DEFORMATION AND FLOW IN BIOLOGICAL SYSTEMS

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INTRODUCTION

The present volume, "Deformation and Flow in Biological Systems", contains some reviews on rheological problems in animal and plant Physiology.

Rheological questions occur wherever movements are observed in biological systems. However, these problems are in general so complex that only an empirical approach is possible. Theoretical interpretations are often hindered by the fact that there are too many variables. As a matter of fact, constants, as used in physical experiments, do not exist. A simple problem, such as flow through a biological capillary, is complicated by the elasticity of the tube causing changes of its diameter depending on temporal or local pressure variations. The pressure itself is regulated by energy consumption and other internal factors which are difficult to control. In addition the elasticity varies with the age and the physiological conditions of the capillary. Also the viscosity of the streaming liquid is not constant, since there are metabolic alterations or concentration changes due to the permeability of the capillary membrane. Furthermore, biological fluids are mostly non-Newtonian liquids and this causes additional complications. Finally, growth intervenes in young tissues so that not even the mass of the system under investigation is constant, and a strict distinction between plastic deformation and change of shape by active growth becomes extremely difficult.

Hence we can study the changes in one definite property depending on the parameters of time and of space only by neglecting the changes in other properties, considering them, as a rather rough approximation, as constants. As a result it is then often found that the physical processes are accompanied by energy-consuming reactions such as acceleration of diffusion by active propulsion (bacteriophage particles), metabolic changes of permeability, active contraction of fibres (muscle) etc. The study and explanation of these phenomena is the typically biological contribution to Rheology. Since it is not clear precisely how the respiration energy intervenes in these cases, our knowledge is still very limited.

As a consequence of this situation the treatment of rheological

problems in Biology consists either in a simple description of observed facts or in attempts at theoretical considerations which generally lead to very complicated mathematical formulations. Therefore, this volume differs from the others of the series of Monographs on the Rheology of Natural and Synthetic Substances by its heterogeneity. The scope of this book is to discuss various types of rheological phenomena in living systems, such as flow, convection, elasticity, plastic deformation, contraction and even growth. Since all biological manifestations are to some extent related to Rheology, no attempt has been made to cover all possible rheological problems.

As the first International Colloquium on Rheological Problems in Biology was held in Lund (Sweden) just at the time when the manuscripts for this book were compiled, an extensive Report of that Colloquium has been added. It gives some idea of the great variety of rheological investigations in Biology and adds some stimulating discussions on the problems treated in this monograph.

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RHEOLOGY IN CELLS

CHAPTER I

THE RHEOLOGICAL PROPERTIES OF PROTOPLASM

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0. Introduction

A rheological study of protoplasm involves all the difficulties which a physicist encounters in measuring and interpreting the flow of a non-Newtonian fluid, and in addition, certain properties inherent to living matter. These properties are not to be regarded as beyond interpretation in physical and chemical terms, but merely as unique to a living system. After all, protoplasm does stand somewhat apart from the substances of the non-living world on which we base our physical laws. Living matter obeys many of these laws, but at times it seemingly ignores them, which may merely mean that it has some laws of its own, or is able to apply laws in a way we have not yet learned.

The study of protoplasm involves techniques. These, whether dissection with a needle, the injection of a salt, or forced flow through a capillary, place the protoplasm in an abnormal situation. We may, under such circumstances, be interpreting abnormal rather than normal behaviour. The student of protoplasm soon acquires numerous criteria by which to judge the state of the material with which he is working. In any case, there is no other way out. The astronomer alone cannot touch the objects he studies, and he loses much thereby. A cell in culture, separated from its normal *milieu* is not the same kind of cell as its counterpart in the body tissue, even though it is alive and thriving. Blood studied outside the living body is not blood in the true sense at all, but blood cannot be readily studied when in the body; the only thing to do is to isolate it. Some properties are thereby changed, whereas others have been modified but little or not at all. A similar situation exists in regard to most

studies on living matter; thus, research on respiration has progressed so far that we can almost say the respiration of living tissues is wholly reproducible in a test-tube; but our knowledge of photosynthesis is still so meager that we cannot yet consider it apart from the living cell. The structural pattern of chlorophyll and the photosynthetic machine within the cell must be kept intact. In all work on living matter it is well to run parallel experiments, one with and one without the living system. Myosin cannot be well studied when in muscle, but muscle as a whole can be, and its behaviour should be compared to that of myosin when isolated from muscle. Similarily, the streaming of protoplasm should be studied both as the flow of a non-living non-Newtonian liquid, and as the flow of living matter which is capable of disobeying certain known laws of flow. Flowing "up hill" is a simple matter for protoplasm. We need not regard this behaviour as in any way involving laws beyond the realm of physics, but it certainly involves mechanisms which are as yet unknown to us.

In presenting the rheological properties of protoplasm, I shall view protoplasmic flow both as the passive movement of inert material and as the dynamic streaming of living matter.

The physicist is often annoyed with the inaccuracy of biological work and suggests a more precise way of doing the experiment. He will want the biologist to run protoplasm through a capillary viscosimeter and thus make direct and dependable determinations of viscosity. He is unaware that living protoplasm is not always obedient to a physicist's wish. The protoplasm is as likely to climb out of the tube as to flow through it. If, therefore, our methods appear at times to be crude and the results inexact, it should be remembered that the task is a difficult one. Considerable ingenuity is necessary, and it may be truthfully said, that the results are often very gratifying ones. The measurements by KAMIYA [1943] of flow pressures, and by PFEIFFER [1940] of the non-Newtonian behavior of protoplasm, impress one not as the results of inexact biological research, but as remarkably accurate determinations, made without greatly disturbing that most fundamental of all protoplasmic qualities, the property of life.

There are two lines of attack on the problem before us; a purely descriptive and speculative one based on direct observations of protoplasmic flow, and an experimental approach, in which quantitative determinations of protoplasmic properties are made, thus, pressures

involved in flow may be calibrated and a coefficient determined. Although the experimental method with its measurements and graphs is to be preferred, the observational and descriptive method may tell more and rest on very sound interpretations. Frequently, it is the only method possible, as in the movement of chromosomes where experimentation is much restricted.

The purpose of this treatise is to show that protoplasm possesses certain very characteristic physical, particularly rheological, properties. To anticipate this purpose by enumerating those properties here may make an anticlimax of the conclusion. But at that, a brief résumé of the final results given in the Introduction serves to point the way. Briefly put, we are setting out to prove that protoplasm is elastic, non-Newtonian, contractile, thixotropic in behavior, possesses a substantial tensile strength, a measurable rigidity even though fluid, and has certain spiral tendencies which permit setting up a state of torsion; these properties are all due to a continuity in protoplasmic structure without which life is inconceivable.

1. The Viscosity of Protoplasm

1. 1. *Viscosity*

Chief among the mechanical properties of protoplasm is *viscosity*. The streaming of protoplasm and the flow of blood are problems in viscosity, so also the amoeboid movement of cells and plasmodia, and the contractility of tissues; mitosis, growth, and rate of metabolic activities are influenced by the viscosity of protoplasm. Indeed, one may say that all vital activities are directly or indirectly affected by the viscosity of the living substance.

Biological research and thinking have their epochs as do all scientific and philosophic disciplines. In my youth every vital process was interpreted in terms of surface tension. Some years later all living phenomena were analyzed in terms of viscosity changes. We were busy measuring the viscosity of protoplasm. There then came an electrical epoch, and today we are in a world of high energy phosphate bonds. These various shibboleths in science are good, for they serve to center attention for a while on one phase of a large problem. It is true that we run the danger of becoming experts, yet much is accomplished by this method even though the broader view presents a larger horizon and therefore yields a more stable advance.

The emphasis laid on viscosity during the decade when it dominated research on protoplasm is indicated by the variety of names associated with it: CONKLIN [1931], CHAMBERS [1921], L. V. HEILBRUNN [1926b], A. HEILBRONN [1922], WEBER [1923], NĚMEC [1901], SCARTH [1924b], FRY and PARKS [1934], MAST [1931], LEWIS [1923], myself [1920], and later, PFEIFFER [1936] and NORTHEN [1946]. Much of this earlier work has been reviewed [1929a].

Interpretations of biological phenomena in terms of viscosity were overdone. They became significant only when amplified by the related properties of anomalous viscosity, elasticity, contractility, and tensile strength; these are more meaningful qualities of protoplasm. However, viscosity, pure and simple, plays an important role in the life of the cell. A change in the rate of a physical or physiological process such as protoplasmic streaming, may be accompanied by, and would then certainly be influenced by, a change in the consistency of protoplasm. Environmental factors such as temperature, salts, and narcotics produce pronounced changes in the viscous state of protoplasm which are rarely the cause *per se* of the pathological condition resulting, but are rather a contributing factor, or more likely, an indicator, a diagnostic property of an abnormal or changed condition. From the viewpoint of the rheologist, the biophysicist, and the polymer chemist, the viscous state of protoplasm reveals less than do non-Newtonian behavior and elasticity.

There are some basic facts pertaining to the viscosity of protoplasm which must be kept in mind. The viscosity of protoplasm is never uniform throughout a cell. Differences in localized regions may be very great. A viscosity value of protoplasm is therefore meaningless unless the physiological state and precise region of the particular protoplast are specified. The viscosity range of protoplasm covers the whole scale of possible values with the exception of extreme fluidity. Protoplasm is never of a watery consistency. Finally, as protoplasm is elastic and therefore non-Newtonian in behavior, no viscosity value can be given without realizing that it is an approximation, or as NORTHEN [1946] would say, it is a value of the *structural viscosity* of protoplasm.

The precautions or modifications in the study of the viscosity of protoplasm were, perhaps, unconsciously realized by the earliest workers on the living substance. What they said a century ago is still the best characterization we have of protoplasm, which is all

the more remarkable in view of the fact that no "test-tube" studies of protoplasm had been made. These pioneers in the study of protoplasm simply observed the living substance, watched its behavior directly through the microscope. VON MOHL [1846] characterized protoplasm as a "viscid mass". He and DUJARDIN [1835] emphasized a high degree of viscosity. Dujardin referred to the "living *jelly*" as a "*glutinous* substance, *insoluble* in water", all of which is true.

1. 2. *Methods and values*

Osmosis

The osmotic method of determining the consistency of cell contents was early used. EWART [1903] estimated the viscosity of the cell sap of vacuoles by comparison with isotonic solutions. He found values which lie between $\eta = 0.01$ and 0.02 at 20° C. In cells rich in sugars the viscosity of the cell sap may rise to $\eta = 0.06$.

Plasmolysis

Under this caption several methods directly or indirectly bearing on the osmotic shrinking of a protoplast may be considered.

The configuration of protoplasts after plasmolysis is said to be an indicator of protoplasmic viscosity. The normal cell, when put in hypertonic sugar solutions, plasmolyzes either with a smooth and convex surface with no or a minimum adhesion to the cellulose wall, or with concave depressions and adhesion to the cell wall at many points (Fig. 1A, B). Occasionally angular plasmolyzed protoplasts are obtained. This form I regard as that of a cell which is not healthy, and therefore should not be taken into consideration as an indication of viscosity. WEBER [1925] is of the same opinion. The protoplasm in a cell with convex plasmolysis is said to be of low viscosity and the protoplasm with concave depressions and many fine strands attached to the wall is thought to be of high consistency (Fig. 1, B).

There is some truth in the foregoing deductions but less than the investigators using this method always realize. The convex surface of a plasmolyzed protoplast indicates not low viscosity but low adhesive qualities, and the concave protoplast with many fine attached threads indicates not high viscosity but a high degree of adhesiveness.

Generalizations on the consistency and glutinosity of the protoplasmic membrane based on the presence and duration of protoplasmic strands have been drawn by PRINGSHEIM [1854], CHODAT and BOUBIER [1900] and HECHT [1912].

SCARTH [1923] finds that the monovalent alkaline metals produce smooth, convex, plasmolyzed protoplasts of Spirogyra without adhesion to the walls, therefore presumably lowering viscosity; that divalent

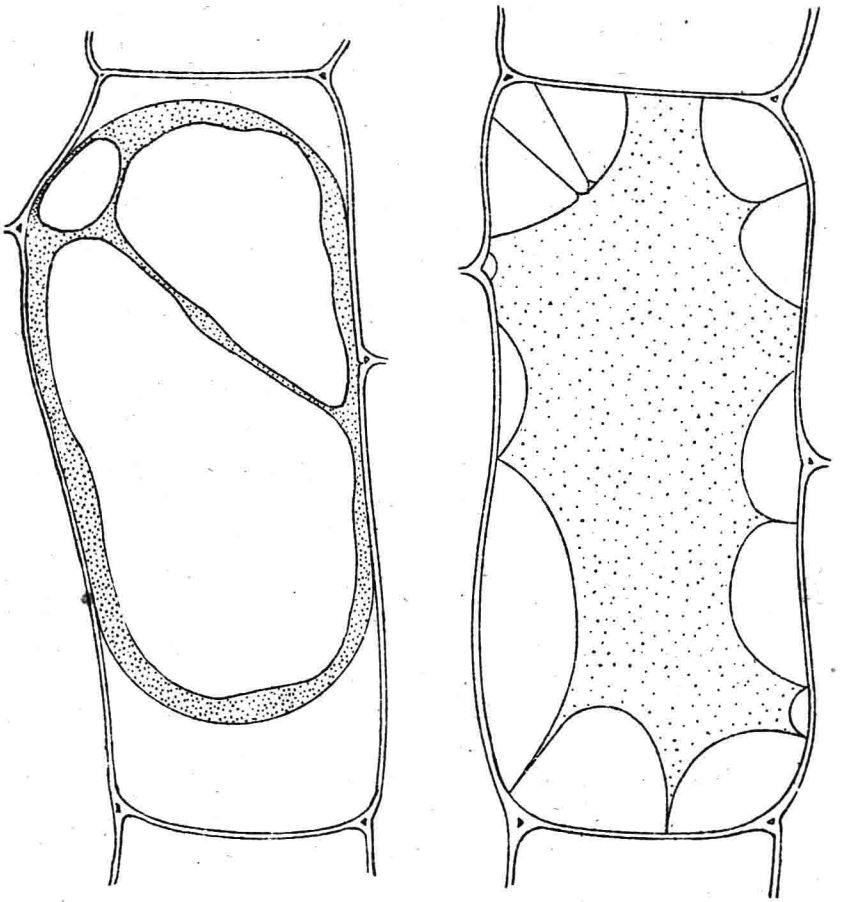

Fig. 1 — Plasmolyzed protoplasts the forms of which indicate tackiness rather than viscosity.

salts of the alkaline earths cause angular or concave plasmolysis, therefore raised viscosity; and that trivalent salts of the rare earths cause the protoplasts to resemble introverted sacs, the protoplasm being adherent over a maximum surface therefore maximum viscosity.

Although there is disagreement on the assumption that adhesion of the protoplast to the cell wall after plasmolysis is independent of

viscosity (SCARTH [1923], CHOLODNY [1924], WEBER [1924a]), it is, in any case, well to bear in mind that glutinosity or tackiness, elasticity, surface tension, and tensile strength are not correlated with viscosity. There is no reason why they should be.

A method for determining viscosity, which though not based on plasmolysis is nonetheless related to the form of protoplasts and the presence of threads, is that used by SCARTH [1924a, b]. He based estimations of the consistency of the cytoplasm of Spirogyra on the shortening of the strands which support the nucleus and the chloroplast. Change in form toward minimal area is thought to be due to a decrease in viscosity allowing surface tension to act. The agents which cause it, electrolytes, heat, narcotics, etc., may also cause coagulation, determined by dark-field study, if their action is prolonged or increased. Where the decrease is moderate the change is reversible.

Gravity

The treatment of a cell with salts or the entrance of needles into protoplasm is likely to set up abnormal conditions. If the plant cell itself is made to serve as a viscometer, these difficulties are overcome. Plant cells sometimes contain freely suspended starch grains called statoliths which normally lie at the bottom of a cell. Statoliths were, by HABERLANDT [1903], presumed to serve as gravitational sense organs. If the cell is turned through 180° the starch grains slowly fall. By exposing a living cell to view, repeatedly reversing the plant, and noting the time of fall of the statoliths, an indication of the consistency of the protoplasm can be obtained. The living cell is a viscometer of the type employed by physicists in which viscosity is determined by the time of fall of a small sphere through a known length of the fluid. The viscosity value is calculated from Stokes' law:

$$V = \frac{2r^2 (D-d)\, g}{9\, \eta}.$$

NĚMEC [1901] was a pioneer worker in this method, but he obtained no actual values. He found the time of fall, and therefore the protoplasmic consistency, to increase with decreasing temperature. He thought dehydration to be the responsible factor. This need not however have been the case.

A. HEILBRONN [1912] applied the gravity method to the living cells of Phaseolus and Vicia. He found the time of fall of the statoliths to vary from 16 to 28 minutes. In further work [1914] he obtained