

The Cytomatrix: A Short History of Its Study

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As early as 1820 and for several decades thereafter the early observers of the cytomatrix worked mostly with the light microscope and very simple equipment. It was not uncommon for the investigator simply to crush a cell between two slides and watch the results. The sole motivation for these studies, as far as I can judge, was the satisfaction of curiosity, for there were no grants. Such observations are valuable to us today, especially those accompanied by imaginative interpretations.

Studies on the cytomatrix fall roughly into three periods, the early, middle, and recent. The early period, which was mostly descriptive, was the longest and filled the years between 1820 and 1910. It was a time when light microscopes were greatly improved, when chemical fixatives were discovered, when microtomes and staining procedures were introduced, and when the community of biologists became aware of cells and how they divide and assemble into tissues. Cell pathology also was pioneered during this period.

The middle period began around 1910 and continued until 1940. The emphasis in this period was on the study of living cells and on experiments designed to answer fundamental questions regarding the properties of the cytomatrix and the structural basis of intracellular organization.

The recent period has witnessed the introduction of phase-contrast microscopy, which greatly facilitated the observation of living cells. It has been also, of course, dominated by cell fractionation and electron microscopy. It has been a period of intense activity, yielding great quantities of information about cells and tissues. Cell biology has now emerged as a distinct science, but the cytoplasmic matrix is still not perceived by all cell biologists as something worth studying.

I would like to begin my discussion with the middle period, then look back at the early period for its background, and finally touch on the recent and more familiar period. Space does not permit me to mention all the details that I would like to.

Middle Period

From the viewpoint of a biologist, the middle period is perhaps the most interesting. A. Fischer and W. B. Hardy, working separately, had just startled the community of light microscopists by publishing evidence claiming to show that the elegant drawings made by their predecessors were full of

artifacts, all products of coagulation during fixation and/or dehydration (15, 17). Earlier cytologists, such as Flemming, Berthold, and Butschli, had already cautioned their contemporaries of the danger of confusing coagulations with genuine structures. Fischer and Hardy provided in their drawings images of asters and spindles found in dead pith cells impregnated with albumin (Fig. 1). For Hardy, the alveolar structure of the cytomatrix, the spongioplasm, was nothing more than a consequence of fixation (Fig. 2).

It may be that these attacks on findings that we now know were meaningful induced enough skepticism in the minds of cytologists and physiologists to encourage greater emphasis on studies of living cells. In any case, there followed a period of intense interest in the study of living protoplasm.

Robert Chambers was a dominant figure in this trend. I doubt that he ever fixed a cell or cut a section. He preferred instead to perform microsurgery on cells and later to inject foreign bodies, particularly oils, into cells and observe the various consequences. Early in his career he went to Cambridge, England, to work on cultured cells with Honor B. Fell. In one experiment, they were able to show that the thrust of a microneedle into the nucleus induced its breakdown and, subsequently, the lysis of the whole cell. If, however, the cell had two nuclei and they destroyed only one, the other survived, as did the whole cell (8). Needle intervention in the life of cells became quite popular. G. W. Scarth, a botanist at McGill University, observed that the nucleus of *Spirogyra* could be displaced several micrometers and that when released it would return promptly to its original position. It appeared that some viscoelastic property of the matrix asserted its influence (37). G. L. Kite was another enthusiast of micro-manipulation. When he interposed a needle between the male and female pronuclei of a recently fertilized *Toxopneustes* egg he found that he could push one of the nuclei about but that it persisted in slipping off the needle to advance toward its mate (7). Again, a mechanism of the matrix appeared to be at work.

Chambers made a fairly systematic examination of what could and could not be manipulated among the structural components of cells. He found striking differences in the ability of structures to withstand his interventions. He found, for example, that "fibrous strands, vesicles and rod-shaped mitochondria may be moved about and disturbed with no apparent loss of integrity. On the other hand, other structures,

such as the aster, when prodded quickly disappear" and that "the physical state of the protoplast resembles that of a reversible sol-gel colloidal system" (7). He also discovered that the transformation from gel to sol, with the subsequent disappearance of structural features, can be induced by various experimental procedures, particularly those that involve the use of hydrostatic pressure or sudden mechanical agitation. I shall return to this topic below.

It is important also to recall some work that Chambers did with M. J. Kopac involving the injection of oil droplets into sea urchin eggs (7, 24). Ordinarily, these fat bodies retain a spherical form. When, however, they are placed within "the radially gelled aster of a fertilized egg, they adopt an ovoid form." Clearly, the structural organization of the aster is sufficiently rigid to distort the oil drop.

Kopac (24) wrote that drops of oil inserted carefully into the immature oocytes of *Asterias* remain spherical and sharply defined as long as the oil causes no perceptible injury to the cytoplasm but that "a pronounced reaction occurs, when, after the oil is introduced, cytolysis is induced. Within 30 s after the cell has cytolized a membrane of adsorbed protein appears at the oil-cytoplasmic interphase and this is observed to crinkle (the Devaux effect) when some of the oil is retrieved." Regarding this, Chambers (7) writes: "Since the Devaux crinkling effect of the oil drop with the oil-retraction method is not noticeable in the living cytoplasm, it is concluded that the proteins in the living cell do not accumulate

on experimentally introduced surfaces while the protoplasm is intact. This suggests that the proteins are not freely diffusible and adsorbable in protoplasm, and that, therefore, these proteins may be bound together to form some kind of continuous phase," and goes on to say that "Probably the strongest argument for the existence of a differentiated layer on the surface of protoplasm is the fact that a colored solution which cannot enter from without will, when micro-injected, spread through the interior but will not pass out of the cell." Apparently, the dye is confined to a water-rich phase.

Discussions of the existence of organization in cells—in particular egg cells—were common, especially in the literature emanating from the Marine Biological Laboratory in Woods Hole. The names of Conklin, Lillie, and Boveri are intimately associated with work done principally to determine whether the so-called organ-forming substances in ooplasm are distributed nonrandomly and fixed in their positions. They found that although such intracellular substances as the yolk and pigment of *Arbacia* eggs could be stratified by centrifugation, the pattern of cleavage was not altered. In other words, a part of the ooplasm that did not move with the pigment went into the formation of the ingredients of asters and spindles and contributed to the content of micromeres and other cells of the 16-cell stage as though the egg had never been centrifuged (11, 40) (Fig. 3).

As E. B. Wilson (43) points out, "The difficulty of conceiving how the prelocalized organization of the egg can be bound up in a liquid or semi-liquid substance, such as the hyaloplasm often seems to be, is obvious. Lillie and Conklin have accordingly argued in favor of a relatively firm condition of aggregation in the hyaloplasm, yet one of such a nature that the cytoplasmic inclusions can still move through it. 'Flowing movements,' accordingly, whether in the normal egg or produced by the centrifuge, are regarded as no more than granule-movements within this semi-solid framework of the ooplasm. Conklin has produced considerable specific evidence that such a persistent framework of more viscid protoplasm (hyalo-

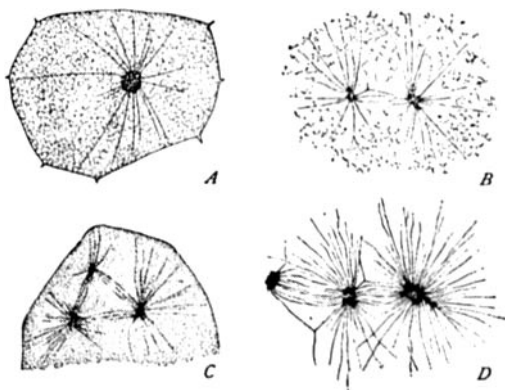


Fig. 24.—Coagulation-artifacts imitating cell-structures (FISCHER). A, dead pith-cell impregnated with 5% albumin and 2.5% hemoglobin and fixed in 1% osmic acid; B, 2% serum-albumin fixed in Flemming's fluid; C, 5% albumose solution in 5% gelatin, fixed in 1% osmic acid and 1% acetic; D, 2.5% albumose solution fixed in 1% osmic acid.

FIGURE 1 From E. B. Wilson (43).

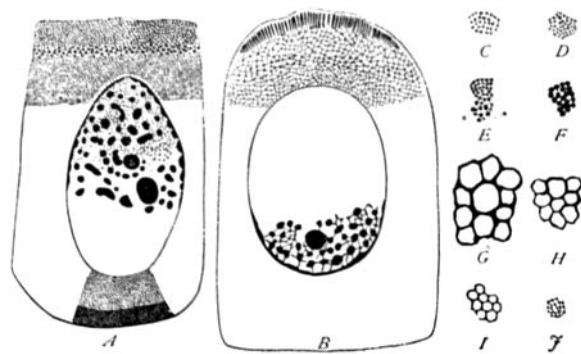


Fig. 25.—Coagulated cells and coagulation-artifacts (HARDY). A, B, epithelial cells, gut of *Oniscus*, A, fixed with osmic vapor, B, with mercuric bichloride; C-F, coagulated egg-albumin; C, 13% solids, sublimate; D, the same, potassium sulphocyanate; E, 30% solids, with included carmine-grains (a, a), sublimate; F, 60% solids, sublimate; G-J, coagulated gelatin fixed with sublimate; G, 10% solids; H, 25% solids; I, 50% solids; J, 4% solids.

FIGURE 2 From Wilson (43).

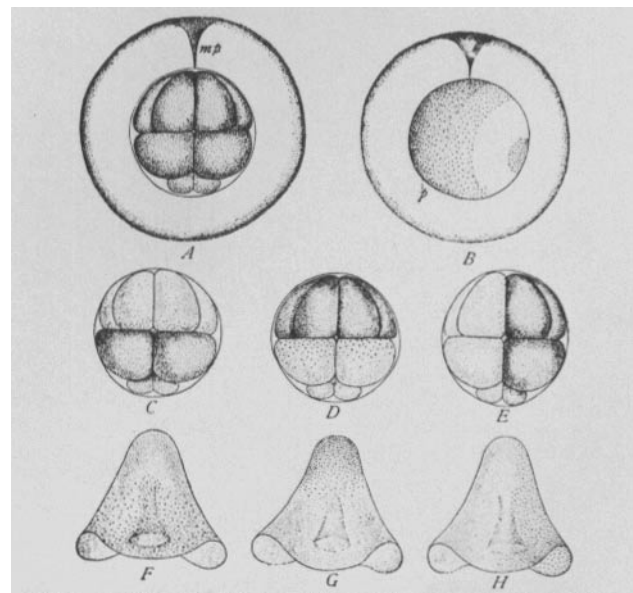


Fig. 521.—Development of centrifuged eggs of the sea-urchin *Arbacia* (MORGAN and SPOONER). A, 16-cell stage surrounded by the thick jelly-envelope perforated by the micropyle (mp) at the animal pole; B, unsegmented egg after centrifuging, showing stratification at right angles to the egg-axis (p, pigment-layer); C, 16-cell stage with pigment in vegetative (micromere) hemisphere; D, pigment in animal hemisphere; E, pigment on one side; F, G, H, three types of larva resulting from the three corresponding types of 16-cell stages (C, D, and E).

FIGURE 3 From Wilson (43).

plasm) exists in the clear substance of the *Crepidula* egg, and that it forms the basis of the true localizing activities. Its substance is assumed by Conklin to be elastic and contractile, and thus to produce the so-called flowing movements of living protoplasm and its contained granules. He emphasizes the fact, also noted by earlier observers, that if the centrifuging acts at a sufficiently early period and is not too long continued the dislocated egg-components tend to return more or less completely to their original position; and this too is ascribed to the action of the contractile framework which has maintained unchanged its original polarity."

These studies remind one of experiments performed somewhat later by H. W. Beams and R. L. King (1, 2). They exposed a wide variety of cells to huge centrifugal forces (400,000 g) and watched for evidence of survival. This was done in a small air-driven centrifuge developed by J. W. Beams, a brother of H. W. Beams. The contents of *Ascaris* eggs stratified into three layers during 1 h at 400,000 g returned to normal distribution over the next 12 h. At the end of 48 h, 90% of the centrifuged eggs had divided.

Other eggs and early cleavage stages centrifuged at 150,000 g for 4.5 d survived and developed at the normal rate. In other experiments, eggs were seen to undergo cleavage while centrifuged at 100,000 g. Apparently, any displacement of the cytomatrix or its contents achieved at 100,000 g is not sufficient to disrupt the organization of the cytoplasm essential for cell division. At the higher forces an interval of recovery appears to be essential for normal cleavage. Perhaps there is something in the concept that characterizes the cytomatrix as "spongioplasm."

The organization essential for mitosis is awesome, and variations in relatively simple conditions have been observed to disrupt it. Thus, as we know from more recent studies by Inoué and his collaborators (21), the birefringence of the mitotic spindle disappears at low temperatures and the chromosomes drift away from their position at the metaphase plate. The amazing thing is that they return to their normal position along with the birefringence when the cells are returned to normal temperature. It is as though some basis of organizational memory survives the experiment and guides the restructuring of the spindle.

In this middle period Marsland introduced and exhaustively used hydrostatic pressures to explore the characteristics of the matrix. These experiments followed the demonstration by Dugald Brown that the central cytoplasm of the *Arbacia* egg is relatively fluid compared with a thick (5- μ m) cortex. When exposed to a weak centrifugal field, the "granular components" of the central cytoplasm were readily displaced, whereas those in the cortex were not. When the same experiment was performed with increasing levels of hydrostatic pressure, the cortex displayed increasing liquefaction. At 10,000 p.s.i. the erstwhile cortical gel was almost undetectable. Subsequent studies showed that hydrostatic pressure induces solation of protoplasmic gels in a wide variety of cells (27, 30). We now know, of course, as first demonstrated by Tilney et al. (42), that microtubules disassemble as part of this phenomenon. The effect is reversible and probably involves solation of more than microtubule components of the cytomatrix. The sol-gel transformation is thought to represent a similar behavior of matrix components under normal conditions. Some observations by Pease (30) on the *in utero* eggs of the nematode *Rhabditis* are pertinent. He found that Brownian motion, ordinarily very limited, increased dramat-

ically in centrifuged eggs: "moderate centrifugal forces solated the gel network, and Brownian movement was unrestricted until the gel slowly reformed, the process taking some minutes to be completed" [see also Kitching and Pease (23)].

Several investigators, beginning with Heilbronn in 1922 (19), including Heilbrunn (20), and ending with Crick and Hughes in 1950 (12), attempted to learn something about the elasticity of the matrix by introducing iron particles into the cytoplasm and then shunting them around with externally applied magnetic fields (20). The experiments sound like fun, but according to Crick and Hughes the results were difficult to quantitate. They succeeded to some degree, where others had not, by taking movies that they could later analyze (12). This work has been summarized elsewhere (35), as follows: "In each case, when a particle was moved there was a small, rapid recoil when the magnetic field was turned off. The recoil never returned the magnetic particle to its starting point; usually recoil was about one-third of the distance originally traveled. Repeated, magnetically induced excursions of particles seemed to generate a larger space for unimpeded motion; ie, eight reiterations of the on-and-off action of the field lowered the viscosity in the space through which the particles had moved. They [Crick and Hughes] concluded from this that the cytoplasm is a thixotropic gel. The limited recoil one might interpret as reflecting the elasticity of a zone of compression that develops in a filamentous meshwork immediately in advance of the moving particle. The distance traversed minus the recoil could represent a part of the matrix in which the structure was damaged and the viscosity markedly altered (a thixotropic effect). Quite obviously, these experiments and others describe the presence of a viscous and structured matrix in the cytoplasm, a matrix with enough elasticity (or structural information) to return it to its undistorted form after some kinds of perturbations."

The ultramicroscope (dark field) introduced in the middle period helped to alert Strangeways and Canti (41) to the presence of small refractile structures in the more central regions of thinly spread cultured cells and also to the striking absence of Brownian motion in a normal uninjured cell. They were original also in showing that the image was not detectably altered by fixing the cells with vapors of OsO₄, which was not true of other fixatives.

The birefringence of cells and especially of certain cell components was pioneered by W. J. Schmidt (39). Fiber systems in the cytoplasm became known for their form birefringence, as did later structures rich in microtubules, such as axonemes, cilia, and mitotic spindles. Flow birefringence was fairly apparent, and this initiated much discussion of non-Newtonian flow as displayed by the viscous cytoplasm.

I should not end my discussion of this period without mentioning two theorists, A. Frey-Wyssling and G. W. Scarth. The former was especially expert in adding it all up. For example, he says of the physical properties of cytoplasm: "The paradox of the cytoplasm is that it shows both fluidity and elasticity. It is a solid and a liquid at the same time to an extent not observed in any other colloid. The task of submicroscopic morphology consists, therefore, of drawing up a structural schema" (16). His scheme shows the matrix as an extremely fine network, the meshes of which contain such interstitial substances as water and salts and glucose in solution. The fundamental difference between nonliving and living gels is that in living cytoplasm the junctions between the filaments are continuously restructured.

Scarth (38) likens the structure to that of a brush heap, and says that "The mystery of cell organization, more perhaps than any other biological problem, seems to conduce a vitalistic viewpoint. However, as Driesch pointed out, if it can be proved that organization may exist without an adequate physical mechanism, a mechanistic explanation of it becomes, ipso facto, impossible.

"The kind of organization that is experimentally discovered in eggs and other cells is that of polarity, localization of substance and, in the nucleus, an orderly serial arrangement of units. For this to exist physically demands a more or less structural basis. Consequently, if it should be displayed by a liquid medium such as protoplasm is said to be—at least in some crucial instances—we are faced with a biological miracle."

Scarth (37) took part in a program organized in 1940 by the American Society of Plant Physiologists (Fig. 4). At the end of his talk he recited a parody on a little light verse written by John Godfrey Saxe a century earlier, "The Blind Men and the Elephant."

It was four fundamentalists to learning much inclined,
Who went to see the Protoplast (though all of them were blind)

That each its structure might observe to satisfy his mind.

The first advancing hurriedly and happening to fall
Right through its soft interior at once began to bawl
"God bless me! But the Protoplast is very like a sol."

The second poked the animal and felt his staff repel
Its tough and springy cortex, so he began to yell
"Tis evident the Protoplast is very like a gel."

The third approaching gingerly did only pinch and squeeze
Its slippery oleaginous hide when he began to wheeze
"It seems to me the Protoplast is just a lump of grease."

The fourth man, having punched and probed and proved its plastic state,
Watery yet indissoluble, did thus asseverate
"The Protoplast is a compound, complex co-a-cerv-ate."

And so these fundamentalists disputed loud and long
Each in his own opinion exceedingly stiff and strong,
Though each was partly in the right and all of them were wrong.

Other volumes grew out of conferences in response to the urge to sum up what had been learned in the 30 years before World War II disrupted normal life and research. One of these volumes that summarized better than others the progress of the middle period comprised 31 essays presented to Sir Frederick Gowland Hopkins (1937) on his 75th birthday. One essay was by Joseph Needham, the author of *Chemical Embryology* (29). He departs from chemistry enough to say "that a whole movement has been taking place in recent years towards the conception of fibre or thread molecules as the basis of protoplasmic organization and since it is easier to conceive of a three-dimensional structure being built of oriented fibers than of cohering spheres the result is of no small importance for morphology." From this point one can easily imagine him working his way to the term "cell skeleton."

A Symposium on **THE STRUCTURE OF PROTOPLASM**

A MONOGRAPH OF THE
AMERICAN SOCIETY OF
PLANT PHYSIOLOGISTS

Edited By
WILLIAM SEIFRIZ

PUBLISHED, 1942
THE IOWA STATE



AT AMES, IOWA
COLLEGE PRESS

FIGURE 4 Title page of a monograph based on papers presented at a symposium sponsored by the American Society of Plant Physiologists and held in Philadelphia on December 30, 1940.

Actually, he had used the term cytoskeleton for the first time in his Terry lectures a year earlier.

Sir Rudolph Peters (31) in his paper expressed impatience with "the current purely colloidal conceptions of the cell." He wandered into fanciful analogies, e.g., that of the existence of an intracellular nervous system composed in some way of cytoplasmic proteins, and emerged at the end with a plea for the study of intact cells. The smooth endoplasmic reticulum and its control of free Ca^{++} would coincide today with Peters' notion of an intracellular nervous system.

Unfortunately, World War II interrupted in England and elsewhere much of what might have been very valuable investigations into the nature of the cytoskeleton.

Early Period

In going back now to the beginnings (the early period), I shall be brief. It is difficult to identify the first and most significant observations on the optically empty matrix of the cell. It may have attracted the sharp eyes of van Leeuwenhoek as early as 1670. Meaningful observations, however, had to await the development of better microscopes, and, especially, of achromatic lenses. Many were attracted to the use of these new microscopes in the first half of the 19th century. The names of some are familiar: C. F. Wolff (1733–1794), B. de Mirbel (1776–1854), Lamarck (1744–1829), Dutrochet (1824), J. P. F. Turpin (1775–1840), Meyen (1804–1840), von Mahl (1805–1872), Brown (1773–1858). All of these, with their observations on the cellular structure of plants and

animals, preceded Schleiden and his *Beitrag* of 1838 by several years.

Felix Dujardin (1801–1860) went further than most of the others with crude attempts to learn something about the physical nature of protoplasm. He called it inclusively “sarcode” (the flesh of the cell) and characterized it as a “clear glutinous diaphanous substance.” Dujardin’s description of the cytoplasmic matrix, published in 1835 (14), was based on microscope examinations and experimental probings of the cell bodies of certain Foramenifera. Whereas other cell biologists of his time had referred to it simply as a living gel (equally true today), Dujardin observed that it was insoluble in water, contracted into globular masses, was sticky in that it adhered to dissection needles, stretched like mucus, and occurred in all the cells (*les animaux inférieurs*) he examined (Fig. 5).

Dujardin was ahead of his time, though not totally alone in making observations on the matrix. I suspect that the importance of what he said about it was largely wasted on his contemporaries.

As the Cell Theory gradually emerged from the observations and thinking of Dujardin’s contemporaries, it was reasoned that the “sarcode” must, in addition to its other properties, be highly organized. This, according to E. Brücke (1861) (4), was essential for the various activities that cells display and set the intact cell above and apart from mere chemical and physical processes. This statement has been repeated many times since 1861. Forty years later Hofmeister suggested in a 1901 paper on the chemical organization of the cell [cited in Wilson (43)] that “the morphologist on the one hand strives to elucidate the structure of protoplasm down to its finest details; the biochemist on the other hand, with his apparently

cruder yet still more searching methods seeks to determine the chemical functions of the same protoplasm; broadly speaking they are only dealing with two different sides of the same thing.”

A lot of ink has been expended on this topic in words and drawings. The morphologist has viewed the cell as possessing an undifferentiated substance, including nucleus, mitochondria, and centrioles, which is able to grow and reproduce, and also a differentiated protoplasm, self-perpetuating and capable of performing special functions. As the techniques of staining improved along with microscopes, the interest in penetrating and defining the nature of the clear viscous matrix continued. The hyaloplasm became the viscid ground substance or matrix. In most cells, this hyaloplasm was observed to be sufficiently viscous to inhibit Brownian motion. Structurally it was thought by some biologists to be fundamentally fibrillar and elegant images (drawings) were produced to illustrate this property (Heidenhain) (18) (Fig. 6). To others (Butschli) (6) it was alveolar, like a sponge. This was labeled the foam theory of protoplasm (Fig. 7). For others, impressed by the sometimes numerous mitochondria, it was basically granular. Dahlgren (13) in his textbook on comparative histology, provided an interesting drawing of the cytoplasmic matrix (my interpretation). The source of the image is not given. If created at Princeton University, where Dahlgren was a professor, it was probably a synthesis of Dahlgren’s and Conklin’s ideas (Fig. 8).

These various discussions attracted the attention of a few who chose to believe none of it and, as mentioned earlier, set about to prove that these various images were artifacts of fixation or dehydration or both.

On the positive side, they probably did a lot inadvertently

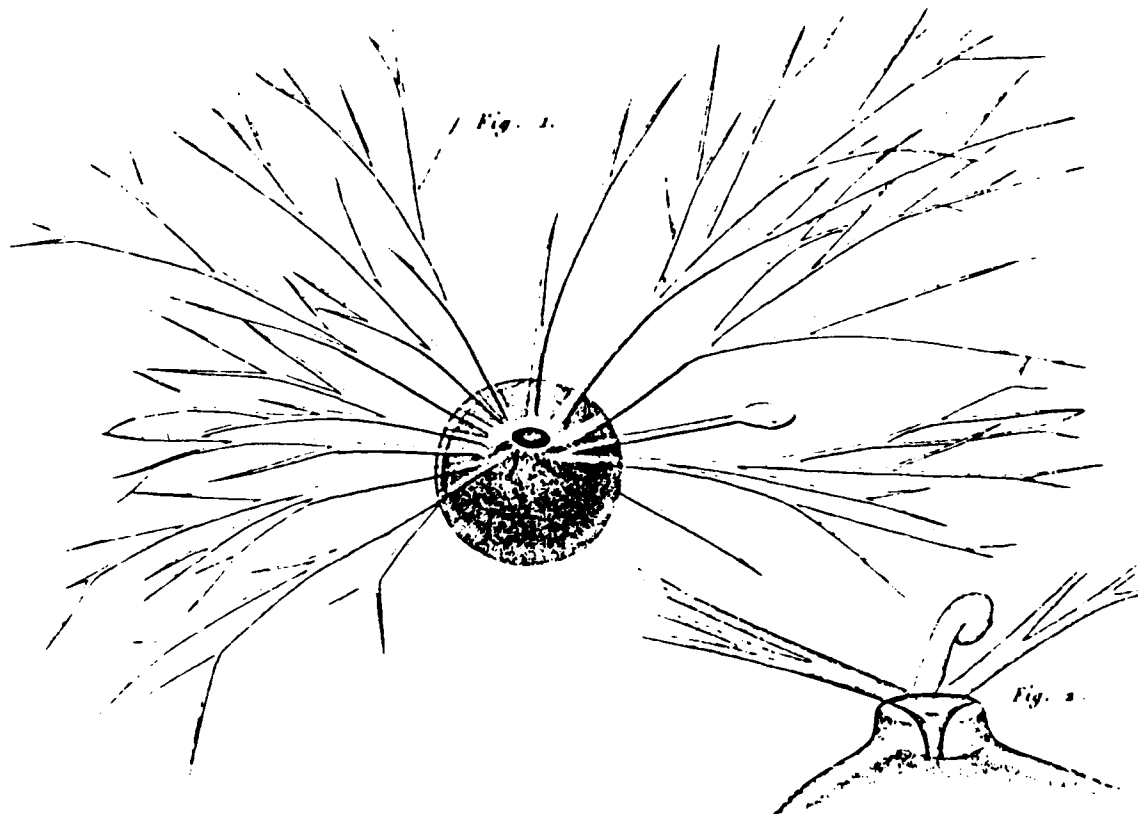


FIGURE 5 One of the organisms (a rhizopod) that Dujardin chose to use for his studies. He named it *Gromia oriformis*. The image here is a copy of Figs. 1 and 2 on Plate IX of his paper (14). Fig. 2 is a detail of the orifice “de la coque membraneuse.”

to encourage the studies of living cells that seemed to dominate the cell biology of the next period. It was at this time that M. Lewis and W. Lewis (25) were watching live cells proliferating and moving about under the conditions of *in vitro* culture. They were able to discern the behavior of mitochondria, the characteristics of cell motion, and some manifestations of differentiation. Their observations were not subject to the criticisms of Fischer and Hardy. On the other hand, the Lewises and their students did not indulge in much speculation on the fundamental nature of the cytoplasmic matrix. That controversial subject at that time had seemingly lost some of its earlier popularity.

Recent Period

The recent period in this arbitrary division of the history of the study of the cytomatrix began just before and during World War II. Albert Claude (9), at The Rockefeller Institute, had been isolating and studying the properties of the chicken tumor I agent, later to be called the Rous sarcoma virus. In those days it was not considered a virus. To obtain control material from a normal source, Claude ground up liver and chick embryos and subjected the homogenates to differential centrifugation. To his surprise the fraction containing the tumor agent and the chick embryo fraction, similarly isolated, had essentially the same properties, except that the fraction from normal tissues did not produce tumors. This prompted

Claude to initiate an extended series of studies in which he developed useful techniques for the isolation of nuclei, mitochondria, and microsomes (fragments of the endoplasmic reticulum). He had had no formal training in cell biology and initially confused mitochondria from liver homogenates with secretory granules (of which there are very few in liver). This error was subsequently corrected by Palade and Hogeboom. The essential fact is that Claude had developed a good procedure for isolating and "purifying" cell components. What he achieved with much patience was a distinct improvement over the procedures published six years earlier by Bensley and Hoerr (3). The cytoplasmic matrix we now address was part of the second or third supernatant, which was usually discarded. It was later named the cytosol fraction. Unfortunately, the term was transferred from the contents of the centrifuge tube to the living cell and equated with the hyaloplasm or cytomatrix which is not a sol, not most of the time, anyway. What has happened since, as far as the cytomatrix is concerned, is current history and will be mentioned by the other authors in this supplement. Their pursuit of an understanding of the matrix has been by biophysical and biochemical approaches.

In spite of the obvious achievement made possible by cell fractionation as practiced by Claude and his followers, there persisted in the minds of some cytologists the thought that the fractions of cytoplasmic organelles must be severely damaged by the procedures involved. This thought, among others, inspired Zalokar (47) to attempt a fractionation within the intact cell. The hyphae of *Neurospora* turned out to be a favorable material. The cells were not destroyed by high-speed centrifugation, and the various components stratified to produce several layers easily identified as mitochondria, nucleus, glycogen, and the basophilic component (ergastoplasm or

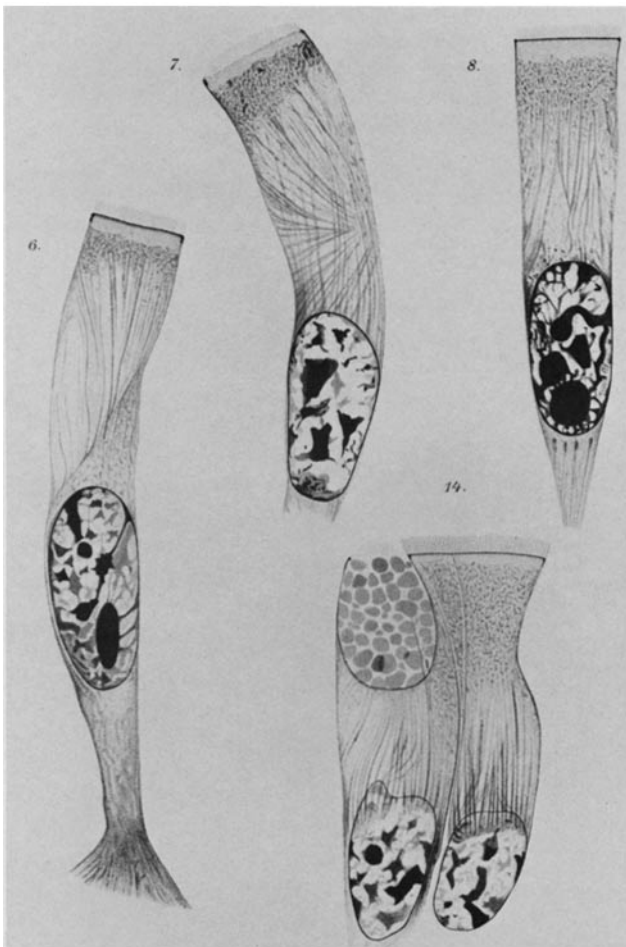


FIGURE 6 A plate of drawings from a paper by M. Heidenhain (18). The cells are all epithelial units from frog intestine. Obviously they contain elaborate unidentified cytoskeletal elements.

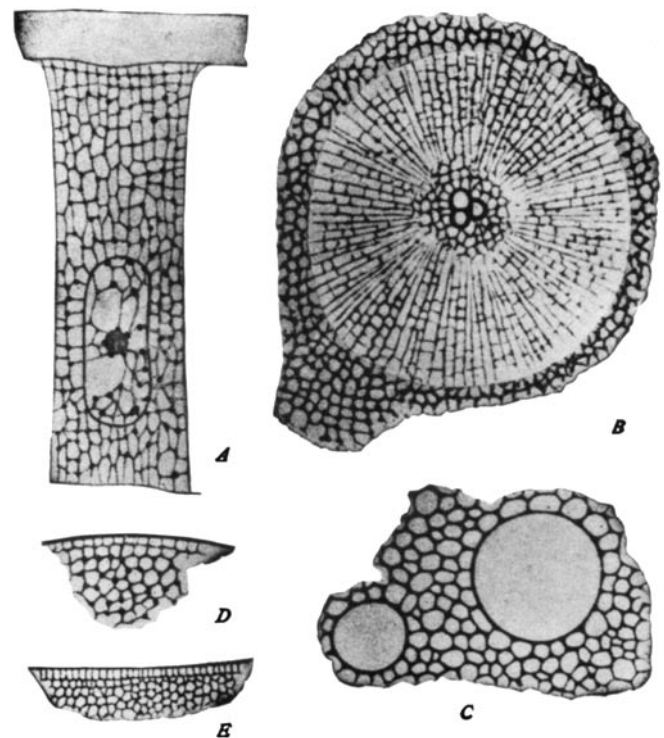


Fig. 26.—Alveolar or foam-structure of protoplasm. (BÜRSCHLI.)
A, epidermal cell of the earthworm; B, aster and central bodies from sea-urchin egg; C, intracapsular protoplasm of a radiolarian (*Thalassioella*) with vacuoles; D, peripheral cytoplasm of sea-urchin egg; E, artificial emulsion of olive-oil, sodium chloride, and water.

FIGURE 7 From Wilson (43).

rough endoplasmic reticulum). There was a fifth or sixth layer that was relatively free of formed structures called supernatant and that had the appearance of a “honeycomb lattice.” It tested positive for the presence of phosphatases. The cells,

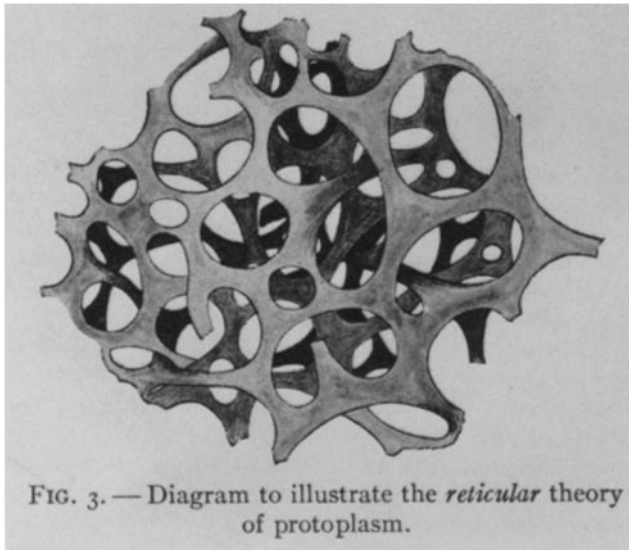


FIGURE 8 Illustration from Dahlgren (13). The drawing was made for Dahlgren not later than 1908, but the artist (author) is not indicated. Clearly our concepts have not changed radically in 75 years.

viable after centrifugation, reorganized their contents and continued to propagate.

Similar studies by Kempner and Miller (22) on *Euglena* yielded similar results. The fifth, or clear layer of their stratifications tested negative for protein and was structure free, as near as they could tell. Following centrifugation, which these cells survived, the various organelles returned to their normal distribution. [See also Clegg (10).]

Another approach has been provided by electron microscopy, which began around 1945 with observations on whole cultured cells (32). The first micrographs obtained were of limited value, but before long, as we learned some tricks of preparation, they got better and we recognized the endoplasmic reticulum and its characteristics and a few small fiber systems (stress fibers of Warren Lewis). The manipulative techniques were difficult, and drying the cells in air, as was then the practice, was destructive. Subsequent studies equated the microsome fraction with the rough endoplasmic reticulum and with the chromidial substance (also called ergastoplasm) of the earlier cytologists. The ready availability of thin sections (around 1953) opened up a vast new world for exploration and discovery. The more obvious things were studied first and included all membrane-limited structures. Eventually, microfilaments and microtubules got some attention, especially after glutaraldehyde was introduced as a fixative.

The cytoplasmic matrix, that part exclusive of identifiable filaments, was least attractive for investigation. It appeared faintly stained, if at all, and essentially unstructured. “Wispy”

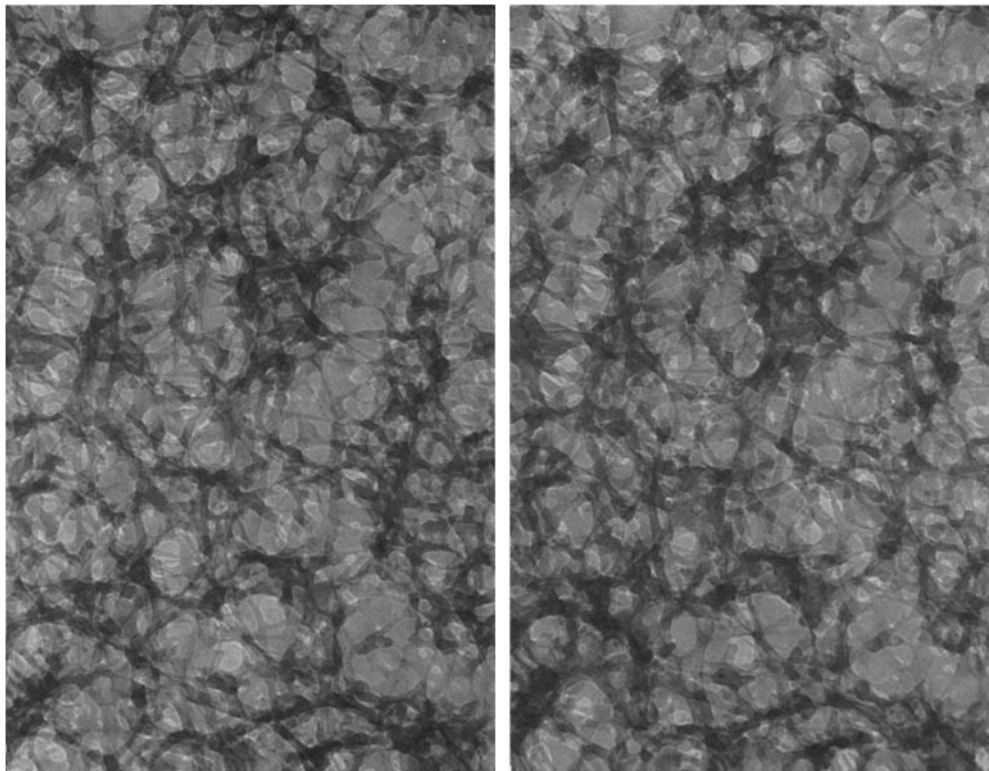


FIGURE 9 Stereo electron micrographs (high-voltage) depicting the structure of the cytoplasmic matrix in a thin margin of a cultured NRK (newborn rat kidney) cell. The cell was fixed in glutaraldehyde, frozen in propane chilled to -185°C and finally dried for 1.5 d while maintained at -95°C to avoid crystallization. In this experiment, we avoided critical-point drying and thus any artifacts it might induce. At the same time we obtained an image of glutaraldehyde-fixed matrix for comparison with that in cells preserved solely by freeze-drying (see Fig. 10). It is evident that the morphology following glutaraldehyde fixation is very similar to that after freeze-drying only. Some shrinkage of individual trabeculae is apparent in the images and in morphometric analysis of their dimensions (33). Microtubules can be identified in this and in the succeeding figures as strands of relatively uniform diameter. $\times 80,000$.

was a favorite adjective for what was there. Electron microscopists habitually included staining with uranyl and lead in their procedures, and, if something failed to stain, it was not there or not worth attention. However, a few desperados in search of excitement entered the fray. Mostly they were interested in the fine structure of axoplasm and the appearance of possible mechanisms for axoplasmic transport. Paul Burton and colleagues (5) formed one group and Yamada, Spooner, and Wessells (46) another. J. Metzels (28), all alone in Ottawa, was doing the same type of work. They all published images showing networks or meshworks of slender strands between neurofilaments and neurotubules. I cannot say that very many of their contemporaries were convinced or even interested.

We find compelling the argument that something in the cells has to account for the nonrandom distribution of formed structures such as the endoplasmic reticulum, the Golgi, the microtubules, and bundles of microfilaments, in other words, for the organization (10). I have always been impressed by the fact that microfilaments (stress fibers) appear and disappear as the cell moves and changes its shape. For me, therefore, there should be a unit structure, a cytoplasm, built around the cell center and including a population of dense bodies (microtubule-organizing centers) distributed in a manner characteristic for the cell type. The cytoplasm contains the more visible components and controls their assembly or disassembly as required. All this sounds a little far-fetched, but we have been encouraged to stay with it by observations of

pigment cells, which tell us that individual pigment granules have fixed positions in the matrix that moves them (34).

At the very least, the arguments for a structured matrix (and there are several, as mentioned in this history) have encouraged us to take a look, not at resin-embedded cells, but at whole cultured cells dried by the critical-point method or otherwise. Having at our disposal a high-voltage microscope has been a great help (Figs. 9–11) (45).

We have found and reported that we can affect the integrity of the lattice by exposing it to low temperatures and that the lattice immediately returns to normal form when the cell is reincubated (36). Its structure changes in response to cytochalasin and to variations in the concentration of Ca^{++} and Mg^{++} in the medium. The return to normal when normal conditions are restored is amazingly rapid. It is, in a few words, a living gel.

Artifacts are present, of course, but they seem not to be gross. The basic design and dimensions are very similar whether the cell is chemically fixed or preserved by freeze-drying or freeze-substitution (33).

It is pertinent that the cilium, as an extension of the cytoplasm, has its own lattice consisting of what are called spokes. They are ordered in their disposition and no one questions their existence in the cilium. There are several lessons to be learned from these spokes.

David Luck and colleagues (26) have shown that in some mutants of *Chlamydomonas* the spokes either do not exist or appear in some abbreviated form. They have determined by

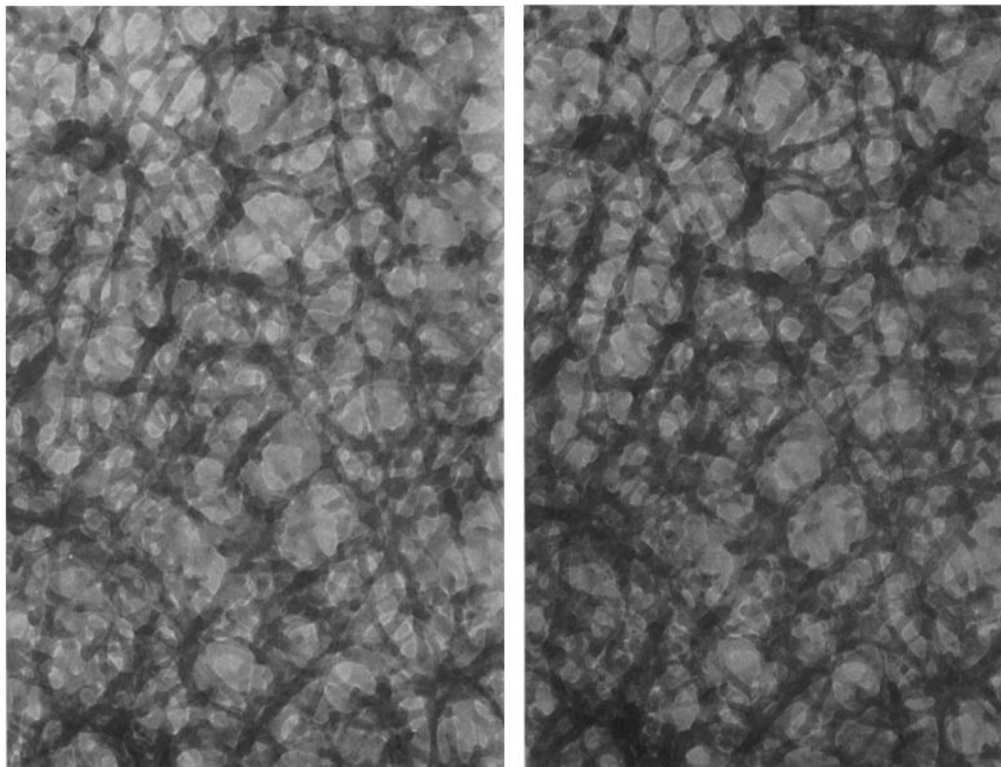


FIGURE 10 Stereo pair of images showing the three-dimensional structure of the cytoplasmic matrix in a cultured NRK cell after rapid freezing and drying from the frozen state. The procedure followed was identical to that used in preparing the cell depicted in Fig. 9, except that here the cell was not fixed with glutaraldehyde before freezing; it was frozen in propane chilled to -185°C and dried while maintained at -95°C . The dimensions of the microtrabeculae show greater uniformity than in Fig. 9. We interpret this image as closely representative of the living structure at the instant of freezing. The variations on this shown in Fig. 9 probably reflect the dynamic properties of the lattice plus the failure of glutaraldehyde to penetrate rapidly enough to stop all motion instantly. $\times 80,000$.

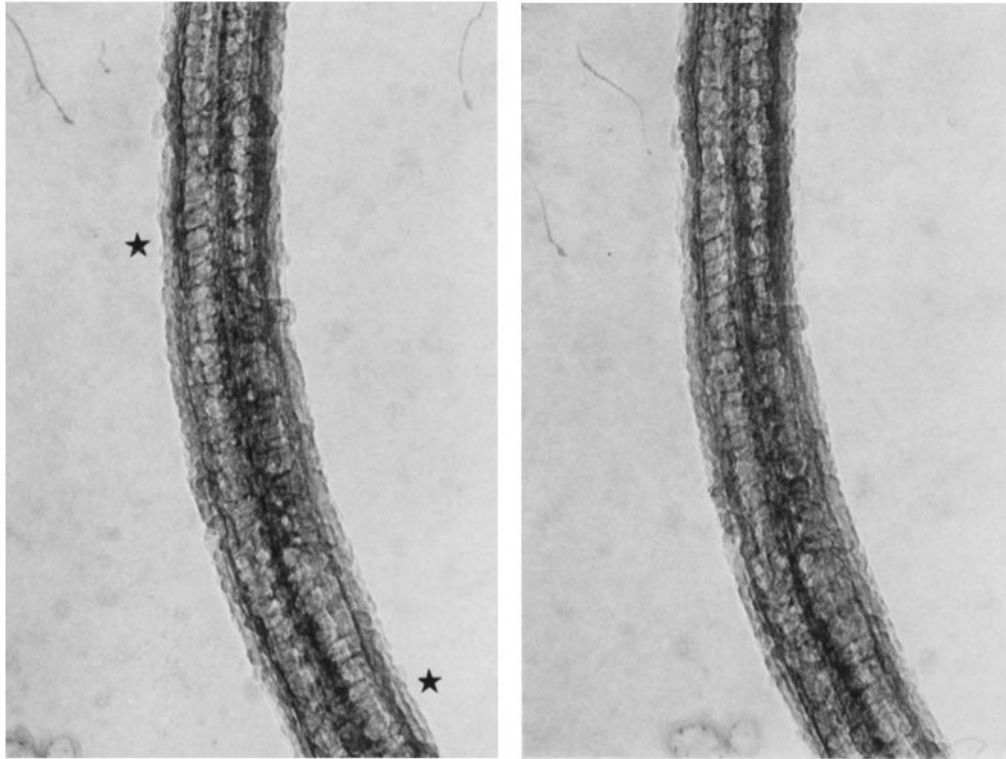


FIGURE 11 Stereo pair of a sea urchin sperm flagellum fixed in glutaraldehyde and dried by the critical-point method. The spokes are evident (see stars) but only in a few places and even there not clearly in the triplet repeating order that characterizes their distribution in thin sections. Obviously the superimposition of structure contributes to the confusion in the whole-flagellum micrographs. That, and the fact that the variable orientation of the spokes brings their full-length profiles into the image plane only occasionally, accounts for fact that the total spoke array cannot be seen. $\times 80,000$.

their dual genetic and biochemical approach that about 17 different polypeptides are involved in the spoke structure. Though the orderly disposition of these polypeptides is readily seen in thin sections, it is discerned with difficulty in micrographs of the whole flagellum (Fig. 11). It should not surprise us, therefore, if some order among the matrix trabeculae is present, though not visible, in the micrographs of whole, thick cytoplasts.

Conclusions

It seems to me that the evidence for a structured cytoplasm (matrix) is incontrovertible. It is structured like a gel that incorporates formed filaments for purposes of giving direction to intracellular motion, anisometry to cell form, and useful variations in viscosity. It is a living gel, at once dynamic and capable of responding by structural changes to numerous stimuli and yet preserving the capability of reverting to a preferred form.

The author is pleased to acknowledge the support of the Fogarty International Center during the preparation of this manuscript and the organization of the Conference.

Thanks also go to Mary Ulrich for work on the illustrations and to Joyce Albersheim for repeated drafts of this manuscript.

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