Is Protoplasm ever Fluid?

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SUMMARY

The viscosity of the hyaline (granule-free) cytoplasm of the immature egg of *Spisula* (Lamellibranchia) was determined by use of the Einstein equation for Brownian movement. The value obtained was 4·3 centipoises. Thus this protoplasm has a viscosity similar to that of a concentrated protein solution. The viscosity of the entire cytoplasm, granules and all, is much higher. This is due to the great concentration of granular material. Study of the movement of the large germinal vesicle through the cytoplasm under the influence of different centrifugal forces indicates that the viscosity of the entire cytoplasm, granules and all, does not decrease as the shearing force increases. Hence, apparently, the entire cytoplasm behaves like a true Newtonian fluid—at any rate there is no evidence of thixotropy. The idea that protoplasm is typically a gel is not in accord with the evidence presented here. Additional evidence for the fluidity of protoplasm is provided by earlier information about the osmotic behaviour of cells, the formation of ice crystals in protoplasm, the rapid dispersion of aqueous solutions injected into cells, the outflow of protoplasm from cells broken in calcium-free media, and by magnetic studies.

FROM the very beginning of the study of protoplasm, biologists have been concerned with the question whether protoplasm commonly was fluid or whether it has some of the properties of a solid. With the rise of the science of colloid chemistry, this question became translated into terms of sol and gel.

Many distinguished authorities have felt it necessary to believe that in view of the complicated processes that occur in any living cell, protoplasm could not be fluid. Thus in his classical book on general biology, Oscar Hertwig (1909) was led to state: 'Wie Nägeli und viele andere Forscher, sind wir der Überzeugung, daß die komplizierten Erscheinungen des Lebensprozesses, vor allen Dingen der Vererbung, nicht aus den Eigenschaften von Flüssigkeiten oder gelösten Stoffen erklärbar sind', and he was of the opinion that the only thing that is fluid about protoplasm is the water it contains. This opinion has persisted to the present. Wald (1954) wrote: 'Lately it has become clear that very little of a living cell is truly fluid.' In 1956 Swann wrote that 'even the most fluid-seeming protoplasm is in fact a gel'. Perhaps in recent years what has been the most convincing argument in favour of a solid protoplasm is the fact that in many different types of cells a complicated reticulum has been discovered with the aid of the electron microscope.

The question whether or not protoplasm is ever fluid is an important one for various branches of physiology. So, for example, for any theory of muscular contraction it is important to know whether the mechanical changes that occur are due to a sol–gel or gel–sol transformation. So too the pharmacologist is

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interested, or should be interested, in knowing what various drugs may do to the colloidal properties of protoplasm, and in order to understand these effects it is necessary to have some notion as to what untreated protoplasm is like. Similarly, the pathologist is concerned, or should be concerned, as to how degenerative changes affect the protoplasmic colloid.

In what follows, no attempt will be made to discuss the voluminous literature concerning the fluidity or viscosity of protoplasm. Such discussion will be found in various books (Heilbrunn, 1928, 1956, 1958). All that we shall do here is to attempt an answer to the argument that protoplasm is never fluid. Actually it is hard to understand how such an argument could ever have arisen. For when Swann (1956) states that the most fluid-seeming protoplasm is in fact a gel, he can scarcely believe that the protoplasm in an amoeba or in a plant cell, such as a cell of *Nitella* or *Elodea*, is not *actually* flowing. And if it is flowing, it is by definition a sol, not a gel. And how can one speak of sol–gel change in protoplasm, as Swann does, if protoplasm is never in the sol state?

We should like to raise the following points, buttressing them by new observations whenever possible. Some of the points may be open to argument, but the sum total of the evidence seems convincing.

1. Living cells show osmotic behaviour. They swell when placed in hypotonic solutions. This, it seems to us, could only happen if the interior of the cell were fluid. Gels do not ordinarily swell in hypotonic solutions; indeed, the presence of certain salts in increasing concentration often favours the swelling of gels. In one experiment, performed recently, eggs of *Spisula solidissima* (Lamellibranchia) were placed in a solution consisting of 60 volumes of sea-water and 40 volumes of distilled water. (This is a hypotonic solution, for sea-water is isotonic for the clam eggs.) In the hypotonic solutions, the volume of the eggs increased on the average from 32,000 to 42,000 cu. μ. On the contrary, eggs treated with osmium tetroxide in sea-water and then immersed in 60 volumes of sea-water with 40 volumes of distilled water did not show any increase in volume. Indeed, their volume, if it changed at all, seems to have suffered a slight decrease—from 41,900 to 38,800 cu. μ. In the osmium tetroxide solution, the protoplasm is quickly converted from sol to gel, as can readily be shown by viscosity tests made by the centrifuge method.

Although modern cell physiologists are generally agreed that living cells show osmotic behaviour, a contrary view is held by Troschin (1958).

2. If frog muscle-fibres are cooled to -1 or $-2°$ C and are then seeded with ice crystals, masses of ice crystals form within the protoplasm (Chambers and Hale, 1932). A gel would not behave in this way, for ice crystals appear in gels only at very low temperatures.

3. When marine egg cells, for example sea-urchin eggs, are placed in solutions from which calcium has been removed by oxalate, and are then compressed until their outer membrane breaks, the protoplasm gushes out in an unbroken stream and then mixes with the surrounding medium. No one watching this unhampered flow would be likely to doubt the fluidity of the protoplasm. Similar outflows can be observed in protozoan cells. In the case

of the giant amoeba, *Chaos chaos*, the outflow occurs not only in the absence of calcium, but also in dilute solutions of heparin (Heilbrunn, Ashton, Feldherr, and Wilson, 1958). Often when amoebae are placed in dilute heparin solutions, the outflow occurs even without external pressure, and the material in an advancing pseudopod disperses into the surrounding watery medium.

4. If an isolated muscle-fibre of a frog is injected with a saline solution coloured with a vital dye, the fluid as it is injected mixes with the interior protoplasm readily. This would presumably not be the case if the protoplasm were a gel, and indeed if the protoplasm is transformed into a gel by one treatment or another, the injected fluid remains as a separate droplet.

5. If specimens of *Amoeba dubia* are made completely quiescent by placing them in dilute solutions of potassium oxalate and are then examined with a horizontal microscope, it can be seen that the small, dark granules of this amoeba fall through the cell under the influence of gravity. There is no evidence of a network, and sometimes the stratification of granular material is so clear that the amoebae look like centrifuged marine eggs. Numerous experiments of this sort were done in our laboratory by Mr. Carl Feldherr. In these experiments it is essential that amoebae be completely motionless, for the force exerted by even a slight amoeboid movement is more powerful than gravity (as can readily be calculated from the speed of the movement compared to the speed of fall under gravity). Studies made on amoebae which are not completely quiescent obviously have no meaning. In oxalate solutions the viscosity of the protoplasm in the interior of an amoeba is increased, as has been shown both by the centrifuge method (Heilbrunn and Daugherty, 1933; Alsup, 1942) and also by the magnetic method to be described in the next section. In the case of marine eggs, it is of course not necessary to immobilize the cells. In the case of sea-urchin eggs and also eggs of the worm *Chaetopterus*, we have frequently observed granules falling through the cytoplasm merely under the influence of gravity; similar observations have been made by Howard (1932).

6. Correct magnetic studies of the protoplasmic colloid indicate fluidity. Years ago, Heilbronn (1922) introduced iron wires into large masses of slime mold protoplasm and then twisted them with an electro-magnet. In this way he obtained low values for the viscosity of the interior protoplasm, values much like those obtained with the centrifuge methods and the Brownian movement method for other types of protoplasm. Only in small slime molds, when the wires impinged on the outer cortex, was there any evidence of thixotropy: otherwise the protoplasm behaved like a true Newtonian fluid. More recently Crick and Hughes (1950) studied the effect of magnetic forces on iron particles taken up by fibroblast cells, and obtained some evidence of elasticity of the protoplasm. They recognized the fact that the method was not properly applicable if the magnetic particle was near a limiting membrane or if it was in a vacuole. Fibroblasts are very flat cells, and it is hard to imagine a particle being anything but close to a limiting membrane. In the one photograph that Crick and Hughes show, the iron particle is directly in contact with

the nuclear membrane. Also it seems clear that particles taken up by amoeboid cells are almost certainly in vacuoles.

The most interesting study is that of Ashton (1957), thus far reported only in preliminary fashion. He found that iron particles within the flowing protoplasm of a giant amoeba (*Chaos chaos*), when twisted by magnetic forces, showed no evidence of elasticity in the medium surrounding them. Moreover, movement of an iron particle with a magnet calibrated so as to give a force exactly equal to gravity at a distance of 1 cm gave values which could be used to calculate the viscosity of the flowing protoplasm. This calculation was made by the aid of Stokes's law. The values thus far obtained are essentially in accord with earlier values for amoeboid protoplasm, obtained both by the centrifuge method (Heilbrunn, 1929) and by the Brownian movement method (Pekarek, 1930). These values clearly show the fluidity of the flowing protoplasm of an amoeba, so that now there is agreement in the results obtained by three different methods.

Ashton has repeatedly attempted to shoot iron particles into sea-urchin eggs by placing a strong magnet under a mass of eggs packed rather closely together as a result of centrifugal treatment. Usually the eggs are completely smashed by this procedure, but in one case an apparently uninjured egg was caused to contain a tiny iron rodlet. This could readily be twisted by a magnetic field, almost as readily as the iron rodlets outside the egg. Then, on removing the magnet, both the rodlet inside the egg and those outside swung around so as to point to the magnetic north like tiny compasses. Anyone observing this experiment, as we did, could not fail to be impressed by the fluidity of the protoplasm. It is a much more significant experiment than the oft-quoted observation of Seifriz (1924). The latter never claimed to have got a magnetic particle into a cell interior, but only in one case into the cortex of a sand-dollar egg. Even this is doubtful according to a statement made to one of us by Robert Chambers, under whose direction the work was done. According to Chambers the nickel ball that Seifriz used only indented the protoplasm, and under the influence of the magnetic field the whole egg rotated. Actually it would scarcely be possible to push a large nickel ball (16 μ in diameter) into a cell merely by poking it with a microneedle. And yet this dubious experiment continues to be quoted, most recently by Giese (1957), who wrongly attributes the observation to Frey-Wyssling (Giese, 1958).

7. Perhaps the most impressive support for the belief that the protoplasm of cells is typically a solid mass comes from many recent studies with the electron microscope. Valuable as this instrument is, it can be used only on dead material. The cells have been treated with drastic fixatives and have then had all water removed from them. The recent work is reminiscent of the older studies of cytologists in the latter half of the nineteenth century. At that time Frommann and Heitzmann among the zoologists and Strasburger and Schmitz among the botanists claimed that the essential nature of protoplasm derived from the fact that it was a network. This older work is discussed in some detail by Berthold (1886) and by Bütschli (1892), and numerous references can be

found there. Various authors, including Hardy (1899), Fischer (1899), and Mann (1902), studied the effect of common fixatives such as osmium tetroxide, formaldehyde, chromic acid, dichromate, &c., on protein solutions and were able to show that these fixatives caused a precipitation which might take the form of granules, filaments, or networks. Moreover, the older observations which indicated the presence of a network in living cells were sharply criticized by Bütschli, who pointed out that the vacuolization which so often accompanies death or degenerative changes was often erroneously described as representing a protoplasmic network. A similar mistake has apparently been made recently. Thus Fawcett and Ito (1958), in their attempt to show the presence of an endoplasmic reticulum in living cells, seem to have fallen into this error in their study of cells removed from the testes of guinea pigs. In cells that the authors state were 'gradually succumbing to unfavorable environmental conditions', they described various structures, some of which are certainly typical vacuoles such as have repeatedly been described in dying or injured protoplasm. They believe that the appearance of what they consider to be a reticulum is not to be regarded 'as a terminal event in moribund cells', for in these obviously dying cells the mitochondria stay intact. This seems to be a rather weak argument, for even in smashed-up cells, mitochondria may stay intact. Moreover, in the cells that Fawcett and Ito tear out of the testis, cells which are presumably already somewhat injured by the isolation process, there is no observable endoplasmic reticulum.

8. If an endoplasmic reticulum, or some structure similar to it, were really present in a living cell, then the protoplasm of such a cell would presumably not behave as a fluid. Because lamellar structures comparable to an endoplasmic reticulum have been described in the immature egg of the surf-clam, *Spisula solidissima* (Rebhun, 1956), it was thought wise to attempt a physical study of the living egg to see if support could be found for Rebhun's studies with the electron microscope. There was also an additional reason for studying the immature *Spisula* egg. As with other immature eggs with large germinal vesicle, the cytoplasmic granules in the cytoplasm do not move readily under the influence of centrifugal force, far less readily than such granules move in the mature eggs of the sea urchin *Arbacia*, the worm *Chaetopterus*, or indeed in the mature egg of *Spisula* itself. Indeed it is generally held that the protoplasm of immature eggs is much more viscous than it is after the germinal vesicle has broken down. Because of the difficulty encountered in getting the granules to move through the cytoplasm of immature *Spisula* eggs, Allen (1953) was led to conclude that the cytoplasm was a gel, and he found support for this opinion in the fact that, after centrifuging, a ring around the nucleus is negatively birefringent.

The work on *Spisula* protoplasm was done at the Marine Biological Laboratory in Woods Hole. Details of our work on *Spisula*, as well as similar work on other marine eggs, will be presented in a paper to be published later. The main facts are these. When *Spisula* eggs are centrifuged so that the heavy granules of the cytoplasm are moved to the centrifugal end of the cell, the rate of return

of these granules can be measured. Then by applying the Einstein equation for Brownian movement, the viscosity of the hyaline (non-granular) cytoplasm can be measured. The Einstein equation is

$$D_x^2 = \frac{R}{N} \cdot \frac{Tt}{3\pi\eta a},$$

in which D_x is the displacement of a particle along one axis in the time t, and at the absolute temperature T, R is the gas constant, N the Avogadro number, η the viscosity, and a the radius of the moving particle. In order to apply the Einstein equation, it is only necessary to know D_x, t, and a. It is not difficult to determine D_x, although no great accuracy is possible. Measurement of the diameter of the heavy granules gave a value of approximately 0·8 μ. This

TABLE I

The viscosity of the hyaline protoplasm of immature Spisula *eggs*

D_x	t	$\eta \times 100$
(μ)	(sec)	(centipoises)
10	360	4
15	300	1·5
10	360	4
15	518	2·5
8	480	8
10	480	5
8	595	10
10	180	2
6	149	4·5
6·5	152	4
13	225	1·5
9	344	4·6
10·5	366	3·7
6	174	5·3
		Mean 4·3

measurement is also somewhat uncertain, for the granules are never completely at rest while the measurement is being made. We centrifuged the eggs for 4 min with a force 9,000 times gravity. In eggs taken from small clams, this was sufficient to move the heavy granules as far as they would go toward the centrifugal end of the egg. The fatty granules moved in the opposite direction; usually some of them formed a ring around the large nucleus, and this is doubtless why Allen found this region to be negatively birefringent.

We measured the time it took for some of the heavy granules to move a given distance (D_x), and thus we were able to calculate the viscosity. Table I shows the results of 14 determinations. The average value for the viscosity was found to be 4·3 centipoises. This is the type of value that one might expect for a concentrated protein solution. After the eggs have been fertilized, the large nucleus breaks down and releases its fluid into the cytoplasm. Then as the concentration of granular material becomes much less, the granules move much more

readily when the eggs are centrifuged. And yet, surprisingly enough, the viscosity of the hyaline protoplasm through which they move remains approximately the same.

The low value for the viscosity of the hyaline protoplasm of the *Spisula* egg is surely an indication of the fluidity of this non-granular protoplasm. It might perhaps be objected that the Einstein equation is not very accurate; and indeed it is not, for in its derivation Einstein took no account of the electrical charges on the moving particles. Although earlier tests of the equation gave results consistent with it, some more recent studies have shown that the observed values for D_x may be less than the calculated values. For example, Bender and Mouquin (1952) found observed values only one-third to two-thirds as great as the values obtained from the equation. But any divergence of this sort would only lower the values obtained in our studies and would thus indicate that the protoplasm was even more fluid than we found it to be. On the other hand, when granules are centrifuged to the heavy end of the egg, their Brownian movement is not perfectly random, at least at the start, for their movement in the centrifugal direction would tend to be blocked. This would tend to increase the value for D_x in the centripetal direction. Fortunately, these two sources of error would tend to balance each other.

Also, it might be argued that the centrifugal force used to move the granules destroys a reticulum originally present in the egg cytoplasm. This is possible, and yet the *Spisula* egg is unusually resistant to centrifugal force, and it can develop normally even after violent centrifugal treatment. Certainly the centrifuge does not cause anything like the damage that fixation and desiccation do. It is also conceivable that the cytoplasmic reticulum has wide meshes and that the small granules sift through it almost as if it were not there. Moreover, in Rebhun's studies, the lamellae which he described occupy only a small volume of the cytoplasm. However, Palade, one of the leading authorities on the endoplasmic reticulum, believes that 'it is present in all animal cells' (Palade, 1956), and he notes that 'it appears that the endoplasmic reticulum is a continuous network of membrane bound cavities permeating the entire cytoplasm from the cell membrane to the nucleus'.

It may be difficult to show with any certainty that no reticulum of any sort exists in the cytoplasm, but if there is a reticulum it must be a weak structure indeed, one without much influence on the physical nature of the cytoplasm. For if one determines the rate of movement of the large nucleus (germinal vesicle) under the influence of centrifugal force and uses this rate of movement as a test of the viscosity of the entire protoplasmic suspension, granules and all, then it can be shown that the relative viscosity determined in this fashion remains the same whether a force 9,000 times gravity is employed, or a force only one-fourth as great. Thus within this range of shearing forces, the protoplasmic suspension behaves like a true Newtonian fluid. Accordingly it seems safe to conclude that if any reticular structures are present, they can be of little importance in influencing the colloidal state of the main mass of the protoplasm.

If our observations are correct, then it is clear that the cytoplasm of the immature *Spisula* egg owes its high viscosity to the high concentration of granules within it. Such a high concentration would undoubtedly lead to high values for the viscosity of the entire protoplasmic suspension (for a discussion of the viscosity of concentrated suspensions, see Heilbrunn, 1958). And yet the hyaline, non-granular protoplasm is quite fluid. Perhaps in all cells with a high concentration of granular or filamentous material, the viscosity of the hyaline protoplasm or ground substance may be low. This is apparently true for the protoplasm of *Paramecium*, as indicated by the work of Larsen, which was discussed in some detail by Heilbrunn (1956). In the near future we hope to investigate the physical properties of the cytoplasm of other types of cells in which the cytoplasm is clogged with granular material.

In conclusion, the important question is really this. If we are to understand the physical properties of protoplasm, are we to depend on the information gained from coagulated, thoroughly desiccated cells, which have lost every vestige of vital activity, or should we not lay more stress on what can be learned from physical studies of cells which are alive and uninjured? The methods we use for a study of protoplasm may not be perfect, but until better methods are discovered, it is to these methods that we must look for proper information. Both centrifugal measurements and measurements of Brownian movement have shown that in various types of cells, both plant and animal, the main mass of the cytoplasm has a low viscosity. We know of no proper measurement for any uninjured cell that has given information to the contrary, and therefore we deny that 'even the most fluid-seeming protoplasm is in fact a gel'.

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