viscosity over small distances by means of a different type of rotor drive which will allow a predetermined speed to be accurately maintained. It seems certain that the average velocity of a body through a large part of the length of the animal is much less than the maximum on account of the numerous obstructions, other food vacuoles, nucleus, and other cell organs which may impede its movement.

## DISCUSSION

The centrifuge technique has been attacked by several workers who rely on other methods. Chambers (1924), for example, objects to "The rather drastic action of centrifugal force on the delicately balanced states of viscosity". Actually the centrifugal force seems to have a surprisingly small effect on the cell. The most remarkable evidence on this point is given by the work of Guyer & Claus (1936), who studied the redistribution of the components of anterior pituitary cells after ultra-centrifuging at 400,000 g. In this case the cells, which were transplanted into the animal after centrifuging, recovered their normal appearance after a few hours, and were otherwise unaffected.

Since Heilbrunn's forces were only about a hundredth of these, and those used in the present work about a thousandth, it seems safe to assume that the injurious effects may be neglected.

Heilbrunn (1926) discusses in detail his figures for *Arbacia* eggs. He measured the specific gravity of the granules by finding the sugar solution in which they would just move centrifugally. It seems important to know what variations in density occur between individual granules. Since the relevant term is so small, a slight variation in it would make a large difference in the velocity. Also, two sets of eggs gave values for the protoplasmic density, differing by about 30%. It seems strange that this difference was not considered to be significant, the measurements being averaged to calculate the viscosity. When eggs of the same species differ in density by 30% it is not unreasonable to expect some other measurable physical or chemical difference.

Heilbrunn, in this work, also makes an estimation of the Cunningham correction for the interference between the granules. He apparently does not take into account the fact that, as the movement proceeds, the granules become closer together and their mutual interference greater. Thus the correction might be more accurately calculated from an average spacing and not, as is done, from the maximum spacing of the granules.

On the whole the experiments show clearly that the viscosity of these marine

eggs is very low, and that it is not therefore comparable to that of glycerine as stated by Seifriz. It is therefore an obvious inference that this author's method of inserting comparatively large nickel spheres into the eggs, has some very marked effect on the physical state of the protoplasm.

It is suggested that these methods, which are dependent on the introduction of bodies into the cell, are only valid in the particular case where the cell is accustomed to ingest solid particles during its normal life, as in some Protozoa and Myxomycetes. The low value obtained for the protoplasm of the latter is evidence that the organism was not seriously affected by the introduction of inert bodies. The same principle is applicable in the present case; *Paramecium* normally ingest solid bodies, so that we need not expect any disturbance to arise from Fetter's method of making them ingest heavy particles.

As previously mentioned, Fetter's results are not comparable to any others obtained by centrifuge technique. It would appear then that Fetter's work needs careful consideration.

Unfortunately her wording is not at all clear on several important points. First, there is no mention of the fact that the iron is in a vacuole. Fetter states that "The density of the iron moistened as it would be in the body was found to be 1·5". Apparently the iron was an amorphous powder, and its density when saturated with water was found. The density of the vacuole as a whole would then be much less. Even assuming that the vacuole was three-quarters full of the water-saturated iron, its density would be 1·12 instead of 1·5. This would make a difference of nearly 600 % in the result. It seems certain that Fetter did not notice the vacuole, since, when using starch, the density used in the calculation is that of the starch; if a vacuole had been allowed for, the amount of water would have been taken into account.

As well as this difficulty the technique itself introduces a serious error. As has been shown, the vacuole is greatly retarded as it approaches the pellicle. It is unlikely that more than a very small proportion of the animals in a wide tube would become fixed in a position with their long axis parallel to the centrifugal force, yet only those few could give even an approximate indication of the true velocity of movement. It is apparent, therefore, that the results may have little or no meaning.

While it is considered that the present work gives a more reliable result, it appears certain that the value obtained is a maximum for the following reasons. The density and diameter of the vacuoles can be determined with some accuracy. In the particular case mentioned the density cannot be less than 1·7 or more than 1·73. The diameter (14 $\mu$) is sufficiently large to be measured without serious error. The applied force can also be known within narrow limits. On the other hand, the technique does not give a reliable value for the velocity of movement, since it does not take into account the retardation caused by various intracellular structures. It does, however, give a minimum value for this speed, and therefore a maximum for the viscosity.

It is possible that the complex structure of a protozoan cell may invalidate the

use of a moving body as large as the vacuole. Fetter suggested that the high viscosity which she found might be due to the presence of a neuromotor system as described by Rees (1922). On the other hand, Lund (1933) has stated that the neuromotor system in *Paramecium* is located almost entirely at the periphery. If this is so, the structure could not have any effect on the viscosity measurement.

Nevertheless, there is a little understood state of organization in the protozoan cell (with great variation between different species) which, unless the movement is studied in different parts of the cell, might cause large and incalculable errors in the viscosity measurement.

One point, slightly outside the scope of this work, on which the microscope centrifuge has given some evidence, is the condition of the material underlying the pellicle. The fact that the vacuole, moving under the action of a low force, stops when it reaches this layer, and requires a higher force to restart it, is evidence that the substance in this region is of an elastic or plastic nature.

## SUMMARY

1. An improved technique for measuring the protoplasmic viscosity of certain types of cell is described.

2. It is shown that the viscosity of *Paramecium* is not greater than 0·5 c.g.s. unit, and that it may be much less.

3. A simplified type of microscope centrifuge has revealed several sources of error in the centrifuge method customarily employed for the determination of protoplasmic viscosity.

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