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# CHEMICAL STRUCTURE OF CYTOPLASM

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IN a recent note written as a supplement to a symposium on the structure of protoplasm, K. H. Meyer[1] summarizes Seifriz's view of the structure of protoplasm as follows: "the ultimate structural units of the living substance are probably linear molecules or micellae so arranged as to form a framework" and "the living substance is composed of a true network of primary valence chains which at several points are tied together by chemical bridges held by molecular cohesion (to-day one would say residual valences or hydrogen bonds)." If Meyer had substituted in the first statement the work "some" for "ultimate" and left out the framework which requires further definition, and in the second statement had substituted the word "contains" for "is composed of" this would be acceptable to the majority of students of cell structure. X-ray diffraction and birefringence studies have brought convincing support to the conception of structural constituents in protoplasm which Seifriz[2] with so much genius and foresight advanced a decade and a half ago.

This theory, however, interprets only some of the properties of protoplasm. These as listed by Seifriz[3] are: "contractility, elasticity, cohesiveness, rigidity, and tensile strength." All these may be possessed by non-living systems. Protoplasm on the contrary respires, excretes, performs complicated chemical operations, uses or liberates energy and reproduces its own substance in kind. This metabolism is mediated by a multitude of intracellular enzymes and carriers and

[1] K. H. Meyer, "The Structure of Protoplasm," Iowa State College, p. 267, 1942.

[2] W. Seifriz, *Brit. Jour. Exp. Biol.*, 1: 431, 1923–4.

[3] W. Seifriz, *Am. Nat.*, 63: 410, 1929.

can be imitated in part *in vitro* but in the cell is characterized by speed, orderliness and rhythm not to be found in a random mixture of chemical substances in solution.

This dilemma in the application of the concept of molecular pattern to the problem of protoplasmic structure has been fully appreciated by Sponsler,[4] Sponsler and Bath[5] and the late Laurence Moyer,[6] who examined the possibilities of the orderly attachment of enzymes, carriers, lipids, etc., to polypeptid chains by hydrogen bonds, von der Waal's forces and salt and sulfur bridges. Sponsler also recognized the presence of microscopic and submicroscopic particulates and discussed the formation of them by folding of polypeptid chains.

This is all very plausible, and useful, in so far as it is capable of experimental test or contributes to the discovery of new methods of investigation. It is a laudable effort to reduce the nature of living substance to a monistic formula.

Protoplasm, however, does not possess either microscopic or submicroscopic homogeneity. Even when it seems optically structureless it may conceal behind the mask of simplicity those local differences of organization which express themselves in determinate cleavage. Often protoplasm is composed of distinct, different and to some extent separable parts. This was demonstrated by Reinke and Rodewald[7] when they pressed out the liquid protoplasm of *Aethalium septicum* and so divided it into liquid and solid fractions, and by innumerable chemists since who have extracted cells with saline solutions and found that only part of them dissolved.

It would seem to be an axiom of analytic chemistry to separate separable things before proceeding to their analysis. The alternative method of mixing all the constituents of the cell as thoroughly as possible and then obtaining from the mixture substances in pure form by skilful chemical procedures has yielded brilliant results in the field of hormones, enzymes, nucleic acids, etc., but has left us in ignorance of the protein structural substrate of the cell and of the distribution and spatial relationships of the active substances.

The possibilities of the method of separating separable things first have been developed in this laboratory and more recently by Claude at the Rockefeller Institute in the separation and preliminary analysis of mitochondria and of several types of submicroscopic particulate components of protoplasm and in the isolation of the structural proteins from the difficultly soluble cytoplasmic components. It is my purpose to review briefly the progress which has been made in this field.

[4] O. L. Sponsler, "The Cell and Protoplasm," p. 166, The Science Press, 1940.

[5] O. L. Sponsler and J. D. Bath, "The Structure of Protoplasm," p. 41, Iowa State College Press, 1942.

[6] L. S. Moyer, "The Structure of Protoplasm," p. 23, Iowa State College Press, 1942.

[7] J. Reinke and H. Rodewald, *Untersach. a. d. bot. Lat. der. Universitat*, pp. i–viii and 1–70, Göttingen, Heft 2, 1881.

## Structural Proteins

The difficultly soluble portions of cells have been much neglected by biochemists, presumably because they were impressed by the ease with which proteins may be denatured and by the fact that such insoluble residues of organs always contain connective tissues. I shared this point of view until Hoerr and I[8] began to work with frozen dried material prepared by the Altmann-Gersh method. This method afforded an opportunity, by the use of freehand sections, to introduce solvent solutions to the interior of a chemically unaltered cell without encountering the obstacle of semi-permeability. In such preparations of the liver of the guinea pig treated with 0.85 per cent. NaCl solution the mobile protein of the cell quickly dissolved but left behind a morphologically complete cell with mitochondria, nuclear chromatin, cell and nuclear membranes. Treatment with $\frac{N}{200}$ ammonia solution removed the mitochondria and nuclear chromatin but left behind a cell with membrane, cytoplasmic substrate, nuclear membrane and linin threads. This residue we termed structural protein. We were first unable to dissolve in it anything but strong alkali. From this solution it precipitated on neutralization in membranous floccules with fibers running through them. On repeated solution and precipitation the substance became more flocculent and less fibrous and evidently had undergone some hydrolysis. To this material we gave the name *ellipsin* in recognition of the long hiatus in our study of the cell during which structural considerations had been jettisoned by those who believed that the liquid state excluded them.

Mirsky[9] reported the portion of the eggs of Arbacia and Strongylocentrotus insoluble in $M_1$KCl solution to be increased after fertilization, and Moore and Miller[10] found that the eggs of Strongylocentrotus which were isotropic became anisotropic in three minutes after fertilization indicating an orientation of structural elements.

In 1938 I[11] reported that a portion of the washed hepatic cell was soluble in stronger salt solution (10 per cent. NaCl) yielding highly viscous solutions with pronounced elastic properties. Those solutions precipitated on dilution or acidification as discrete fibers of great length which tended to assemble side by side

[8] R. R. Bensley and N. L. Hoerr, *Anat. Rec.*, 60: 251, 1934.

[9] A. E. Mirsky, SCIENCE, 84: 333, 1936.

[10] A. R. Moore and W. A. Miller, *Proc. Soc. Exp. Biol. Med.*, 36: 835, 1937.

[11] R. R. Bensley, *Anat. Rec.*, 72: 351, 1938.

into fascicles. Soon after formation the mass of fibers contracted into a knot. To this substance I gave the name *plasmosin,* because of a fancied resemblance to myosin, reserving the name *ellipsin* for the residual substance not soluble in 10 per cent. NaCl.

In 1940 Banga and Szent-Györgyi[12] by extracting washed kidney tissue with a 30 per cent. solution of urea obtained a similar viscous extract which when precipitated in fibrous form gave an x-ray diagram similar to myosin B. The insoluble residue, insoluble either in saline solutions or 30 per cent. urea solution, when dissolved in alkaline solution exhibited streaming birefringence.

I am not sure that the fractions obtained by Banga and Szent-Györgyi are the same as those of Hoerr and the writer, because urea solutions are under suspicion by reason of their capacity of converting corpuscular proteins into fibrous proteins,[13] but the general trend of their work is the same and these distinguished chemists are not at all embarrassed by the possibility of denaturation.

My first preparations of plasmosin were highly contaminated by other substances since I found it difficult to purify by repeated solution and precipitation. Lazarow, my assistant, however, working in a cold room at + 2° C. and transferring the balloon of fibers to fresh solvent before it had contracted into a mass was able to redissolve and reprecipitate several times. Also we found that unless the water used in dilution was buffered to pH 6.8 with citrate or phosphate buffer much protein was adsorbed by the fibers.

The purified product contained 3.7 per cent. of phosphorus and gave positive pentose and purin base reactions. It was obviously a nucleoprotein. This fact would suggest to many minds its origin from the nucleus. The idea that nucleoproteins are confined to the nucleus has, however, been breaking down owing to the discovery that many active components of cells have a nucleotide structure and the discovery of Claude[14] that the particulates contain nucleoprotein. Casperson and Schulz[15] also have demonstrated the presence of nucleoprotein in cytoplasm by spectrophotometric methods. I would not wish to reject the idea that the nuclei contain plasmosin, but the rapid rise in viscosity of suspensions of washed cells in 10 per cent. NaCl solution compared with the slow rate of extraction of the nuclear chromatin in the same solution and the rapid extraction of plasmosin from frozen dried cells without much loss of nuclear chromatin inclines me to the opinion I previously expressed that it is in large measure a constituent of cytoplasm. Whether it originates there or is produced in the nucleus is of course an interesting topic for speculation and research.

Plasmosin in solution in 10 per cent. NaCl dialysed against distilled water lightly buffered to pH 6.9 with phosphate buffer first precipitates as fibers, then the precipitate swells into a gel, and finally redissolves as the electrolyte is progressively reduced. In this state it has an isoelectric point of about pH 3.2. Thus, plasmosin may be extracted from cells by strong solutions of NaCl at a level of pH at which most of the mobile proteins are insoluble. Plasmosin is also soluble in 30 per cent. urea solution.

The portion of the liver cell which remains after removal of mobile proteins, mitochondria, submicroscopic particulates, nuclear chromatin and plasmosin represents cell and nuclear membranes, substrate of cytoplasm and linin threads. It is completely soluble in $\frac{N}{2}$ sodium hydrate and is reprecipitated on neutralization. The preparation carries about 25 per cent. of its dry weight of lipids. The preparation is phosphorus free and thus my original suggestion that it was a denatured form of plasmosin can not be sustained. I have suggested that the name *ellipsin* be retained for this material, although it seems hardly possible that it represents a single substance.

It is obvious that plasmosin is the substance associated with the variable solation-gelation phenomena and possibly in an oriented state with spindle and aster formation, while ellipsin is concerned with the more stable structural substrate of the cell such as membrane and perhaps intracellular fibril formation. The fact that we have produced membranous sheets which included fibers and have a lipid content of about 25 per cent. of dry weight should add weight to Schmitt's[16] conception of alternating protein and bi-molecular phospholipid components in cell membranes.

The amount of these structural proteins in cells in spite of the impressive showing which they make under the microscope is small. As is well known the fibrous macromolecules are capable of producing high viscosity at low concentration.

## PARTICULATE COMPONENTS OF PROTOPLASM

The fact that certain of the particulate components of protoplasm have sufficient stability in ordinary saline solution to permit their isolation should have been apparent from Plosz's[17] (1872) experiments with which I had been familiar for many years, but I did not perceive this at first and had to learn it by the

[12] I. Banga and A. Szent-Györgyi, SCIENCE, 92: 514, 1940.

[13] W. T. Astbury, *Symposia on Quantitative Biology,* Cold Spring Harbor, 6: 120, 1938.

[14] A. Claude, SCIENCE, 91: 77, 1940.

[15] G. Casperson and J. Schulz, *Proc. Nat. Acad. Sci.,* 26: 507, 1940.

[16] F. O. Schmitt, *Physiol. Rev.,* 19: 270, 1939.

[17] P. Plosz, *Arch. f. d. ges. Physiol.,* 7: 371, 1873.

hard way of study of frozen dried material and fresh cells. Warburg[18] too had partially separated mitochondria in 1912 and noted their participation in the oxygen uptake of saline extracts of liver. However, Hoerr and I[19, 20] succeeded in 1934, 1937, in separating mitochondria from liver cells and making preliminary analyses. I had no suspicion at first that still smaller particulates were present in the liver cell until Lazarow, by long-continued centrifugation, obtained a glassy cherry red pellet composed of particles so minute that they were quite invisible under the microscope, but showed in the dark field of the cardioid condenser a shimmering field of light in which individual particles could with difficulty be distinguished. We were investigating this particle when Claude[14] announced his discovery of the presence of submicroscopic particulates in clarified saline extracts of embryo chick. Claude's particles contained nucleoprotein and phospholipid, the latter in part acetal phosphatid, which we were later able to confirm for mitochondria and the red pellet from liver. Claude thought that his particles were mitochondria which clearly could not be the case since mitochondria are not submicroscopic in size. The fundamental idea of kinship between these two components is, however, not to be so summarily rejected, since we know nothing of the antecedents or the products of either.

It is noteworthy that Claude[21] had previously found the virus of chick sarcoma associated with a similar particulate and that Stern and Wyckoff[22] recovered a pigmented pellet from liver extracts which had catalase activity.

In case any one should be inclined to regard mitochondria and the submicroscopic particulates as unimportant and casual products of cell metabolism or the result of temporary flocculation of cell constituents I would recommend that they suspend judgment until they learn of the lipid and enzyme content of these structures and their extraordinarily complex composition.

The analysis of particulate components of cytoplasm is only as good as the species-purity of the preparation and much effort has been expended on this phase of the work. It is quite certain that the original preparations of hepatic mitochondria which were separated at rather high speeds were contaminated with glycogen which is also particulate[23] and with the red submicroscopic particle and it is equally certain that the fractions obtained by Claude[24] by time fractionation at high speed were mixtures. The general trend of Claude's analyses of his fractions, however, accords well with our analyses of preparations made with much greater care, but the quantitative results are different.

Both mitochondria and submicroscopics have a high water content which, however, we have not been able to determine with accuracy owing to the unknown factor of dilatancy operating while the centrifuge is coming to rest. The results were for mitochondria 82.5 per cent., for submicroscopics 89.8 per cent., which for the reasons stated are probably too high.

Mitochondria and submicroscopic particulates are stable in 0.85 per cent. NaCl solutions but swell and lose substance if the electrolyte content is much reduced. They dissolve in water on the alkaline and on the acid side of their pH stability range. The latter has not been determined with accuracy but is known to be dependent also on electrolyte concentration. We have not been able to find that the stability is much influenced by the substitution of NaCl for the normal inorganic constituents of the cell water, but the enzymatic activities apparently are.

Qualitatively, mitochondria and submicroscopic particulates are similar in composition but there are quantitative differences.

The following substances have been identified chemically in both groups: protein, nucleoprotein, flavoprotein, triglycerides, lecithin, sterol and vitamin A, the latter by Goerner and Goerner.[25] The submicroscopics have a higher content of lipids, nucleoprotein, flavoprotein and water than mitochondria. The yellow color of mitochondria and the cherry red color of submicroscopic particles are due in part at least to flavoproteins. This has been confirmed by extraction of riboflavin and its conversion by the action of light in alkaline solution into chloroform-soluble lumiflavin.

The succinoxidase system has been demonstrated in both mitochondria and submicroscopics by Lazarow and Barrón.[26] Both give a moderate positive reaction for cytochrome oxidase with the nadi reagent, but the presence of Cytochrome C has not yet been confirmed by spectroscopic study. Both catalyse the decomposition of hydrogen peroxide.

Kabat[27] has demonstrated the greater concentration of phosphatase in a particulate from kidney separated from clarified suspensions at 27,000 RPM and has made interesting suggestions as to the function of particulates in the orderly assembling of members

[18] O. Warburg, *Arch. f. d. ges. Physiol.*, 54: 595, 1912.
[19] R. R. Bensley and N. L. Hoerr, *Anat. Rec.*, 60: 449, 1934.
[20] R. R. Bensley, *Anat. Rec.*, 69: 341, 1937.
[21] A. Claude, SCIENCE, 90: 213, 1939.
[22] K. G. Stern and R. W. G. Wyckoff, *Jour. Biol. Chem.*, 124: 573, 1938.
[23] A. Lazarow, SCIENCE, 95: 48, 1942.
[24] A. Claude, *Symposia on Quantitative Biology*, Cold Spring Harbor, 9: 263, 1941.
[25] A. Goerner and M. M. Goerner, *Jour. Biol. Chem.*, 123: 57, 1938; A. Goerner, *Jour. Biol. Chem.*, 122: 529, 1937–38.
[26] A. Lazarow and E. S. G. Barrón, *Anat. Rec.*, 79: 41, Suppl.
[27] E. A. Kabat, SCIENCE, 93: 44, 1940.

of enzyme carrier systems which recall earlier suggestions made on the same topic by Stern.[28]

The high lipid content of the mitochondria and particulates calls for some comment. Dry mitochondria contain about 34 per cent. of lipids; dry particulates as high as 51 per cent. Both figures are much higher than the average content of the whole cell. Therefore other portions of the cell contain much less than the average. Recent quantitative studies of this distribution reveal that structural proteins and particulates in the liver together carry about 90 per cent. of the total dispersed lipids and as high as 98 per cent. of the phospholipids. These determinations must be made on cells without an oil phase. Plasmosin contains when purified little fat, about 4 per cent., the ellipsin residue about 25 per cent. Thus, in the liver the interparticulate liquid contains little disperse fat and almost no phospholipid. These substances are largely contained in these little packets which I have called particulates and bound in the membranous and fibrous portion of the cell.

On the other hand, the interparticulate portion of the cytoplasm contains much protein, probably for the most part of the corpuscular or globular type. It also contains some flavoproteins but does not oxidize succinate, indicating that some essential member of the succinoxidase chain is missing. I do not know what the content of Wyckoff's macromolecular substances is or where they fit into this conception of protoplasm.

The fat distribution in mitochondria and in particulates does not differ in any important respect from that of the whole cell. Our previous estimates of lecithin were too low owing to the use of the unreliable acetone precipitation method. Phospholipid estimated as lecithin from phosphorus determinations show a content of lecithin of 45 to 58 per cent. of the total lipids. A positive Schiff reaction indicates a content of acetal phosphatid. The distribution as to lecithin kephalin and sphingomyelin has not been determined. We have not yet determined the inorganic constituents of the particulates.

Cytoplasm thus has no ultimate structural unit but consists instead of several perhaps many different types of units, all cooperating in an orderly fashion to produce that ensemble of properties which we call life. At the present time our knowledge is very incomplete but we can recognize the following categories:

(1) Those units upon which the integrity of the cell as a unit of structure, the maintenance of its organization, and those properties enumerated by Seifriz, depend. In these units the fibrous proteins and nucleoproteins with associated lipids, etc., described play an important role.

(2) Particulates, microscopic and submicroscopic, of highly complex composition mediating special chemical processes.

(3) The interparticulate liquid menstruum also of complex composition but at present little understood.

The methods and quantitative results upon which the foregoing statements are based will be published elsewhere in collaboration with Dr. Lazarow, who for several years has assisted me in the work.

Obviously the possibility of separating mitochondria and particulates and of isolating the structural proteins for chemical study opens up a rich field for further research. The localization of enzyme and carrier systems, vitamins and hormones, and the viruses, functional changes in composition, the tracing of radioactive isotopes into the interior of the cell and the further fractionation of the submicroscopic particles by more refined methods all offer inviting opportunities to the inquiring mind.

It is a pleasure to acknowledge that my work has been much helped by the loyal and generous attitude of my colleagues and former students in the Department of Anatomy and by the generous contribution of funds from the Rockefeller grant and from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

It is a pleasure also to reflect that the funds at my disposal have never been large enough to tempt me to abandon investigation for direction of others, and thus to miss in these years of retirement the joys that come, in fullest measure, only to those who satisfy their desire for knowledge by a direct and personal appeal to nature by research.

# WARTIME MAINTENANCE OF SCIENTIFIC PRODUCTION

By Dr. J. S. NICHOLAS

YALE UNIVERSITY

MANY theses have been founded on the relationship of supply and demand. When the President made his wartime demands on industry, few thought that the stated objectives could be attained. Although in some cases there has not been complete attainment, in the majority and dominant aspects of the program

[28] K. G. Stern, *Symposia on Quantitative Biology*, Cold Spring Harbor, 7: 312, 1939.