THE PHYSICAL PROPERTIES OF CYTOPLASM

A STUDY BY MEANS OF THE MAGNETIC PARTICLE METHOD

Part I. Experimental

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A. INTRODUCTION

THIS PAPER has two aims: first to describe our experimental work on the physical properties of the cytoplasm of chick fibroblasts in tissue culture, with a short discussion of its implications and secondly to present such details of the magnetic particle method as to enable other workers to use it.

A second part will be devoted to the theory underlying these methods.

B. TECHNIQUE

In this section we shall describe in outline the methods we have used, leaving the details to later sections.

Our basic technique, which was suggested to us by Dr. Honor B. Fell, is to allow cells growing in tissue culture to phagocytose small magnetic particles, and then to move these particles in the cytoplasm by means of external magnetic fields. It is possible to make certain limited deductions about the physical properties of the cytoplasm from the character and magnitude of the movements.

The magnetic materials we have used have been in a finely divided form. The material is added to the culture medium of the original explant. The cultures are then allowed to grow for three days after which they are subcultivated into the normal medium. In the subsequent outgrowth, cells containing phagocytosed particles can be observed under high power. The explants were usually from the frontal bone of an 11 or 12 day chick embryo.

In applying the magnetic field to the particles within the cells we have experimented with three types of movement, which can be described as

(a) twisting, (b) dragging, and (c) prodding. We shall amplify these in turn. Our work on the two last has been preliminary only.

(A) TWISTING

We have done this in two ways; firstly, by applying a large field parallel to the length of the particle, and then turning this field through a small angle; secondly, by making the particles within the cells into little permanent magnets (by magnetising them in an initial very large field, applied momentarily) and then observing their motion when a relatively small field is applied, usually perpendicular to their length. This latter method has proved to be the more useful, and has been adopted for the greater part of our work.

(B) DRAGGING

A uniform magnetic field produces a pure twist on a magnet. In order to drag it from one place to another a non-uniform field must be used, and to produce appreciable movement, a very high field-gradient is necessary. This implies that the poles of the external magnet must be as near the magnetic particle as possible. To do this so that we can observe the particles during the movement, Dr. H. B. Fell was able to produce tissue cultures on cover-slips no more than 4 mm. in diameter. The polepieces can then be brought up to one side of this culture, and the cells can then be observed with a $\times 20$ objective.

(C) PRODDING

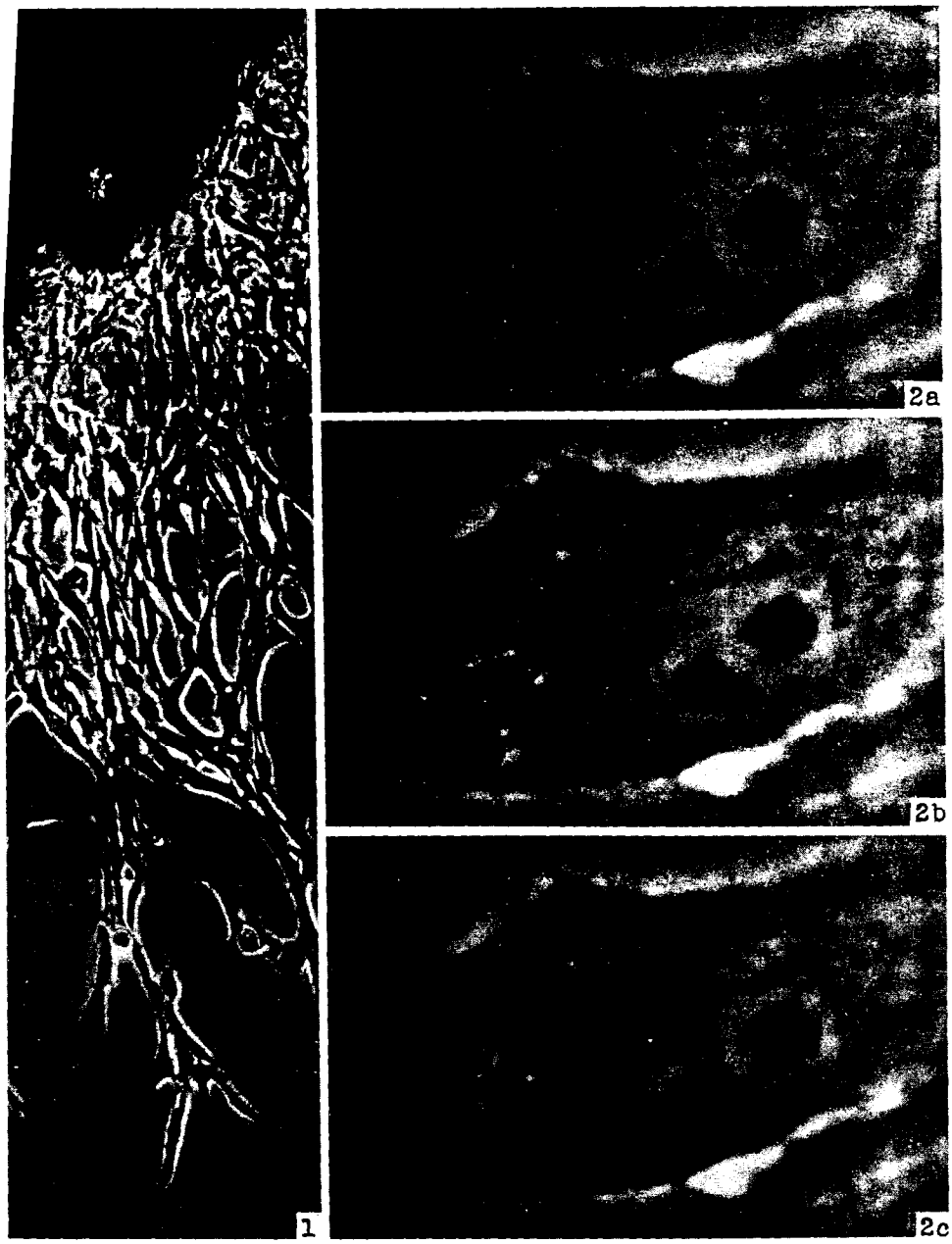
This is really a special case of the first form of twisting. If a cell contains numerous magnetic particles, a strong magnetic field will loosely unite the particles into a rod. This rod, which may be longer than the width of the cell, can be rotated by the field to bear upon the structures of the cell. We have thus been able to prod the cell membrane outwards, and the nuclear membrane inwards.

The descriptive part of the paper is presented under these three headings. However the greater part of it falls under Twisting, so this has been subdivided into a section on the details of the method and one describing most of our experimental results.

PLATE I

Fig. 1. Part of a culture of embryonic chick bone containing magnetic material to show normal outgrowth. Phase contrast $\times 200$. The magnification refers to the *unreduced* plate.

Fig. 2. One cell in the outgrowth shown in Fig. 1 containing a magnetic particle. Phase contrast $\times$ 4,900. (a) before actuation by magnet; (b) after magnet was moved across culture: (c) magnet again moved but in reverse direction. In this cell, the particle is unusually close to the nucleus). The magnifications refers to the *unreduced* plate.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2a
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C. SUMMARY OF THEORETICAL CONCLUSIONS

(To be given in full in Part II)

(1) On the scale of our experiments, inertia delays the attainment of equilibrium by only a very short time (less than 1 milli-second) and can therefore be entirely neglected. This is because the particles are so small.

(2) Consider a particle within a fluid, the whole enclosed by a boundary as in Text Fig. 1, then if the scale of the system is varied, and the relative proportion of the particle and boundary is kept constant, the effect of size is as follows:

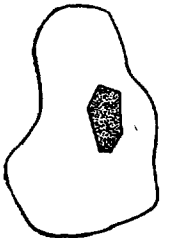

Fig. 1. Diagram to illustrate effects of boundaries.

(a) For twisting: none. The angular velocity for given magnetic conditions is independent of size. This is true for both newtonian and non-newtonian liquids.[1]

(b) For dragging: the velocity decreases as the size decreases. For a newtonian liquid the velocity is proportioned to the square of the linear dimension.

(3) The effect of boundaries at a short distance from a short particle (say at a distance $\frac{1}{4}$ the greatest dimension of the particle) is to produce an increased resistance. We consider a newtonian liquid.

(a) For twisting: the resistance is increased moderately. (Probably by less than a factor of 1.6 for the case quoted). This remains true unless the boundaries are put very close to the *ends* of the particle.

(b) For dragging: the resistance is increased much more. For the particle moving inside a cylinder, parallel to the axis, with a clearance equal to the average dimension of the particle, it is probably increased by a factor of about 2 or 3.

(4) The forces vary in a complicated way with the *shape* of a particle, but approximate methods are given in Part II for estimating them for newtonian liquids.

[1] A liquid is said to be newtonian when its viscosity is independent of shear-stress. A simple account of viscosity, thixotropy, etc. is given in Heilbrunn, 1943, page 61.

In our experiments there is also a variation due to the magnetic properties of the particles. The combined effect of these two factors is to make absolute measurements on the twisting of the individual particles only approximate (see page 55).

(5) For a non-newtonian liquid it is not possible to obtain any but the crudest estimates of the variation of viscosity with shear stress with these methods. The values given by the newtonian formulae are some indeterminate average values.

(6) The behaviour of the particles in an elastic medium can be calculated for small strains from the corresponding formulae for a viscous medium. In a visco-elastic medium it is possible to calculate the "relaxation time" (if this is constant with stress) without knowing the precise nature of the particle and the boundaries.[1]

(7) In dragging experiments, the force on a magnetically saturated particle is proportional to the field gradient. A particle is not necessarily pulled parallel to its length. A magnetically saturated particle can never be in true stable equilibrium under magnetic forces alone.

(8) To obtain a large drag on a particle, it is essential to have iron not far from the particle. The value of the field-gradient is of the order of $(B-H)/Xo$ in well-designed systems, where $(B-H)$ refers to the iron near the particle, and Xo is the distance of this iron from the particle.

(9) Air-cored coils cannot compete with magnets for producing sustained field-gradients, because of the heating effects.

(10) On *small* particles the shear stresses in the fluid due to twisting magnetically are, in general, rather bigger than those due to dragging magnetically. Both these stresses are much greater than those caused by gravity. The shear stresses produced by centrifuging natural inclusions of radius 1 μ can only equal those produced by twisting magnetically if the centrifugal fields are of the order of 100,000 g. This is in the region of the fields given by the ultracentrifuge.

D. TWISTING: APPARATUS, TECHNIQUE AND CALIBRATION

1. *Biological Technique*

The explants were obtained, unless otherwise stated, from the frontal bone of 11 or 12 day chick embryos. They were grown in hanging drop pre-

[1] The terms in this paragraph are explained in the Appendix, page 80.

parations on $1\frac{1}{4}''$ square No. 2 coverslips over $3'' \times 1\frac{1}{2}''$ hollowground slides. The medium consisted of equal parts of chick embryo extract and fowl plasma. When required a small amount of a dense suspension of the magnetic material was added to the fowl plasma. In the early experiments it was sometimes impossible to see the explant clearly because of the black magnetic material.

After incubation, usually for three days, each culture was transplanted, generally into two fresh hanging drop preparations. No more magnetic material was added to the new embryo extract — plasma medium. The outgrowth was cut away except for a narrow margin round the original explant. This time a No. 1 coverslip was used, which was mounted on a special metal ring, with another coverslip on the lower side of the ring, to facilitate observation. Both were sealed on with paraffin wax (Hughes and Fell, 1949).

The explants still contained magnetic material, and many of the fibroblasts growing out into the medium contained one or more magnetic particles which they had phagocytosed. The growth appeared normal and vigorous, and it was possible to sub-cultivate several times satisfactorily. On each occasion some of the cells migrating into the new medium contained magnetic particles.

This particular method of preparation and mounting allows easy observation of individual magnetic particles in cells migrating into the clean medium.

The special cultures developed for the dragging experiments are described on page 70.

2. *Possible Biological Artefacts*

One of the advantages of the method is the comparatively small disturbance of the cell which results because the particles enter the cell 'naturally' without forcible introduction as in the experiments of Seifriz (1924). We have not made quantitative studies of the rates of growth of cultures with and without the magnetic material, but we can state that there is no obvious difference. Cultures with a layer of magnetic material so dense that it was difficult to see the explant have been known to grow as actively as control cultures. In such extreme cases it is difficult to place the knife cuts when subculturing, and this would account for certain cases where the growth was poor on one side. However, in the cultures with smaller amounts of magnetic material this caused no difficulty, and the rate and amount of growth appeared very similar in both treated and control cultures. Moreover, the appearance of the cells — the amount of mitochondria, fat, vacuoles and so forth — though naturally very variable, showed no consistent difference due

to the particles, nor in a given culture did cells without particles appear obviously different from cells with them. Cells containing much magnetic material passed normally through mitosis.

Although in general we have only used the first subcultures on one or two occasions we have for special purposes subcultivated several times, when the growth was always normal. It is thus fairly certain that the magnetic material produces no gross interference.

It is possible that the particle inside the cell is within a cytoplasmic vacuole. If this were so, our twisting experiments would measure some property of the vacuole rather than of the cytoplasm itself. Such vacuoles, which show clearly in the phase contrast microscope, can accumulate neutral red, and show active Brownian motion in older cultures during the second day after sub-cultivation. Small magnetic particles appear to become attached to the surface of these vacuoles. (If they were free inside they would quickly fall to the bottom.) It is not easy to see whether they are just inside the surface, or just outside, because one is working near the limits of resolution of the optical system.

Particles attached to vacuoles behave in a characteristic way when a magnetic field is applied. Firstly they show translation in addition to the usual rotation. This is presumably because they are constrained to move only in the surface of the vacuole. Secondly, the movement is typically very irregular and abrupt. For example, they may not move at all when the field is switched on, and then suddenly move rapidly to another position. We have not studied this movement in any detail.

Although we can be sure that a magnetic particle apparently free in the cytoplasm is not attached to a large vacuole, it is not absolutely certain that we could detect optically a vacuole which just surrounded a medium-sized particle. The vacuoles, however, are typically spherical and we could probably detect a vacuole around an elongated particle. Moreover, we might well expect the vacuole to grow with time, so that even if we could not see it at the beginning of an experiment it would be visible several hours later. This we have never observed.

In two cases after measurements had been completed on a particle we stained the living culture with neutral red. It is not certain that this method would disclose a tightly-fitting vacuole any better than phase-contrast, and there is the additional danger that the neutral red itself may produce a vacuole. However, in these instances no enveloping vacuole was apparent, although in one cell a very small watery vacuole was demonstrated near the fairly large particle, as had been suspected from the phase-contrast observations.

We think it improbable, therefore, that the particles we have selected for measurement were actually in watery vacuoles. In our later experiments we were careful to select cultures and cells which as far as possible were free from vacuoles. We have found, incidentally, that cultures from the same batch may vary considerably in degree of vacuolation of their cells.

There remains, however the more profound objection that the particle, while not producing a vacuole, may modify the physical properties of the adjacent cytoplasm. This is clearly impossible for us to refute. The only approach would be to repeat the measurements by an entirely different method, and this we have not been able to do.

It may be worth while to note that certain modifications might only have a small effect on the rate of twisting. For example, if the cytoplasm solidified (or liquified) around the waist of an elongated particle, but not at its ends, this would have little effect, as the greater part of the viscous couple comes from the forces at the ends of the particle. This is not, of course, a very likely alteration, although a small vacuole might conceivably form there. Again, if the protoplasm formed a solid layer a fraction of a micron thick all round the particle, this would only increase the viscous couple slightly, as the shearing would simply take place a little further out.

3. *Magnetic Materials*

We have regularly used two magnetic materials, and tried two others. The first was magnetite, an oxide of iron, prepared in colloidal form. It has ferromagnetic properties, but much less than those of iron or steel. The material we have used most was originally prepared as finely divided iron, but by the time we came to use it was largely oxidised. However, it had a much greater retentivity than the magnetite, and was thus more suitable for our second method of twisting.

The other magnetic materials we have tried were finely divided nickel, which proved to be toxic to the cultures, and powdered 'Alcomax', which proved variable in its magnetic properties, so that no further work was done on it. We shall only describe here the experiments with the first two materials.

MAGNETITE (Fe_3O_4)

This was prepared by Lefort's method, given by Welo and Baudish (1927). That is:

"281 grams of $Fe_2SO_4 + 10H_2O$ and 134 grams of $FeSO_4 + 7H_2O$ are separately dissolved in 750 cc. and 500 cc. of water, respectively. They are then mixed, and poured into a boiling solution of NaOH containing 250 grams of the hydroxide in 3,200 cc. of water. The black mixture formed on the addition of the sulphates is boiled for an hour. The mixture is near colloidal, so that the precipitate does not settle readily."

It was then washed with distilled water and centrifuged, repeatedly until neutral to litmus paper, after which it was dried in a desiccator.

We have not determined the B/H curve for this material, as we only used it for our preliminary experiments; where necessary we have used the one given by Welo and Baudish. It is possible that the ordinate of their curve should be multiplied by a factor to allow for the looseness of packing.

OXIDISED IRON

This was supplied to us by Dr. K. Hoselitz, Director of Research of the Permanent Magnet Association, for whose assistance and advice we are very grateful. He has sent us the following account of the method of preparation:

"We made an alloy of 50 per cent by weight of aluminium in iron in the high frequency furnace. This alloy, which is hard and brittle, was easily crushed and ground in a planetary ball mill. After grinding, the powder was graded and 100 gm of the following grading was selected:

$\frac{1}{2}$ passing 200 mesh.
$\frac{1}{6}$ passing 160 mesh, resting on 200 mesh.
$\frac{1}{6}$ passing 90 mesh, resting on 100 mesh.
$\frac{1}{6}$ passing 30 mesh, resting on 60 mesh.

This sample was well mixed, and then slowly added to an NaOH solution consisting of 100 gm of caustic soda in 400 gm water. This caused the aluminium to be dissolved out of the alloy. The reaction is exothermic and the solution soon reaches the boiling point, which accelerates the reaction and no attempt at cooling is made.

After the reaction has ceased the solution is filtered through a Buchner Funnel with 540 filter paper and washed with clean water until the washing water is no longer caustic. The wet sludge retained on the filter is scraped into a bottle and covered with distilled water for storage."

We kept the material under water and when a sample was required, the bottle was shaken and allowed to stand for a couple of minutes to permit the coarser material to sink. The top layers were pipetted off, and the iron was washed in glass distilled water and centrifuged repeatedly. It was then dried in a desiccator and used as required. Dr. Hoselitz has pointed out to

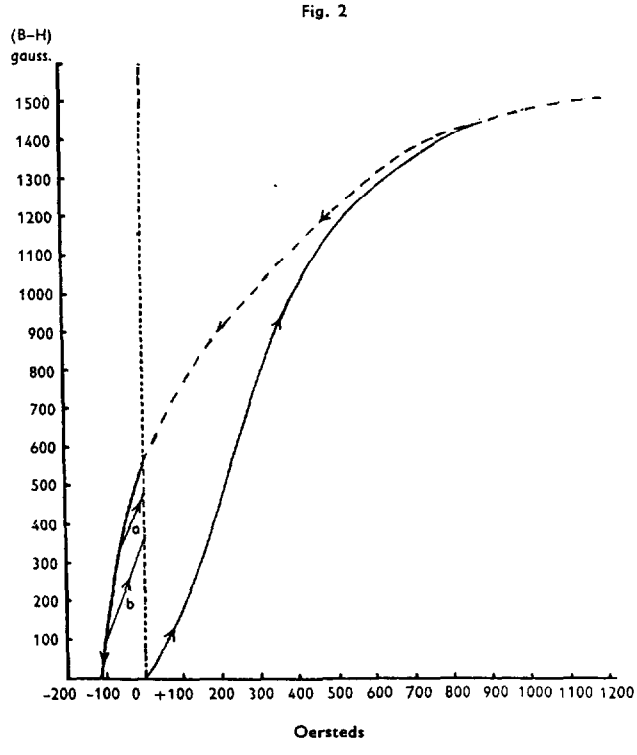

Fig. 2. (B–H) Curve of oxidised iron. (a) and (b) are recoil curves.

us that if the powder is dried it oxidises visibly, so that as we used it the material was substantially oxidised iron. An analysis by Messrs. Geochemical Laboratories has confirmed this. The percentage composition is as follows:

Iron	61.0
Aluminium	5.4
Combined oxygen	28.3
Water	2.1
Silica, manganese	2.2

The B/H curve was found by pressing the powdered material into a glass tube so that it formed a cylinder 15 cm. long and 0.4 cm. diameter. The length/diameter ratio was therefore so great that demagnetising effects could be neglected. The method used was the ballistic one, a flux coil being wound on the glass tube, and the whole placed inside a long solenoid. The curve of (B-H) v H is given in Figure 2. The values have been corrected for the effect of packing, by increasing the (B-H) figures in the ratio of

$$\frac{\text{apparent density}}{\text{true density}} = \frac{4.2}{2.0} = 2.1$$

so that they should represent average values for individual particles.

Owing to the low permeability the results are not very accurate, but they are good enough to show the essential features of the curve. The permeability is low (maximum about 3.6), and the material saturates at (B-H) equal to about 1,500. The back part of the curve shows that the effective value of (B-H) for ellipsoids of different length/diameter ratios does change, but not enormously.

TABLE I

Length/diameter ratio	1.0	2.0	3.0	5.0	∞
Effective (B-H)	225	325	390	460	550

The effective value of the slope of the curve over the part which interests us, is about 4½. The recoil curves show that a field of 50 oersteds applied in reverse momentarily to a short particle (axial ratio 2 : 1) may permanently lower its magnetic moment, probably by about 20 per cent. It would therefore be inadvisable to use fields much greater than this. Fields of this magnitude would not give exactly the usual variation of couple as cos θ (where θ is the angle between the applied field and the magnetic axis of the particle), but for our purposes the differences are not important.

4. *Methods of producing magnetic fields*

Our first method consisted in mounting a large U-shaped Alcomax magnet horizontally with the objective in between the poles. The size of the faces was 1.9 × 2.2 cms and the distance between them 2.2 cm. The mean length of the U was 13 cm. The bottom edge of the magnet was a few millimeters above the level of the culture. This magnet could slide horizontally in and out, so that the strength of the field could be varied from about 800 oersteds to 20 oersteds. It could also be rotated about the objective to change the direction of the field, over an arc of about 150°.

This magnet was used for the original experiments with magnetite. By simple mechanical means the magnet was made to perform oscillations of ±15°, with periods of the order of 5 to 15 seconds. This was done by means of an electric motor, a reduction gear, and a series of levers. This magnet was also used for the experiments on prodding, where a large field was re-

quired. In this case a different mounting was used which enabled the magnet to move round the full 360°.

Such a method suffers from the disadvantage that the magnetic field cannot be turned on and off either conveniently or quickly, so for most of our experiments we have used fields produced electrically. The main field was produced by a horizontal electromagnet. This consisted of a μ-metal rod, diameter 1.4 cm, length 14 cm, wound with about 900 turns of wire. This was mounted with one end of the rod 2.75 cm from the axis of the objective, and the end of the microscope stage was cut away to give necessary clearance. This electromagnet proved very satisfactory. The magnetic field was linear with the current up to about 50 oersteds, after which the metal saturated rapidly. The maximum field was 65 oersteds. There was no detectable hysteresis, as might be expected. In the experiments an air-cored coil was used to produce a small horizontal field at right angles to the first. This was mounted on the back of the hot-box about 8 cm from the objective. It gave a field of only 10 oersteds for a current of 4 amps, and it was thus not really strong enough. In our later experiments, therefore, we have used a second horizontal electromagnet mounted on the stage of the microscope. This has the same kind of μ-metal core as the first one. At a point near the centre of the magnet, but 3 cm from its axis, it gives a field of 60 oersteds for 1.2 amps, after which it saturates very rapidly. As now mounted, the second electromagnet moves with the mechanical stage, so that the field it produces under the objective for a given current varies a little. However, as it is only required for moving the particles into position or waggling them violently, this does not matter. Exact measurements have always been made when the first electromagnet was used. To produce vertical fields a small air-cored coil in the shape of a ring was mounted on the substage condenser, with its axis in line with that of the optical system. This had a mean radius of 3.6 cm, and was wound with 75 turns of 30 S.W.G. wire. This again was not fixed in position, and it was only used for manipulation. It gave a field of about 20 oersteds for 1 amp.

The calibration of these fields was done with a search coil and a galvanometer. The second electromagnet, as might be expected, slightly increased the field due to the first.

The heating effects for the kinds of currents used in the various coils was negligible except for the coil mounted on the sub-stage condenser. This produced a rise of $\sim\frac{1}{2}^\circ$ C in the culture for a current of 2 amps for 1 minute. It was thus necessary to use this coil for short periods only, but in practice this has not proved a disadvantage.

5. *Residual Fields*

These fall into two classes: permanent fields, like that of the earth which can be compensated for; and varying fields, which alter as the different fields are turned on and off in the various coils, and which are due to the effects of iron, or other ferromagnetic material in or near the objective.

Early in the work it was found necessary to remove the steel springs and a steel rod from the movement of the microscope stage. As our experiments became more precise we were compelled to replace the steel ring in the substage condenser by a brass one. This proved fairly satisfactory until we began to use the phase condenser and objective. Both were appreciably magnetic. The major part of the effect due to the condenser was, rather surprisingly, removed by taking off electrolytically the plating on the brass ring which fits into the condenser holder. The adjusting rods were replaced by brass ones. The condenser still has magnetic material in the centre of the large phase ring, but this is far enough away for its effects to be very small.

The magnetic effect of the objective (due to the plating?) is quite appreciable, and we have temporarily overcome this in our most recent experiments by changing the objective to an ordinary one which is non-magnetic when we wish to make or record exact measurements. This is not as great a disadvantage as it might seem because the actual particle is more easily visible without phase-contrast than with it.

Unfortunately we had recorded some of our results before the magnitude of the residual field from the phase-contrast set-up was realised. We have not discarded these results, but as will be explained later, certain features of the curves must be regarded as possible artifacts.

The fixed fields are more easy to compensate. A ring-shaped coil, mean diameter about 14 cm, mounted on top of the hot-box with its axis coinciding with the optical axis, compensates for the vertical residual field. It has 30 turns of 24 S.W.G. and its usual current is about 0.1 amps. The remaining horizontal fields are compensated by a bar magnet (two at right angles would be more convenient) outside the hot-box. Naturally this must be done with the μ-metal electromagnets in position. The vertical compensating coil must also be roughly adjusted, as it produces a horizontal component due to its action on the μ-metal rods.

It is interesting to note that the large iron feet of the microscope, the top of the hot-box heater, and the tall steel pillar on which the camera is mounted,

produce a negligible effect on the *variable* part of the residual field. It is clearly only magnetic material which is both near one of the field-producing coils and not too far from the objective which causes trouble.

The actual adjustment was done by making up a slide of some of the magnetic material in a mixture of 90 per cent glycerine and 10 per cent water (if at room temperatures), magnetising, and then observing the behaviour of individual particles under the microscope. The particles must of course be very widely spaced, and not near any big fragments. The residual horizontal field is then found by applying a small field due to the main electromagnet until the particle roughly bisects the angle between its position with no applied field, and its position in a very large applied field. The small applied field necessary to do this is then equal in magnitude to the residual field.

The vertical field is adjusted roughly first. After the first compensation of the horizontal field this adjustment is refined. The technique is to choose a long particle, and move it so that it is subtending the maximum length to the eye. This can only be approximate, but is quite adequate if the horizontal residual field is already very small.

Under the best circumstances the horizontal residual can be reduced to a field of about $\frac{1}{10}$ oersted, which may vary in direction as the various coils are used. In practice it is probably safe to assume that the residual is less than $\frac{1}{5}$ oersted. With the phase objective, however, a change of 0.6 oersteds can be observed after the main field has been full on in one direction, and then in the other. Before the phase condenser ring was deplated the effect must have been bigger than this.

It is not however sufficient to eliminate external magnetic fields due to the microscope, etc. Since there is magnetic material in the culture itself it is important to know how big an effect this can produce. Before undertaking exact measurements, therefore, we made preliminary experiments to estimate this. At the time it was usual to use a large amount of magnetic material in the explant. Two cultures were chosen, one with such a large amount of magnetic material that the culture could hardly be seen, and the other with rather less, but still more than average. The cultures were magnetised in the usual way — that is, by a very large field perpendicular to the plane of the coverslip. They were allowed to grow, and then dried by exposure to the air. The new growth was cut away, leaving the magnetized explant in position. Glycerine, with about 10 per cent water, and containing a small number of particles of the usual magnetic material, was added. The behaviour of these particles in the fluid near the explant was then ex-

amined. As might be expected, particles far from the culture were pointing approximately vertically (as the culture had been magnetised vertically), whilst those nearer to the explant pointed in various directions. The strength of the field was estimated by the "bisection of angle" method just described though naturally the result was only approximate. There was a considerable vertical field ($1\frac{1}{2}$ oersteds) at distances of 150 μ from the edge of the explant. However, in the case of the less densely magnetised culture this residual field fell to $\frac{1}{2}$ oersted, or less at about 300 μ from the explant. We therefore decided to reduce considerably the amount of magnetic material in the culture, and to work only with particles at some distance from the explant. Moreover, under these conditions any small residual field still remaining will almost certainly be vertical, and is thus not as serious as if it were horizontal.

There remains the question of the *changes* in the residual fields produced by the culture due to the external fields used. This might be expected to be small, and with moderate amounts of magnetic material in the culture it was shown to be certainly less than that due to the remainder of the system, i.e. less than $\frac{1}{10}$ oersted.

Finally the possible effect of magnetic particles in the zone of growth must be considered. These are very sparse, and confined to a plane, so that we need regard only the nearby particles. We can estimate crudely the effect of such a particle by treating it as roughly equivalent to a sphere of diameter equal to its greatest dimension (which we will call l) since this can hardly give an underestimate.

The maximum field due to such a particle at a distance r is:

$$\frac{2\,M}{r^3} = \frac{2}{r^3} \cdot \frac{(B\text{-}H)}{4\pi} \frac{4\pi}{3} \frac{l^3}{8}$$

$$= \frac{(B\text{-}H)}{12} \left(\frac{l}{r}\right)^3 \text{ oersteds.}$$

Taking (B-H) = 225 we see that for the field to be $\frac{1}{10}$ oersted or less, we must have $\frac{r}{l} > 6$. This gives a rough working rule for gauging the effect of neighbouring particles. It should be noted that these particles may be rotated by the applied fields, so that their fields may change direction in a complicated manner.

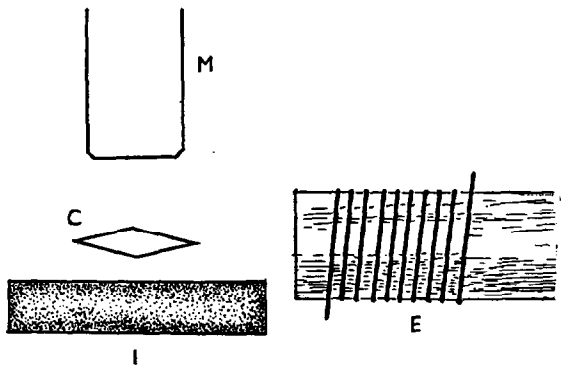

Fig. 3. Diagram to explain pseudo-elastic effect.

6. *Magnetic pseudo-elastic effects*

The residual magnetic effects discussed above are naturally rather small, and if merely regarded as a small percentage error in the main field applied they would be quite negligible, in view of the very low accuracy to which we are working. It is the fact that they persist after the main field has been switched off which makes them important, and, in particular, that their direction may be opposite to that of the main field. This can easily be seen by considering Text figure 3 which shows diagramatically an electromagnet, E, a compass needle, C, under the microscope, and a mass of iron, I. Imagine that the electromagnet produces a field with direction right to left. This will magnetise I in roughly the same direction. The compass will also point to the left. If the electromagnet is now switched off, it will produce no further effect, having no hysteresis. The iron, I, on the other hand, may possess some residual magnetism, with direction right to left. This will now deflect the compass to the *right*. It can easily be seen that the direction of the effect depends on the relative position of compass, iron, and electromagnet.

The effect on the particle in our experiments, if the direction of field is reversed, is to simulate an elastic recoil. However, even this might not be serious, as the residual fields are rather small, were there not evidence that the effect is exaggerated by the thixotropic nature of the cytoplasm. When the couple on a particle in a cell is reversed, it moves initially much more easily than at the moment before reversal. This suggests the possibility that small reverse fields might produce quite appreciable recoils. The matter is discussed more fully later. A group of particles may show a pseudo-elastic effect if they rearrange themselves, e.g. straighten out, when the field is re-

moved, and moreover the effective properties of composite particles would change with rearrangement. For these reasons, measurements have been restricted to single particles within a cell. We should perhaps mention that with single particles in castor oil we have never detected any elastic recoil.

7. *Calibration*

The magnetic properties of the particles were measured by observing their behaviour in castor oil when under the action of magnetic fields. A slide was prepared with a very small quantity of the material, which was crushed with a glass rod, and mixed with castor oil. Two no. 1 coverslips, one on either side, served as spacers, and a third coverslip was put over the top, and lightly waxed down. A $\times 40$ dry objective was used so that the lower surface of the castor oil could be observed.

The particles were carefully selected so that they were not near others, nor above a large collection of material on the bottom, nor near either the top or bottom surfaces of the preparation. They were also selected within arbitrary limits of size, namely between 2 μ and 10 μ for their greatest dimension. Only single particles were chosen, and the slide was used as soon as possible after preparation, so that few particles, except the very large ones, had time either to come together or to fall to the bottom.

To measure the angles an eyepiece with an angular graticule, produced photographically, was used and sometimes also a device for measuring the angle through which this eyepiece was turned.

In the later method in which the field was applied approximately perpendicular to the particle, the particles were magnetised before measurement, by applying a permanent magnet with conical pole pieces for a moment to the slide. This gave a field of several thousand oersteds. For timing, a small field produced by the main electromagnet was used, and the time to move through a preselected angle was measured with a stop-watch. The field was usually $2\frac{1}{4}$ oersteds in strength and the particles were timed from 45° to 135°, where 90° represents the position perpendicular to the applied field. The scatter of times was fairly great. An example of a set of 20 particles of the oxidised iron from the same batch of material gives (arranged in order): 1.6, 1.8, 2.3, 2.3, 2.4, 2.7, 2.9, 3.4, 3.7, 3.7, 3.8, 4.2, 4.3, 4.8, 5.0, 5.1, 5.5, 7.4, 9.2, 9.9 seconds. Each time is the mean of 3 readings (standard error $\sim 1/3$ seconds). This variation naturally includes variation in shape as well as in magnetic properties. The median is 3.75 seconds. As to the variation with the passage of time, the above figures were taken in July 1948, from

the material received in November 1947, which had been standing in water since then. A check in November 1948 gave a median of 4.4 seconds for a sample of 10, but at a lower temperature. Correcting for this brings the figure to 3.1 seconds, so that there was certainly no gross change in the material with time. Intermediate checks gave similar agreement. We have not considered it worth while for our purpose to press the accuracy further. The mean value of the greatest dimension of the particles used was estimated as 5 μ.

We have also made measurements on a few particles to check that the magnetic couple is roughly proportional to the field. The movement of the particles in castor oil was recorded photographically by the method used for the particles in the tissue culture (see page 55). The fields ranged from $2\frac{1}{4}$ oersteds to 36 oersteds, each being double the previous one. The slopes of the curves of angle versus time, at the 90° position, were proportional to the applied field to within ±15 per cent. The only measurement at 80 oersteds also falls within this limit. The experiments were done at an early stage before the technique was refined, and it might well be possible to improve this figure which, however, is quite satisfactory for our purpose.

The viscosity of the castor oil was checked by the falling sphere method at two temperatures. The values found, 16 poise at 14.7° C and 7 poise at 24.8° C, are reasonable.

The variation observed between the behaviour of particles in a set is bigger than would be expected if it were solely due to variation of shape. Application of the standard theory, as set out in Part II, gives the following relative angular velocities for various ovary ellipsoids.

TABLE II

Length/diameter ratio	1	2	3	5
Relative angular velocity	1.0	0.86	0.64	0.27

Most of our particles had length/diameter ratios of 2 or 3 to 1, and never as much as 4 to 1, so that even allowing for the simplifications of the theory we should not expect a variation of much over 2 to 1 in the angular velocity, whereas we actually get about 5 to 1. This must be due in part to the variability of the material, and a closer examination of the results supports this, since particles of apparently similar shape can vary considerably in angular velocity. The variation could be quite serious if we were concerned with obtaining accurate values rather than orders of magnitude but in the present

study we have included the shape variation with the other variants. For more precise measurements it would be better to use a more homogeneous material.

This grouping together of results is justified to some extent by the reasonable agreement between the value of the viscosity of the castor oil found by the magnetic particle method and by the normal method. If we take our average particle as having a length/diameter ratio of 2:1, and calculate the value of η by using the ellipsoid theory and the B/H curve, we obtain from the figures quoted above a value of $11\frac{1}{2}$ poise (at 19.7° C) whereas the falling sphere method gives $10\frac{1}{2}$ poise by interpolation.

8. *Photographic recording*

After our preliminary experiments it became clear that the velocity and to a certain extent the amount of movement of a particle could not be estimated sufficiently accurately by eye. We have therefore used cinematographic recording, usually at 9 frames per second, but occasionally at 15 frames per second. An 'Ensign' 16 mm camera was used, with a viewing eyepiece in a side-tube, into which part of the light beam from the microscope was deflected by a semi-reflector. This camera has previously been used to record at low magnification the beating of the heart of a chick embryo (Hughes 1949). A rough check showed that the speed of running of the camera was sufficiently constant for our purpose. The film used was usually Kodak R. 55, which is rather coarse-grained, but a more finely grained film would have needed more light, which we felt it would be better to avoid. The light was only used at full intensity for the actual time of recording apart from the necessary focussing.

During the course of the work a marker eyepiece was devised, by means of which a spot of light could be projected on to one side of the film. The time at which the circuit of the electromagnet was opened and closed could thus be recorded on the film.

The overall magnification with the 1/12th objective was about $\times 330$. The particle was photographed using the normal illumination.

To measure the angle of displacement, the film was projected downwards onto a horizontal table with a total magnification of about 5,000. A sheet of mm graph paper was pinned to the table, and the projector adjusted so that the edges of the frames of the film were parallel to the lines of the graph paper. A small loose sheet of white paper, with a straight edge, was now placed so that the image of the particle (white on a dark background) fell

on it. Holding the paper fixed, the observer traced a faint pencil outline of the particle on the paper. The procedure was then to fit this outline to the image of the particle from each subsequent frame of the film in turn; the angle between the straight edge of the paper, and the lines on the underlying graph paper was measured with a large circular protractor. The zero of the angle was rather arbitrary, but the 90° position was chosen such that the major axis of the particle appeared to be roughly perpendicular to the magnetic field.

The largest error in this technique is the fitting of the pencil outline to the projected image. This error varies with the size and shape of the particle and the accuracy of focus. There is also occasionally the difficulty that if the particle does not always remain in the horizontal plane it may appear to change shape. Accurate measurement in such cases is clearly impossible. The root mean square deviation was usually between 1° and 2°. The angle was in general only measured once for any frame, and in passages where the particle was effectively stationary, only every fifth or tenth frame was measured.

The procedure is tedious and time-consuming, the maximum rate of measurement being about 120 readings an hour. For this paper about 8,000 frames have been measured.

The above remarks about errors apply to a slowly moving particle. There is an additional error in measuring the velocity of a swiftly-moving particle, due both to the blurring caused by movement during the exposure, and to the time lost between frames. These are best regarded as errors in the time to which the measured angle corresponds. It is difficult to estimate them accurately, but a rough guess can usually be made in any particular case.

9. *Other details*

The arrangements for controlling the temperature were quite straightforward. The hot-box consisted of a roughly rectangular wooden box, 34 × 24 × 16 cm, with the top and front of thin transparent plastic. The heater was in the metal base of the box, and was supplied from the A.C. mains. The lower half of the microscope, from a point 3½ cm above the stage, was enclosed in the box. A subsidiary heating coil was wound at the bottom of the microscope tube, just above the objective, to prevent a large temperature gradient at the level of the culture. The heating current was controlled by a bi-metal strip mounted behind the microscope and the temperature was measured by a thermo-couple in the immersion oil.

The controls for the focusing of the objective and the movement of the stage could be operated without opening the box. The large magnet, when used, could also be moved from outside, and all the currents in the coils were similarly controlled.

The electric circuits require no special comment. They were run from two 12 volt car batteries.

E. TWISTING: EXPERIMENTS

1. *Preliminary experiments*

The first experiments were done at 37° C, magnetite particles within the cells being used. We will only sketch the details here. A large horizontal magnetic field (600 oersteds) was applied by means of the U-shaped magnet, which lined up the particles in the cells. This magnet was then oscillated mechanically in a horizontal plane through $\pm 15°$, with a period of about 12 seconds. Pictures were taken with phase contrast, at a rate of one every 3 seconds and from these the amplitude of oscillation of the chain of particles was measured. A rough calibration was made under similar conditions with castor oil. A simple theory made it possible to deduce how the amplitude of oscillation should vary with magnetic field, damping force, and the period of oscillation. If taken at its face value it gave a high figure for the viscosity of the cytoplasm, similar to that for castor oil.

These preliminary experiments suggested that the physical properties of the cytoplasm were very different from those of water. It was not possible to interpret them any more accurately because of the difficulties of calibration when long chains of particles are used, and because it is not possible to distinguish between viscous and elasticoviscous effects from the amplitude of the oscillation alone.

We therefore did a few experiments using rather smaller particles in which the magnet was moved suddenly from one angle to another. These proved difficult to interpret mainly because the couple on the particle was dependent on the angle between the magnetic axis of the particle and the field. It thus varied in an uncontrolled manner, and was difficult to measure as it depended on a small difference between two angles. Finally the method of moving the magnet was rather clumsy and difficult to control. We have omitted our results of these experiments, although they did suggest quite strongly that the resistance forces were not characteristic of a simple viscous fluid, and that there might be some elastic effect.

To improve the method it was necessary to use a relatively constant couple, and to apply it suddenly. For this reason we have developed the technique of using single magnetic particles as little permanent magnets, and applying a field, produced electrically, perpendicular to their length.

Our initial experiments, with this method, without photographic recordings, showed that the majority of single particles of the oxidised iron could be moved by the magnetic fields we could apply. They also showed that small fields would often move the particle hardly at all, and that if a large field were applied to a particle for a short time, it appeared to recoil at the moment when the field was switched off. This suggested that elastic effects were probably present. There was also a distinct indication that after the particle had been moved once or twice, it became rather easier to move than before, though it was difficult to demonstrate this unambiguously. Movement of the particles in the absence of an applied field was also noticed. These movements were both rotational and translational; they may have been partly Brownian but they were certainly not entirely so, as they were often not sufficiently random. This feature of the movements of the natural inclusions of the cell, such as small mitochondria, is usually attributed to the streaming of the cytoplasm, though there is some doubt whether this is a sufficient explanation.

2. *Photographic experiments at 37° C*

Early in the course of the work it became clear that to obtain results which we could interpret it was necessary to use only those cells and particles which fulfilled a number of distinct conditions. These have tended to become stricter. They are as follows.

(I) The cell must be a healthy intermitotic fibroblast, from young cultures (24 to 36 hours after sub-cultivation), without large vacuoles and containing few lipoid granules.

(II) The cell must not be far from the edge of the zone of outgrowth so that phase contrast illumination could be used if required.

(III) The cell must not be near other magnetic material, either in neighbouring cells or in the explant.

(IV) The cell must contain only a single particle.

(V) The particle must be of medium size and be fairly short. (The majority of the particles photographed were from 2 to 3 μ in length though one was as small as $1\frac{1}{2}$ μ and one as big as 4 μ. Their shape was usually irregular. Most of them were short, that is, their length/diameter ratio was less than 3.)

(VI) The particle must not be very close to the nucleus or to the side of the cell, nor crowded by cell inclusions.

Preferably it should be possible, with the vertical field, to turn the particle on end, showing that it is not hemmed in by the top and bottom walls of the cell. For this reason very flattened cells were avoided. A spindle-shaped cell was usually chosen with the particle in the middle of the cytoplasm near, but not very near the nucleus.

As might be expected, not many cells fulfil all these conditions, probably on an average about one per culture, so that a fairly extensive search has to be made to find a suitable one. It is difficult, however, to see which condition could be relaxed.

The time-scale of the movements and the magnitude of the angles involved made it difficult to obtain accurate estimates by eye, and it was therefore decided at this stage to use photographic recording.

In our first set of measurements we recorded successfully the movement of particles in 7 cells, though not all of these were entirely satisfactory. The number of records made with each particle varied, but averaged about 10. It is not easy to select with confidence the general features shown by these curves, because there are so many variables, but the following tentative conclusions were reached. The figures (Nos. 4–8) have been selected to illustrate these features.

(1) When the field was switched on, the particles began to move with a fairly high angular velocity. Any initial lag was too small to be detected by our methods. This velocity decreased as the deflection of the particle increased. The extent of the rapid part of the deflection generally increased with the size of the applied field (Text Fig. 5).

(2) After the rapid part of the movement, the angular velocity usually approached a steady value, provided the initial deflection had not been so big that the couple was no longer constant, but on other occasions the particle went more and more slowly, or even stopped (Figs. 4 and 5). In a few cases it appeared to start moving slowly again after having stopped, but the limits of accuracy in measuring the angle made it difficult to be certain of this.

In one case only did the particle start to move very fast after it had been moving steadily, and there were other reasons for regarding this example as exceptional.

(3) When the field was turned off there was, in general, a small fast recoil, followed occasionally by a slow drift in the opposite direction to the original movement (Figs. 4, 5, etc.). This rapid recoil was often of the same size as

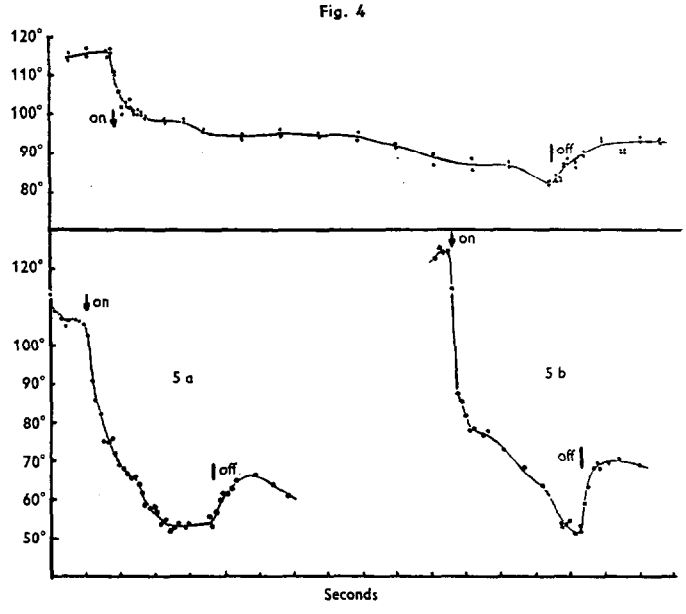

Fig. 4. Response curve of particle in cell. 37° C. 24 oersteds.
Fig. 5. Response curves of particle in cell. 37° C. (a) 8 oersteds; (b) 16 oersteds.

the initial rapid deflection, was never bigger, and was quite frequently smaller. It was sometimes difficult to be certain that it was there at all, especially in cases where it might have been expected to be small, since the marking eyepiece (p. 55) had not yet been fitted, and the natural movements and the experimental error confused the curves.

(4) When a large field was turned on for a short time, a procedure which we call a 'twitch', the subsequent recoil was, in all cases but one, incomplete (Fig. 6). It was usually between $\frac{1}{2}$ and $\frac{1}{3}$ the initial deflection; this could not be accounted for merely by the change of couple with angle. Again there was sometimes a subsequent slow drift which increased the total return.

(5) There was evidence to suggest that if the particles were deflected in one direction, then allowed to recoil, and then, within a few seconds, deflected in the opposite direction to the first, it moved further and possibly more quickly than on the first occasion (see Fig. 7).

(6) In the case of one particle the cell produced considerable natural movements which made it very difficult to obtain consistent readings, and in most of the others there were measurable perturbations (see Fig. 8).

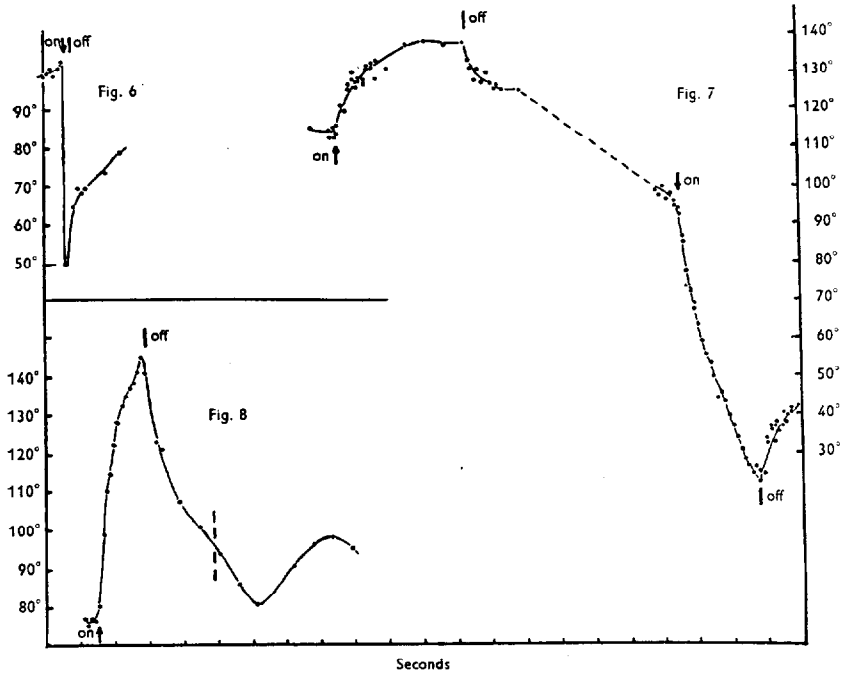

Fig. 6. Response curve of particle in cell. 37° C. 32 oersteds; scale on left.
Fig. 7. Response curve of particle in cell. 37° C. 48 oersteds applied successively in each direction. Scale on right.
Fig. 8. Response curve of particle in cell. 37° C. 32 oersteds. Natural movements follow actuation. Scale on left.

It can be seen from the figures that the results are by no means clear cut. This was partly because the effects of the previous history of the cytoplasm were not clearly realised, and partly due to a general lack of refinement in the technique.

Of these experiments all except one were done without phase contrast, so that it was less easy to be certain of the immediate surroundings of the particle. Moreover the natural movements of the cell were changing the environment of the particle, as well as producing rotational perturbations.

During one of these experiments the temperature had been taken down to room temperature and up again. The results suggested that the physical properties of the cytoplasm at the lower temperature were not greatly different from those at 37° C. The intracellular movements, on the other hand, were very much less active. We decided therefore to undertake a series of experiments at room temperature, and using phase contrast.

3. *Photographic experiments at room temperatures*

These were all done between 15° and 20° C. The cultures were incubated at 37° until required, and were then allowed to cool down, care being taken to avoid the condensation of moisture on the lower coverslip. Measurements were not made until at least half an hour after cooling.

Observation on particles by eye and by time-lapse photography with phase contrast showed that the non-random movements were reduced at room temperatures, but not entirely prevented. Though brownian motion cannot with certainty be detected by eye at room temperatures, the time-lapse films show that there is still some apparently random movement present.

Reliable data was obtained from 4 cells. The average number of recorded actuations for the first 3 was about 12. For the last cell over 50 actuations were filmed. As has been stated the residual field due to the phase contrast apparatus was not negligible in these experiments.

The initial rapid movement, the subsequent rather ill-defined slowing down, the almost universal fast recoil, and the fact that this was usually incomplete were all confirmed. We must make the reservation that when a particle was moving rapidly over large angles, after repeated actuations, it was impossible to estimate the variation of velocity accurately, or to distinguish between genuine slowing down and that due to the couple being less at the extreme angles.

Further evidence was obtained for the effects of repeated actuations. Thus one particle was given a series of twitches, with intervals in between. The results were roughly the same. It was then waggled violently and twitched again. This was done twice. On both occasions it moved faster than in the first series. In addition the recoils were less. Figure 9 shows the effect of twitches before and after waggling.

A further experiment was done with the same particle. After violent waggling with a big field it was allowed some seconds to settle, and was then actuated with a medium-sized field for a few seconds. This was repeated at intervals of the order of minutes. The results showed that over a period of minutes the material appeared to harden somewhat. Figure 10 shows the initial actuation and that 10 minutes later.

Of our other results, for two of the cells we have curves bearing on this point, but although they do not contradict those given in text Figs. 9 and 10, they are open to other interpretations, as the couple was not always constant. Finally we have a series of observations on our fourth cell.

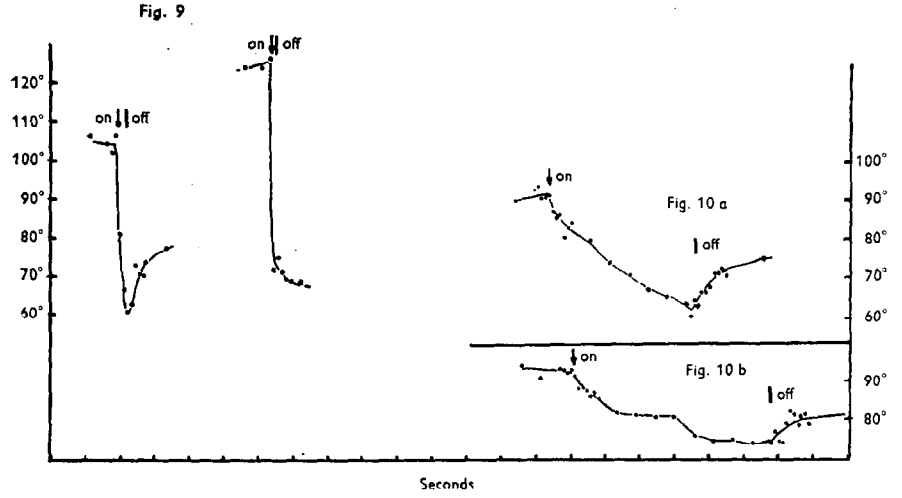

Fig. 9. Response curve of particle in cell. Room temperature. 60 oersteds. (a) before; (b) after waggling. 'Twitch' shorter in (b).
Fig. 10. Response curve of particle in cell. Room temperature. 8 oersteds. (a) immediately after 'waggling'; (b) 10 min. later.

This particle was left undisturbed for 10 minutes before the beginning of the experiment. It was then actuated with a moderate field (about 24 oersteds in this case) for some seconds; after a pause of a second or so, the field was applied in the reverse direction. The cycle was repeated several times until the movements became rapid.

Figure 11 shows the results. It can be seen that repeated actuation undoubtedly allows the particle to move more easily. An attempt was made with this particle to demonstrate the hardening of the material on standing, but the results are too irregular to be worth reproducing, although they suggest that after an 8 minute interval some hardening had taken place.

Our experiments on dragging (see page 70) and our general experience in moving the particles suggested that small stresses often do not produce a yield even if continued for a long time. This is difficult to check at 37° C because of the perturbations, but we have done a few experiments at room temperatures. A typical result showed that for a certain particle $\frac{1}{2}$ oersted for 12 minutes, or 2 oersteds for 3 minutes produced no perceptible yield, whereas 8 oersteds for 45 seconds gave immediate movement followed by a slow yield of about 15°. It was also shown for this particle that even after waggling it gave practically no movement with 2 oersteds, but an immediate movement and a gradual yield with 8 oersteds.

It is not possible to show this behaviour clearly with all particles, especially

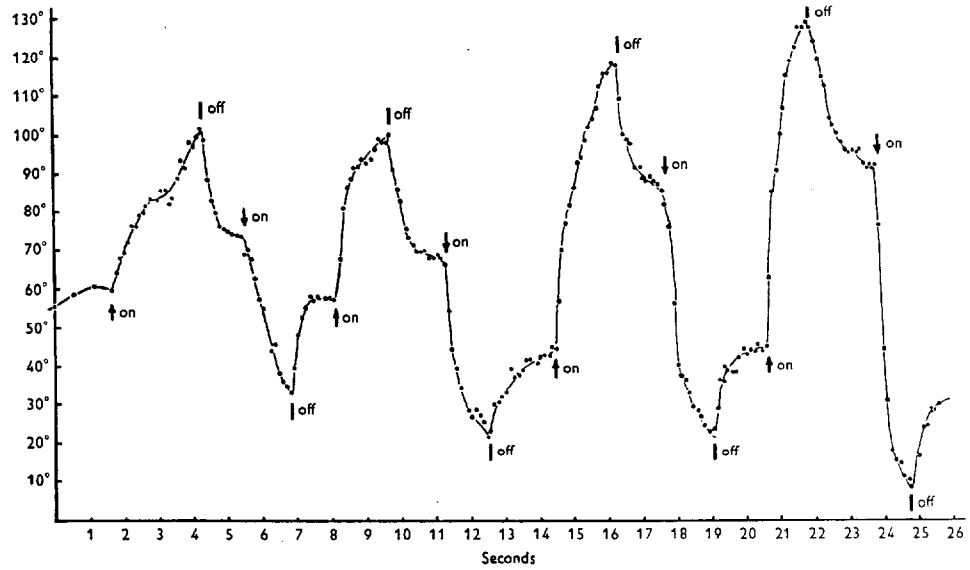

Fig. 11. Response curve of particle in cell. Room temperature. 24 oersteds. Repeated actuation.

if they move under small fields, in which case there are experimental difficulties due to the residual field.

We have made a rough estimate of the order of magnitude of the modulus of rigidity given by our results. Taking as a typical figure a deflection (or recoil) of 12° for a field of 10 oersteds, we obtain

$$n \sim 10^2 \text{ dynes/cm}^2$$

(Recorded values range, all told, from about 20 to 500 dynes/cm^2, but some of this scatter is due to the magnetic material).

The rate of movement after waggling, which can only be obtained very roughly, corresponds to a "viscosity" of the order of 10 poise.

The effect, if any, of boundaries would be to lower both these figures, but almost certainly by less than a factor of 2.

4. *Subsidiary experiments*

Experiments on bias. In interpreting our results we have considered the fast recoil to be a genuine effect, and the slower part to be a possible artefact. To confirm that the recoil is in fact genuine we have devised a special technique which we think rules out all possible magnetic artefacts. This consists

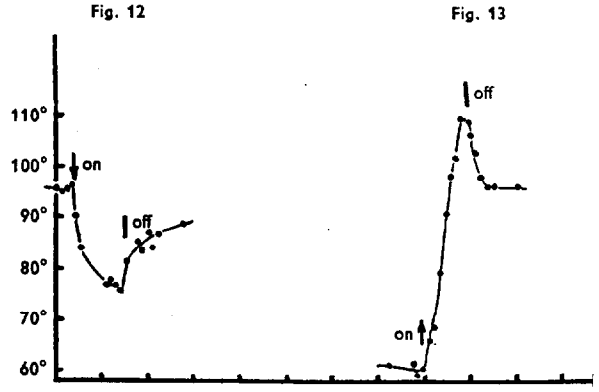

Fig. 12. Response curve of particle in cell. Room temperature. 32 oersteds. Bias: 1 oersted.
Fig. 13. Response curve of particle in cell. Room temperature. 20 oersteds. Bias: 1 oersted.

in applying to the electromagnet a small bias current which remains when the main current is switched off. This bias current can be adjusted to have different values of either sign, and it is thus possible to neutralise or increase any residual field. Examination of numerous cells by eye shows that in general a bias of, say, 1 oersted can prevent or produce an after drift according to its direction, but that there is always, or almost always, a small fast recoil which occurs with either bias. We have made a few recordings of this. Figure 12 +13 show the results for two different cells. The recoil in these cases is certainly genuine, since any magnetic artefact would be less than 1 oersted.

It should be noted, however, that the fact that the bias can prevent a slow drift back does *not* prove that the latter is necessarily an artefact. It may well be that the slow drift is produced by the material near the waist of the particle, which since it has experienced smaller strains will probably be relatively intact. However it can only exert a small couple. If the structure near the ends of the particle had been broken down by the movement, this small couple might be enough to overcome slowly the viscous effect of the broken-down material, and thus produce a slow drift back. This could easily be prevented by a small magnetic field.

Possibilities of this sort greatly complicate the interpretation of experiments with variable stress.

Drifting downwards experiments. At an early stage we had considered the possibility that the particles normally fell under gravity until they rested on the bottom of the cell. We considered this unlikely because the stresses due

to gravity are very small, and the cytoplasm at very low stresses appeared plastic rather than truly fluid. Moreover the movements of the particles due to the cell were often considerable. However to make sure that it was not an important feature we grew some cultures in a vertical position after the first subculture, and after one or two days' growth in the incubator we examined them (at room temperature) in the same orientation. Two cultures were examined, one on the first day and both on the second day. Every intermitotic fibroblast which was growing within 30° of the horizontal and whose outline could be clearly seen was scrutinised. All such cells with particles were roughly sketched. The particles were then grouped into 3 classes, according to whether they were in the top, middle or bottom thirds of the cell (allowance being made for the inversion due to the microscope). The results were:

top third	16
middle third	18
bottom third	15

There is clearly no great tendency for the particles to fall down to the bottom of the cell when the cells are grown sideways.

We have also checked that there is no perceptible drift down under gravity after the particles have been waggled violently in all directions. This was also done by fitting up a microscope horizontally, so that the cultures could be mounted vertically, and single particles in spindle-shaped cells were observed. The experiments were done at room temperatures.

Five cells were observed. One was rejected because the particle showed large angular perturbations due to the cell. Two showed no detectable movement during the time observed, which was 20 minutes from the cessation of waggling. One showed no movement for 10 minutes, and then moved *upwards* about 1 μ in a few seconds. The last one showed a slight brief upward movement after about 5 minutes, but no further movement in the remaining 15 minutes. Thus, as expected, there is no steady drift downwards, but at these temperature (20° C) the natural movements of the cell are not entirely suppressed.

BIREFRINGENCE

We have made a brief attempt to detect birefringence in the cytoplasm in the neighbourhood of a twisted particle. This was done with Mr. J. M. Mitchison of the Zoological Laboratory, Cambridge, on his polarising microscope, for which assistance we are very grateful. The phenomenon was

complicated by polarising effects due to the particles themselves. This was confirmed by observing the particles in a mixture of equal parts of glycerine and water. It was therefore difficult to observe small amounts of birefringence near the particles, which is the only place in which we should expect it.

The experiments were done at room temperatures. Although several cultures and a number of cells were tried, some with fairly large particles, no effect could be detected.

A negative result is not really surprising because if there were an effect it might be expected to be small.

5. *Sodium oleate and bentonite*

We have made some observations at room temperatures on magnetic particles in two materials of known composition. The same technique were used as for the previous experiments, except that rather larger particles were chosen in order to increase the accuracy.

The first material was an elastic soap solution, very kindly supplied to us by M. Dervichian of the Institute Pasteur, Paris, and prepared by him as follows.

Concentrated soap solution:

36.0 gr Na-oleate
180 cc KOH 2 N.
820 cc H_2O

KCl solution:

3.8 N (that is, 283.3 gr/liter)

The elastic soap solution itself was prepared as follows.

20 cc. Concentrated soap solution
22.5 KCl solution
17.5 cc H_2O

Thus the final concentration of sodium oleate was about 1.2 per cent by weight.

The results showed that when the magnetic couple was applied, there was an initial rapid deflection, followed by a constant rate of yield. When the field was removed, there was a swift recoil equal in size to the initial fast deflection. This is illustrated in Text Fig. 14 a. Moreover both the recoverable deflection and the rate of yield were approximately proportional to the applied couple. The "relaxation time" was about 0.9 seconds.

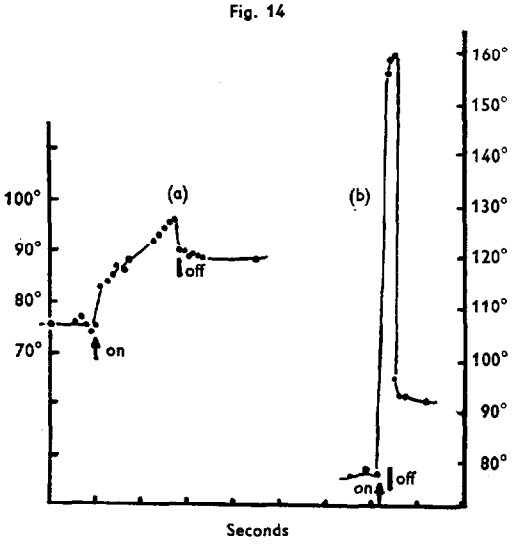

Fig. 14. Response curve of particle in sodium oleate. (a) 3 oersteds; (b) 24 oersteds.

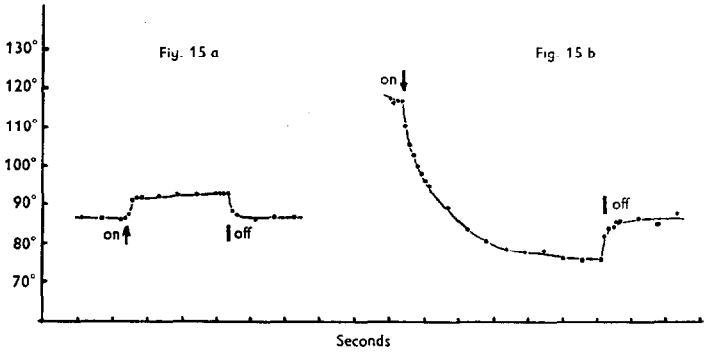

Fig. 15. Response curve of particle in Bentonite. 8 oersteds. (a) before; (b) after 'waggling.'

The effect of a large field for a short time (a twitch) gave a high degree of recovery even for large angles, as illustrated in Fig. 14 b.

The second material was a dilute gel of the clay, Wyoming Bentonite, given to us by the kindness of Dr. M'Ewen and Mr. Mould of King's College, London. It had been roughly fractionated by centrifuging. The particles are believed to be thin plates, which could not be seen by the phase microscope. The concentration was about 1.8 per cent by weight, and no extra

salts or NaOH had been added, so that the material was a feeble thixotropic gel.

After having been allowed to stand, this material also gave a swift initial response, which was approximately proportional to the applied couple. The modulus of rigidity was $1\frac{1}{2} \times 10^2$ dynes/cm^2 approximately. If the couple were small, there was little or no yield, but if the couple were increased, the yield went up rapidly. Thus, in one case, $\frac{1}{2}$ oersted for 16 minutes, or 4 oersteds for 2 minutes, gave no significant permanent deflection ($< 2°$), whereas 8 oersteds for 1 minute gave a yield of 6°. Repeated actuations by large couples appeared to destroy the gel structure, so that small fields, which initially gave small recoverable deflections, would give large rambling permanent ones. (See Fig. 15.) On standing, the gel would harden again and a series of small actuations taken 5, 10, 20 and 40 minutes after vigorous stirring show increased hardening at each stage.

We have not succeeded in twitching a particle through a large angle without producing a permanent deflection. When the particle was deflected through a large angle in one direction, so that a big yield was produced, it moved even more when the couple was reversed, as might be expected on theoretical grounds, since in effect it had made the beginnings of a small hole for itself in the gel, through which it could move back easily on reversal until it came near fresh unsheared material.

A few measurements on a gel where concentration was threequarters that of the first gave substantially the same type of results, though the coefficient of elasticity was rather less than before, being roughly 0.3×10^2 dynes/cm^2. Material with only half the original concentration did not gel when put on the slide, and showed Brownian motion.

Thus under the conditions we have used these two materials show behaviour of a very different character, although their elastic moduli are of the same order of magnitude. These differences are, in fact, quite obvious in the test tube. The soap solution, when shaken, shows lightly damped elastic oscillations. When poured it pulls out into long semi-liquid filaments. On the other hand, the bentonite hardens into a rigid gel, which is easily destroyed by tilting the test-tube. Probably the best way to describe the difference between them is to say that the "relaxation time" of the bentonite is a function of stress, while that of the elastic soap solution is roughly independent of it.

Though these experiments are of little value for finding the structures of these substances, they do establish experimentally several points about the behaviour of twisted magnetic particles in different types of material.

F. DRAGGING

1. *Biological technique*

As stated above, for our dragging experiments specially small culture vessels were devised. The cultures were made in the usual way, with magnetic material in the medium, but they were subcultivated twice. On the second day of the last passage they were transferred to specially prepared chambers.

Each chamber was made from a strip of platinum 0.25 mm thick, about 2 cm long, and 1 or 2 mm wide. This strip was bent round, a glass rod being used as a jig, into the shape shown in Fig. 16, and then fixed together at

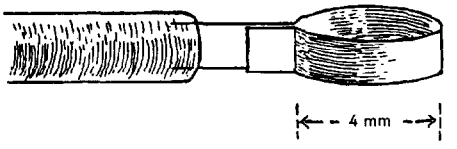

Fig. 16. Diagram of mount for small cultures.

the join with a small piece of glass, by heating in a bunsen flame. A glass rod was then fused to the free end of the platinum strip to act as a handle. The top and bottom surfaces of the platinum chamber were made approximately flat by rubbing them on fine emery paper on a flat surface.

The small coverslips, about 4 mm diameter, were cut from ordinary No. 1 coverslips with a diamond. A hole in a piece of perspex was used as a jig. The top coverslip, on which the culture was to be mounted, was fixed to the ring with paraffin wax. This was done by dipping the platinum into hot wax, after which it was withdrawn shakily to remove surplus wax, and allowed to cool. The coverslip was placed lightly in position, and the whole warmed over a hotplate till the wax melted and ran into the corner round the edge of the coverslip. The hot plate was then turned off, and the wax allowed to cool very slowly, so that large crystals were formed. It was found that the seal produced by quick cooling was unsatisfactory, and allowed water vapour to leak out, but the slowly-cooled wax was water-tight. A plastic material was also tried. This was satisfactory, but it was more trouble to prepare than the wax, as it had to be baked.

For the lower coverslip which was not in contact with the plasma clot, vaseline was used as a seal, as this joint had to be made with the living culture in position. The platinum chamber and the coverslips were sterilised *before* waxing, and thereafter kept under sterile conditions.

The technique for the final subculture, which was done for us by Dr. Honor B. Fell, was as follows.

The chamber was mounted upside down, so that its single coverslip formed a floor. The explant, instead of being divided into two, was cut into five or six pieces, each about $\frac{1}{2}$ mm square. Originally each tiny fragment was added to the usual mixture of embryo extract and plasma, and then pipetted into the small chamber. This was rather a race against clotting, and it was found more satisfactory to pipette the explant in with the embryo extract and add the plasma afterwards. Any excess was then removed with another pipette. The technique was tricky, but there were only occasional failures.

When the medium had clotted, vaseline was smeared round the lip of the chamber and the other coverslip added. The chamber was then turned the right way up and put in the incubator.

2. *Apparatus and calibration*

Our experiments were made with very simple apparatus. The U-shaped Alnico magnet described previously (page 47) was used with a single soft-iron pole-piece of tapering shape. Several types were tried, and using a simple test we selected the one which gave the greatest force. The test consisted of finding the force on a small speck of the magnetic material embedded in a blob of glue and fixed to the end of a glass filament. The deflection of the filament and the distance of the magnetic material from the pole face were observed under a low power (2/3″) microscope, and measured with a micrometer eye-piece. This method enabled rough relative measurements to be made, giving the curve of force v. distance from polepiece, and enabling difference pole-pieces to be compared. A crude estimate of the absolute value of the force per unit volume of the magnetic material was made by calibrating the filament with a 10 mg weight, and estimating the volume of the material. This gave the force per unit volume as about 2×10^6 dynes/cc at about $2\frac{1}{2}$ mm distance, but this figure should probably be increased by a factor of 2 to allow for the real volume being less than the apparent volume. For a more accurate calibration it would be necessary to record the velocity of the particles in castor oil.

3. *Experiments*

Once the initial difficulties with sealing had been overcome the cultures grew quite well, although the tissue deteriorated rather more quickly than in the usual preparations. The material was, as usual, frontal bone of 11 or 12 day chick embryos, incubated at $37\frac{1}{2}$ C. The culture was examined by means of a $\times 20$ objective and a $\times 15$ eyepiece. The magnetic particles could be seen very clearly, and also the general shape of cell, though naturally not all the finer details.

The magnetic field was applied by simply sliding the magnet into position along the stage. This had the disadvantage that it could not be done suddenly. The pull was horizontal; that is, parallel to the cover-slip. No particles were observed to move quickly. Some particles never moved at all during about half an hour's observation. A few particles could be seen to be moving if watched carefully. A typical rate of movement was the particle's own length in half a minute. The movement was not always regular. We recorded two sequences photographically.

These results were much as expected, as the stresses applied were low. To obtain better results the pole-pieces should be redesigned and made of more suitable material to increase the gradient, and that the field should be produced by an electromagnet, so that it could be switched on and off suddenly. This we have not yet done.

G. PRODDING

We record these observations for the sake of completeness but we have not developed the technique very far.

As has been stated, we apply in this case a fairly large field of about 600 oersteds. The cultures are made with a relatively large quantity of magnetic material in the medium cells containing many particles are chosen. Under favourable circumstances the particles can be lined up by the field and if this is done with the field parallel to the length of the cell, a composite rod of length somewhat greater than the width of the cell can sometimes be formed. If this rod is rotated by rotating the magnetic field it will eventually come up against the walls of the cell. Further rotation frequently causes the rod to break up into its component parts, but occasionally we have succeeded in making it push out the cell wall. It can be made to protrude some distance without apparently causing any break in the wall. It was not pos-

sible to see any displaced membrane, even under phase-contrast. If the magnetic field were then reduced in size without its direction being altered the particle appeared to be pushed back by the cell wall.

We have also been able by a similar technique to prod the nucleus inwards. This is not easy to do, and still more difficult to establish that one has done it. The only conclusive test seems to be the movement of adjacent nucleoli. This we have observed on several occasions.

It is obvious that the forces that can be applied by this method are considerably bigger than in our usual twisting technique, but we have not as yet evaluated them.

H. DISCUSSION

1. *Previous work*

We do not propose to review in detail all the literature on protoplasmic viscosity. A fairly recent account has been given by Heilbrunn (An outline of general physiology, 2nd Edition, 1943). His monograph (The colloidal chemistry of protoplasm, 1928) covers the earlier work in more detail. It will suffice to say that in certain materials, such as the protoplasm of the sea-urchin egg (*Arbacia*), and the interior of *Amoeba dubia*, the viscosity is not many times that of water, while in other materials, such as the ectoplasm of *Amoeba proteus*, the cytoplasm appears to be more in the nature of a gel.

We will consider, mainly, previous work in which the magnetic particle method is applied. Its first recorded use is in the classical experiments of the botanist Heilbronn, who inserted small pieces of iron into slime moulds. These are discussed by Heilbrunn (1928, p. 62). It appears to us, if we have understood the description of the experimental technique correctly, that whatever else was being measured, it was certainly not the viscosity. To measure viscosity it is necessary to measure a *rate* of movement, whereas Heilbronn found the field that would just move the particle. Moreover, although he gives no details of the magnetic properties of his iron rods, it is very probable that the forces he applied would have moved freely suspended particles very rapidly, especially in the calibration experiments in water and ether. These experiments did, in fact, show unexplained deviations. There seems little doubt that the particles were resting on the bottom, and that Heilbronn was measuring something which might be called adhesion.

Both Heilbronn and Heilbrunn discuss this possibility of adhesion, but they do not seem to have realised that in all probability such forces were

entirely swamping any effect due to viscosity. The difficulty would have been avoided if an estimate had been made of the absolute value of the couples applied, instead of relying entirely on a "calibration" in known fluids.

It is all the more remarkable that such consistent results should have been obtained, and a repetition of their experiments might be well worth while.

Freundlich and Seifriz (1922) inserted nickel spheres into gelatin and other gels, and attracted them with an electromagnet. These experiments are unobjectionable, except for the fact that these workers also made no estimate of the absolute values of the properties measured. The gels used were probably rather stiffer than those on which we have experimented. Seifriz's attempts to apply the technique to the egg of the Sand Dollar *Echinarachinus* (Seifriz, 1924) — were less fortunate and Heilbrunn's criticisms here are more to the point. Although Seifriz claimed to have demonstrated an elastic cortex, it is not certain that the observed recoil was not due to the cell membrane. Moreover, he probably damaged the egg while inserting the nickel ball. His estimate of the interior viscosity lacks precision.

Pfeiffer (1936) has made experiments in which protoplasm has been forced through a capillary tube, and has shown that the "viscosity" varies with the rate of shear, but here again it is not clear how much of the effect may not be due to the pellicle or to the damage produced by rough handling.

Many workers have shown that different structures such as the erthyrocyte membrane, or giant chromosomes of the salivary glands of Dipterous larvae, are elastic, probably highly so, but their properties are not directly relevant to the question of the elasticity of the cytoplasm itself. We include in this category the spindles and asters of dividing cells. To use the term protoplasm in a loose way to cover all these materials as well as the hyaline cytoplasm, as Seifriz does (1936, page 233) may occasionally be convenient, but it makes nonsense of any discussion of the structure of the cytoplasm.

No quantitative work appears to have been done on vertebrate tissue cells, although some have been centrifuged (Beams, 1943, p. 71) and some have been microdissected (Chambers 1924). Chambers and Renyi (1925) in a study of vertebrate epithelial cells by microdissection state "In consistency the protoplasm of the different cells studied varies from the very tough, jelly-like solid of the squamous epithelial cell to the very fluid cytoplasm of the gland cells." It is probable that the differences observed in microdissection do correspond to differences in the physical properties of the cytoplasm, but it is not possible to make the results as they are stated, even approximately quantitative.

2. *The present experiments*

We are now in a position to survey the results of our experimental work. We have established that when a magnetic particle is deflected in the cytoplasm of a chick fibroblast

(1) there is usually a small fast recoil;
(2) this recoil is usually incomplete, and always so for very large angles of twist;
(3) the order of magnitude of the modulus of rigidity is 10^2 dynes/cm^2;
(4) particles can frequently be made to move more easily after repeated movement;
(5) some particles cannot be moved appreciably by very small stresses, even if applied for a long time.

We have only attempted to demonstrate the last point at room temperatures, and not at 37° C.

The question now arises: are these effects due to the cytoplasm itself, or to other factors, such as the cell inclusions or boundaries?

We have argued (page 42) that the results are not due to the particle being in a vacuole, nor do we think that they can be produced by the proximity of the natural inclusions of the cell. It is possible that the elastic recoil is due to the cell membranes, but we think it improbable. Our experiments on bentonite encourage us to attribute the phenomena to the cytoplasm itself, which we believe to be a thixotropic gel, showing feeble elastic properties.

There is little new in these conclusions. It has been advanced for many years, though often on rather inadequate evidence. However we believe that this is the first time that the order of magnitude of the elastic modulus has been established. The value shows that the gel is very feeble, so feeble in fact that in bulk in a test-tube it would probably be classed as a sol or an elastic liquid rather than a gel.

It is difficult to interpret point No. 2 above because of the complications involved when measurements are not made at constant shear. The results certainly suggest that chick cytoplasm may not be "highly elastic", in contrast to the sodium oleate solution, and that the relaxation time is a function of the shear. In this respect it appears to resemble the very feeble gel of bentonite, though the analogy should not be pressed too far, as the similarity may not extend to all features of the behaviour.

It is often assumed that normal cytoplasm is "highly elastic" but it is doubtful if there is any really convincing evidence for this. What has some-

times been observed is the elasticity of surface membranes. Our observations on prodding, and all other experimental evidence, suggest that in these chick cells the physical properties of the surface membrane are quite different from those of the interior.

Attempts to demonstrate highly elastic behaviour by drawing out strands of cytoplasm are open to the criticism that in doing so a new surface layer is formed, and that this, rather than the cytoplasm itself, may be giving the effect.

It is probable that a quantitative investigation of certain other biological gels, such as the ectoplasm of *Amoeba proteus*, would show elastic properties of a similar type. Indeed the centrifuge data suggest that the elasticity of this material is rather weak (Heilbrunn, 1943, p. 67).

Incidentally it should be noted that our technique is not sufficiently precise to enable us to say whether the inside of the chick cell is uniform in its physical properties. For example, we cannot rule out the possibility that the middle of the cell may be rather more fluid than the regions nearer the cell membrane.

Can we, from our results, deduce anything about the underlying structure of the cytoplasm? The analogy with bentonite is in some ways fortunate, because nobody will be tempted to think that the cytoplasm *is* bentonite; but we must consider whether there is a real content in the analogy.

At present the only conclusion is that dilute gels cannot show elastic properties unless they contain highly asymetrical particles or aggregates. This is probably true. For more concentrated solutions, such as cytoplasm, it is not clear how far this idea can be applied. Presumably the more "highly elastic" the material is, the higher is the assymetry required.

At small stresses, the approximate constancy of relaxation time with stress probably indicates a purely thermal relaxation, while for materials which are relatively plastic the relaxation is probably due to the shear itself. This suggests that in the latter case the shear is carried by fewer links than in the former instance, assuming that in both there is roughly the same temperature dependence. However our results are for comparatively large strains, and perhaps reflect more the configuration of the materials.

The difficulty here is that there is as yet no general theory of the structure of substances of this sort, so that it is not yet possible to relate features of their behaviour with features of their structure. Much work has been done on rubber-like substances and gelatin gels on the one hand, and dilute solutions of very large molecules on the other, but no studies on elastic liquids, other than of an exploratory nature, have yet been published.

One approach to this problem might be to take certain biological materials,

such as crude protein solutions, or possibly a suspension of Claude's microsomes (Claude, 1943) etc., and see how magnetic particles behave in them. We believe this method might lead to misleading interpretations due to superficial similarities in behaviour. It is important to discover which features of a model are essential to give the properties measured, and which are accidental. To do this it is necessary to have a theory, however crude, to account for at least some of the observable features.

This can best be constructed by using, in the first instance, chemically defined materials in test-tube quantities, so that a large variety of methods (birefringence, light scattering, X-rays, etc.) can be brought to bear on each particular substance. There is then some chance that the structure can be determined unambiguously, so that there will be a firm foundation for any theory.

When this has been done, it might be profitable to reinvestigate the properties of the cytoplasm. It is unlikely that all the methods available for test-tube amounts could be used on the cytoplasm, both because of the size of the cell and also owing to the biological limitations. However, from the limited data which could be obtained it might be possible to establish some features of the structure. The magnetic particle method might prove very valuable at this stage.

We must consider the theories of cytoplasmic structure which have been put forward in the absence of such an approach. Of course it may well be that no general theory is possible; different biological materials may differ so radically that their cytoplasm has little in common.

If we neglect hypothesis of merely historical interest, there are at present two main theories of cytoplasmic structure: the "brushheap" theory of Seifriz (1936, p. 247) and the "frame work" theory of Frey-Wyssling (1948). Both are necessarily vague because of the absence of precise data and where they attempt to be more definite they are open to criticism. For example Frey-Wyssling states "The members of the fibrous framework are extraordinarily thin: the order of magnitude of their thickness is that of the molecular cross-section of a single polypeptide chain". This may be true, but there does not appear to be any evidence for it. The argument really amounts to saying that they cannot be so thick that they can at present be detected. Does he regard 50 Å, for example, as being of the order of magnitude of a *single* polypeptide chain?

Nor is it obvious that the elements of the framework must necessarily join to each other as postulated by Frey-Wyssling. In a fairly concentrated solution, they may be linked more often by the other molecules present. Finally although presumably there must be some asymetrical units present, it

is not obvious that they are necessarily flexible. Are bentonite plates flexible, for example? This brings us to a picture rather like Seifriz's brush-heap, which, if we understand correctly the implication of his photograph of a pile of matches, contains rigid rodlets.

It is clear that if these two models are compared, there is no real evidence on which to decide the points on which they differ. All that can reasonably be said is that in the cytoplasm of some materials there are probably asymetrical units or aggregates present, which are not large enough to be easily detected.

If we were compelled to suggest a model we would propose Mother's Work Basket — a jumble of beads and buttons of all shapes and sizes, with pins and threads for good measure, all jostling about and held together by "colloidal forces".

Finally we would plead that the evidence we have presented for elasticity, and therefore a "structure" in the cytoplasm will not be regarded as evidence for some "cytoskeleton" in the chick cell. This implies, as we understand it, a fixed and characteristic spacial relationship between the highly specific components of the cytoplasm, extending throughout the cell. Such evidence as we have is against such a conception as applied to the cytoplasm as a whole (in contrast to individual parts of it, such as the mitochondria) since the cell produces considerable relative movement of many of its natural inclusions, especially at 37° C.

We particularly deprecate statements such as that of Frey-Wyssling (loc. cit. page 115) that "the structure of plasm must be of a wonderful coordination. The framework cannot represent an unordered pile, but must possess an organised and well-defined structure". We do not see why the presumably coordinated chemical actions of the cytoplasm *must* imply an *organised* frame-work in the cytoplasm, except possibly in special cases. The "structure" we have established may well be a rather non-specific, transient affair for a normal cell.

3. *The magnetic particle method*

We summarise here briefly the advantages and disadvantages of the magnetic particle method as they appear to us.

The paramount advantage is that it is possible to apply a stress which varies swiftly with time. This has enabled us to distinguish small elastic effects which would be difficult to demonstrate by any other method.

Secondly, the stresses can be applied locally; that is, it is not necessary to apply them intensely to the cell as a whole, as in centrifuging.

Thirdly, the stresses in the case of twisting, and also for the dragging of large particles, can conveniently be made high, and for twisting, though less conveniently, very high.

Among the disadvantages are the difficulty of getting the particles into the cell, since only certain types of cells will phagocytose them; the possibility that they may modify the neighbouring cytoplasm; and the care needed to perform some of the magnetic measurements without artefacts. For small particles the accuracy is low, though this could probably be improved for larger particles. The method of recording and analysing the results is rather laborious.

The fundamental disadvantage that the properties cannot be measured at constant stress applies to all methods for living material with which we are familiar.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. H. B. Fell for her continued help during the course of the work, to the Medical Research Council for a studentship held by the senior author, and to Mr. L. J. King for his skilled preparation of most of the tissue cultures used in this work.

SUMMARY

1. A magnetic method is described for the study of the consistency of the protoplasm of living cells in tissue cultures.
2. Such protoplasm is not purely viscous, but has elastic properties.
3. The order of magnitude of the modulus of rigidity is 10^2 dynes/cm^2.
4. There is evidence of thixotropic behaviour.
5. The bearing of these results on current theories of protoplasmic structure is discussed.

APPENDIX

In order to understand the meaning of the terms used, consider text Fig. 17. This represents the deflection of a body in a viscoelastic medium, plotted against time, when a constant stress is suddenly applied and later suddenly removed. If the viscosity were very small, the deflection would go from A to B when the stress was first applied, and AB would represent the recoverable, elastic, deflection. The actual viscosity, however, delays this, and so the deflection follows AC. When the stress is removed, at D, then deflection DE (equal to AB) is recovered. Viscosity again delays the response. There has been, during the time the stress was applied, a steady, irrecoverable

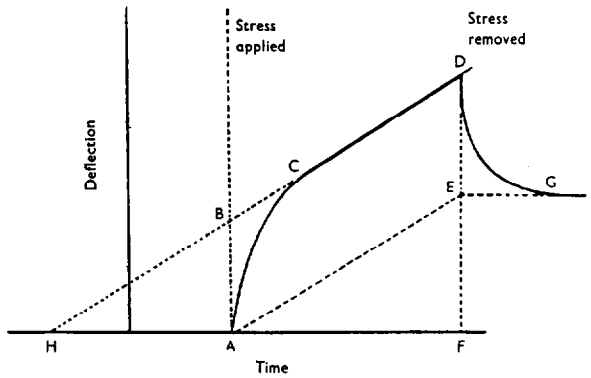

Fig. 17. Explanation of visco-elastic yield (see Appendix).

yield, and this has amounted in all to the deflection FE. This yield has been called the pseudo-viscous yield.

The relaxation time is the distance HA. A simple way of thinking of it is as follows. Suppose that a stress is applied to give a recoverable, elastic, deflection (AB) of 5°. Then suppose that if this same stress is maintained for t seconds the pseudo-viscous yield (EF) also just amounts to 5°. Then the relaxation time is t seconds. The faster the yield rate, the smaller the relaxation time.

It can be seen that if we apply, say, double the previous stress, and if both AB and EF are just doubled (i.e. the initial recoverable deflection is twice as big, and the pseudo-viscous yield twice as fast), then the relaxation time, HA, is the same as before. In some materials, however, although AB us doubled, EF is much more than doubled, so that the relaxation time is then not constant with stress.

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