54. Cell Operation in Nitella. II. Behaviour of Isolated Endoplasm

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Among the studies on the naked endoplasm obtained from the internodal cell of *Nitella flexilis* by our technique (Kamiya and Kuroda, 1957), we would like to begin our description with the behaviour of the continuous thread of endoplasm coming out of the cell into an artificial medium consisting of 0.08 M KNO$_3$, 0.05 M NaCl, and 0.004 M Ca(NO$_3$)$_2$. As reported in the foregoing paper, the endoplasm falls down from the opening of the cell in the form of a thread onto the bottom of the cuvette where the endoplasm forms an ever-growing sessile drop. When the amount of endoplasm coming out of the cell and that going into the drop on the bottom are equal, the phenomenon is stationary; it appears in this case as though the opening of the cell and the endoplasmic drop on the bottom are connected with an endoplasmic column.

It is not difficult to change the distance between the cell opening and the bottom of the cuvette by raising or lowering the cuvette while the position of the cell is fixed. A series of pictures in Fig. 1

Fig. 1. Behaviour of the endoplasmic column connecting the amputated cell and sessile drop when the latter was moved upward and downward repeatedly

which were reproduced from a strip of cinematographic film show how the endoplasmic thread behaved when its two terminals came closer to each other or became more distant. Fig. 1a represents an
endoplasmic column seen before the bottom was moved. The distance between the lowermost tip of the amputated cell wall and the bottom of the cuvette was 1.0 mm, the average diameter of the endoplasmic column being 130 µ. When this distance was reduced to about 1/2 within 1 second, the endoplasmic column is seen to be bent (Fig. 1b). The following pictures show how the endoplasm behaved as the bottom of the cuvette was raised or lowered alternately. In Fig. 1e in which only 2/3 of its total length is shown, the endoplasmic column was stretched to about 2 mm, i.e., twice the original length. By raising the bottom of the cuvette until the distance to the lower tip of the cell wall became 0.67 mm, or 1/3 the elongated column, the thread was bent aside like a loop-worm without having become much shorter (Fig. 1f). A similar figure was also seen in Fig. 1d. Such a procedure can be repeated many times without giving any harm to the endoplasm.

Though our observation was so far rather qualitative, the above behaviour of the endoplasmic column reveals that the endoplasm itself is very poorly elastic if at all. We notice that the endoplasmic column is rather different in its visco-elastic properties from the myxomycete plasmodium which is known to be highly elastic (Norris, 1940; Seifriz, 1942). Obviously the plasmodial strand has a cortical gel while in the endoplasmic column of Nitella there is no continuous gel structure except the 'surface precipitation membrane' demarcating the endoplasm from the external solution. So far as the authors' knowledge goes, the endoplasmic column such as we obtained has never been a subject of study before.

The next problem to be dealt with is the behaviour of the endoplasm in respect to its motion. To begin with, we shall direct our attention to the condition in the endoplasmic column outside the cell. The endoplasm falls down gently as a whole like a plug without giving rise to any appreciable velocity gradient inside. The only visible movement in the falling column of endoplasm is the Brownian motion of the small particles and the independent rotation of chloroplasts around their own axes.

The investigation of protoplasmic motion in a sessile drop on the bottom of the cuvette was carried out by taking advantage of the inverted microscope which enables us to observe in detail the minute motion in the drops of protoplasm while they were left as they were. Both nuclei and chloroplasts being somewhat greater in their specific gravity than their matrix (hyaloplasm) they tend to sink in the lower part of the drop. This fact further makes the use of the inverted microscope advantageous as they can be observed clearly in one focal plane (Fig. 2).
We could ascertain no mass streaming in these isolated drops of endoplasm. Absence of streaming in the endoplasmic drop is, however, not due to any mechanical injury, since chloroplasts as well as nuclei, both of which are often included in the drop, rotate most actively each around its own axis. Chloroplasts found in an isolated drop of endoplasm have been suspended in the endoplasm before the cell was cut and have not been stripped off from the cortical gel layer as a result of operation. The rate and direction of rotation vary with individual chloroplasts, the rate being generally within the range of 0.5–2 revolutions per second (Fig. 3). The rotation of the chloroplasts usually lasts 10–50 hours in the isolated endoplasmic drop kept in the mixed solution described before.

Fig. 2. An endoplasmic drop sunk on the bottom of a cuvette seen from below. Diameter of the drop: 210 μ (reproduced from a 16 mm cinematographic film)

Fig. 3. Rotating chloroplasts containing several starch grains which are strongly light-refractive. The pictures were taken from the same endoplasmic drop as that shown in Fig. 2 under higher magnification. b represents the same portion as a, taken 1/4 second later (reproduced from a strip of 16 mm cinematographic film). Exposure: 1/50 sec. each. c shows direction and time in second required for one revolution of individual chloroplasts in a and b.
An important question arises here as to why there is no mass streaming in the drops of endoplasm which was in a state of active motion when it was in the cell, while individual chloroplasts rotate furiously around their axes. We think that the lack of cortical gel is responsible for it.

Recently we (Kamiya and Kuroda, 1956) pointed out that the seat of the motive force generation is confined to the boundary region between cortical gel layer and outermost endoplasmic layer. This conclusion was derived from the analysis of the intracellular velocity distribution of the endoplasmic flow in *Nitella flexilis*, the same material as was used for the present work. In other words, the endoplasmic sol alone is not potent for inducing the streaming. That there is no appreciable velocity gradient within the falling column of endoplasm and that no rotationary mass streaming is seen in an isolated fragment which consists solely of endoplasm, are good evidences supporting the above conclusion.

If, on the other hand, the plasmagel exists in the form of freely suspended particles instead of existing in the form of fixed cortex, and if the shearing force is developed at the interface between plasmasol and plasmagel, what may happen is not the mass streaming of protoplasm, but the free motion of the individual particles. Thus individual rotation of the gel particles (chloroplasts and nuclei) in the isolated endoplasmic drop is a further support of our conclusion in our previous work (Kamiya and Kuroda, 1956) that the endoplasm alone is inert in respect to streaming, and that the seat of the motive force is confined to the interface region between the plasmasol and the organized plasmagel. We think that the motive force of the protoplasmic motion in *Nitella* cell is the shearing force which tends to shift the sol and gel phases against each other along their interface. Therefore, it is well to be understood that when the gel phase is fixed, endoplasmic streaming takes place, and that when the gel phase is freely suspended, the gel phase itself moves.

The phenomenon which shows this relation still more clearly is a minute, local streamlet occurring in the region very close to the surface of the gel particles, whether nuclei or chloroplasts. The direction of the streamlet is always opposite to that of the rotation of the particle. This minute, but rapid stream of plasmasol in direct proximity of a nucleus or chloroplast was noticed a long time ago by several workers, stressed by Valkanov (1934), and has been studied most exactly by Jarosch (1956) quite recently. We also confirmed this phenomenon clearly both around nucleus and chloroplasts in our ‘pure’ endoplasmic drop.
References

Plant Physiol. (Ames-Iowa), 245.