MOLECULAR STRUCTURE AND MECHANICAL PROPERTIES OF PLANT CELL WALLS IN RELATION TO GROWTH

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INTRODUCTION

Interest in the mechanical properties of botanical material has been manifest for a very long time, and a wide variety of associated problems have been examined.

The following very brief review, indicates this wide range of interest. It is not exhaustive, but is intended to furnish examples of various aspects of the problem which have been investigated.

According to Haberlandt, Schwendener (1874), who was both botanist and engineer, was the first to undertake an experimental investigation of the elasticity and tensile strength of mechanical cells. He published a treatise on the subject in which he examined the properties of certain types of I-beam and of hollow cylinders, particularly from the point of view of their contribution to the rigidity of a structure with a minimum expenditure of material. Plant organs were separated into groups with similar structural arrangements and a qualitative description was given of the advantages of these arrangements in meeting the particular demands made upon the structure. Bower (1930) has also drawn attention to the fact that mechanical requirements need to be met all through the ontogenetic history.

Greenhill (1881) examined the elastic stability of very tall trees, and calculated the greatest height to which a tree of given proportions could grow. Opatowski (1944) has also considered problems

related to the form and strength of trees, and Easer (1946) has considered the problem of tree trunks and branches as optimum mechanical supports of the crown.

Jacobs (1945) and Boyd (1950) have investigated tree growth stresses taking into account changes in shape of cells immediately after differentiation, tree weight, sap stream tension, osmotic forces, etc.

The elastic properties of wood have been investigated from quite early times. It is interesting to note that the first experiments on wood were carried out in order to verify and establish the theory of elasticity, particularly in bending, rather than to determine the properties of wood itself (Hearmon, 1948). Since then a very great deal has been done on the elastic properties of wood, particularly in connection with the use of wood in aircraft (Jenkin, 1920). Wardrop has investigated the elastic properties of sections of conifer wood and contrasted the behaviour of the whole tissue with the behaviour of the individual tracheids comprising it.

The mechanical properties of those plant materials which are of technological importance, e.g. cotton, flax, ramie, hemp, sisal, etc., have been studied extensively over the years. Some very notable work was done on the elastic properties of cotton, for example, by Pierce and by Meredith.

This very short summary provides examples illustrating the wide range of interest in this subject.

The present investigation is, however, concerned with yet

another aspect of the problem; the effect of the mechanical properties of the plant cell wall on the growth of the cell. It is an apparent paradox that the wall which surrounds the growing cell is a fairly rigid envelope, which is capable of sustaining comparatively high stresses with only moderate strain; but which, in the course of growth, can undergo enormous increases in area in an ordered way, and yet be capable of sustaining these high stresses before, during, and after the process of enlargement. The mechanism of this process is not yet well understood, and it will be the aim of this investigation to see if some further light can be shed on the problem by investigating the elastic properties of the growing wall.

Organisation of Thesis

The thesis will be presented in four main Parts. They will be organised as follows:-

Part I - A review of theories of cell wall growth.

Part II - Determination of the wall structure of Nitella opaca (on which most of themeasurements have been made).

Part III - Mechanical properties of the wall of Nitella opaca, with a brief section on Valonia.

Part IV - A Theory of Spiral Growth.

PART I.

REVIEW OF THEORIES OF CELL WALL GROWTH

Broadly speaking cell wall growth may be classified into three stages,

(a) Division growth - the cell is dividing and increasing in volume by synthesising new cytoplasmic material, taking in water insofar as is necessary to maintain the consistency of the cytoplasm more or less constant.

(b) Extension growth - the wall is undergoing rapid increase in surface area associated with rapid water intake in large volume.

(c) The Cell Wall is increasing in thickness rather than in area.

The follow ng review is mainly concerned with those aspects of cell wall growth which have a bearing on the present problem, viz., stage (b) above.

Composition of the Wall

Before proceeding to discuss the various theories of growth it might be as well to briefly outline the structure of the wall.

The plant cell wall can be separated into two distinct phases; a highly crystalline phase consisting of the microfibrils and an 'amorphous' phase which is the matrix in which the microfibrils are imbedded. 'Amorphous', as used above, is meant to imply that the material of the matrix is not highly crystalline with a well established lateral order, but it is not meant to imply that it is necessarily isotropic at either the macroscopic or microscopic levels. Northcote (1958) has classified

the material of the cell wall in the following provisional manner;

(1) Continuous Matrix:

(a) Pectic substance

(b) Lignin

(c) Possibly polysaccharides of the hemicellulose group

(2) Microfibrils:

(a) Polysaccharides containing glucose (cellulose)

(b) Polysaccharide (s) containing xylose (Cronshaw, Myers & Preston, 1958)

(c) Polysaccharide (s) containing mannose (Cronshaw, et al., 1958; Meier, 1956)

(d) Possibly polysaccharides of the hemicellulose group

It is thought that the bulk of the growing primary wall is made up of the amorphous matrix and water. Estimates of the volume of the primary wall occupied by cellulose microfibrils have been made and it seems that the fraction is of the order of 5 to 10% of the wet wall volume. This calculation is made on the assumption that all microfibrils are composed of cellulose. If one allows the possibility of microfibrils of the hemicellulose group, or of mannose or xylose, then this estimate of the microfibrillar component may be rather low. However, there seems reason to believe that the microfibrils are in fact well separated by the amorphous matrix.

Theories of Cell Wall Growth

A very great deal of work has been done on the structure and organisation of the wall and the changes which take place as the wall increases in area. This work and the study of cell physiology during growth and, in particular, in the presence of plant growth substances, have resulted in various suggestions being put forward as to possible mechanisms of cell elongation. Some of these are outlined below. (A very good review of the subject up to 1940 has been given by Heyn, 1940).

(i) Mosaic theory

Frey-Wyssling and Stecher (1951) and Stecher (1952) in an electron microscope study of actively growing tissue, observed that in the normally loose network of microfibrils in the primary wall, there were in addition small open perforations ranging up to about ½ to 1 micron in diameter. On the basis of this observation, they suggested that the cell wall is transiently penetrated by protoplasm at points on the surface and that the microfibrils in this region are pushed aside. New microfibrils are subsequently woven into these areas, the total area of the wall having been increased.

This is an example of growth by intussusception. One attraction of this theory is that it is possible, with very little extension of the theory, to postulate that it could be the mechanism by which adjacent cells increase in size at the same rate (symplastic growth).

Roelofsen & Houwink (1953) have raised the objection that in the case of the Phycomyces sporangiophore, for example, the comparatively large perforations postulated are incompatible with growth in air and the measured turgor pressure of two atmospheres.

Wardrop (1954) proposed a rather similar mechanism of growth but, instead of growth proceeding from random points over the cell area which had been transiently penetrated by the protoplasm, he suggested that it proceeded from the primary pit fields where the plasmodesmata penetrate the wall.

Later, however, Wardrop himself (1955) and also Setterfield and Bayley (1958) showed by autoradiographic studies of surface growth of Avena coleoptiles, that the deposition of cellulose takes place over the entire surface of the wall, and that there is no obvious localisation at specific points on the wall; for example, there is no localization of deposition of cellulose in the region of the pit fields. Setterfield and Bayley point out, however, that the limit of resolution of radiography is about 5 microns, and that the addition of microfibrils may therefore, take place at sub-microscopic "islands of synthesis" (Preston and Kuyper, 1951), by transient intussusception as in the mosaic theory, or by apposition as in the multi-net theory (see Below).

(ii) Bipolar tip Growth

On the basis of electron microscopic examination of cells isolated from elongating coleoptiles by maceration, Mühlethaler (1951) concluded that extension growth in parenchyma took place by bipolar tip growth.

He observed that at an early stage the parenchyma showed thickenings, at the corners of the cell, composed of longitudinally oriented microfibrils. To quote Frey-Wyssling (1953) in summarizing Mühlethaler's theory; "It is evident that a wall fortified by numerous parallel textured ribs cannot be extended in the longitudinal direction. Therefore, an extension growth, in the classical sense, of such a cell is not possible." Mühlethaler also observed that in some cells the walls were thinner and looser in texture towards the ends and the long bands were absent. On the basis of this latter observation, and on the assumption that the wall could not be extended in the regions of the longitudinal bands, he proposed that extension took place by penetration of the protoplast through the attenuated tips and new wall material was deposited behind the advancing protoplast.

"It was further claimed that the epidermal cells of the oat coleoptile proved more conclusively that cell growth really occurs by tip growth. In these cells, which elongate some 150 X during four days growth, the exterior wall is very thick from the beginning. It was considered that this outer thick wall, in which the microfibrils tend on the whole to be longitudinally, rather than transversely, would prevent any considerable cell extension, and that in this case a fortiori growth must occur at the tips" (quoted from Preston, 1958).

The autoradiographic studies referred to above which showed that the deposition of cellulose takes place over the entire surface of the cell, does not lend support to this theory. It can, however,

be criticized on other grounds. In a study of the number and distribution of pit fields in the wall of the elongating Avena coleoptile, Wardrop (1955) found:

(a) that the number of pit fields per cell does not change in coleoptile parenchyma at different degrees of extension.

(b) The number of pit fields per unit area of cell surface decreased in cells of increasing length.

If it is assumed that primary pit fields are not transient structures then the conclusion that growth must take place over the entire surface of the wall seems inescapable. This seems to rule out bipolar tip growth of Mühlethaler unless some pit fields in the non-extending portion of the wall are eliminated and new ones created in the newly formed parts of the wall. This does not appear probable.

A similar conclusion has been reached by Wilson (1957) who examined the cortical parenchyma of Elodea, Glyceria aquatica, and Hippuris vulgaris. He found the number and spacing of pit fields to change in an orderly way as the tissue grows and his results were not consistant with bipolar tip growth.

Perhaps a note can be added here. If, as seems likely, growth proceeds over the entire surface of the cell and not by bipolar growth, then the assumption that these longitudinal bands could not extend seems to be incorrect. No doubt this conclusion was reached by comparing the bands of thickening with mature dry fibres which also have axial orientation of microfibrils, and by assuming that they had

similar elastic properties. It might well be true, in the growing wall concerned, that the microfibrils are widely spaced and separated by pectic substances which will allow flow and relative movement between fibrils. Whatever the true situation, however, it does highlight the danger of basing a theory on the assumed elastic properties of this sort of material.

(iii) Multinet theory

Roelofsen & Houwink (1953, 1954) carried out an electron microscope examination of the cell wall of Phycomyces sporangiophores, and of growing hairs of Gossypium, Ceiba and Asclipias, staminal hairs of Tradescantia and root hairs of Zea mays.

Roelofsen & Houwink were struck with a feature which was common to all of these hairs, viz., that on the inner wall there was a compact transverse arrangement of microfibrils while on the outside, there was a very loose texture with the microfibrils either oriented in an approximately axial direction, or in what appeared to be a completely random manner. They suggested that this difference in the arrangement of the microfibrils was due to the process of wall extension during growth. They suggested that the appearance of the wall could be explained if one assumed,

(a) that new fibrils were deposited on the inner face of the wall (apposition growth) and that they were oriented at the time of deposition in an approximately transverse direction,

and (b) that as the wall extends the fibrils are passively

oriented so that they behave like an expanding net. The twines of the net are not imagined as being tied together with fixed knots (cross-links) but are able to slip over and past one another. While this process is going on more fibrils are being deposited on the inner surface in an approximately transverse direction.

Thus the general orientation of the microfibrils changes from transverse to a more longitudinal direction as the wall changes in length. There are no distinct layers but rather a gradual change in orientation. The degree and direction of reorientation would depend on the amount and polarity of growth. This process is illustrated in Fig.1., which is taken from Roelofsen & Houwink's paper.

The 'multi net' theory is not so much a theory of growth as an attempt to explain the microfibrillar morphology of the plant cell wall. However, although it has nothing to say about the mechanism causing the wall to extend, the assumption that the microfibrils are passively reoriented by the non-fibrillar matrix and that they can move relative to one another does impute certain physical properties to the matrix and microfibrils. Further, there seems to be an underlying assumption in the theory, that it is the non-fibrillar matrix which is the 'active' component of the wall so far as the mechanism of growth is concerned. Finally, nothing is said of the mechanism by which the microfibrils are oriented transversely at the inner face of the cell.

There are some difficulties which can be raised against the theory. In Valonia and Cladophora the fibrils are arranged in a

"crossed fibrillar" manner and the interstriation angle remains almost constant during growth. In cases where the form of the cell does not change during surface enlargement growth of the wall can still be interpreted in terms of the multinet theory, but in the case of elongating filamentus algae with a crossed fibrillar arrangement the situation is more difficult.

Further Scott, Hamner, Baker and Bowler (1956) in an electron microscope study of cell wall growth in the onion root, find no evidence of a gradual transition from parallel texture to an interwoven mesh as would seem to be required by the multi net theory. Setterfield & Bayley (1957) point out that the layers in the walls of epidermal and subepidermal cells of coleoptiles, young cortical cells of onion roots, and collenchyma are difficult to explain on the multinet theory alone. So also, are the ribs of longitudinal fibrils in coleoptile parenchyma. These extraneous layers are often classified as secondary wall layers but all appear to be present during cell elongation. Belford (1958) working with root hairs has also found that the transition from transversely oriented microfibrils to random fibrils is not as uninterrupted as the multinet theory demands.

Although there are some difficulties in the way of this theory which have to be looked at more closely, it has a good deal to recommend it. In fact it is difficult to imagine a process of surface growth in which some such mechanism of reorientation does not take place;

unless one assumes that the wall is being continually dissolved and recreated during the process of growth. Further, it is in general agreement with recent ideas on the mode of action of auxins and other plant growth substances in cell wall extension. These ideas will be briefly summarised below.

Effect of Plant Growth Substances on Cell Wall Extension

Many hypotheses have been advanced as to the mode of action of auxin in cell elongation. Bennet-Clark (1955) has classified these into three broad classes:

(a) The cell wall is rendered more plastic and is thus subjected to turgor stretching.

(b) Active water and possibly solute uptake is promoted.

(c) Polysaccharide synthesis is stimulated and the wall 'grows'.

There seems to be a good deal of support for the hypothesis expressed in (a) above, viz., that it is the pectins and related compounds rather than the cellulose which strengthen the young growing wall in the longitudinal direction and that increased pasticity of the cell wall during growth is brought about by disturbing the intramolecular lattice of the young wall (Van Overbeek, 1939; Kerr, 1951).

In other words, the pectins and related compounds provide a plstic matrix and the cellulose microfibrils might be considered as providing structural reinforcement. The idea is in harmony with the central idea of the multinet theory, viz., that the fibrils are passively reoriented in a plstic matrix.

The idea that the growth promoting effect of auxin is associated with an increase in plasticity was first put forward by Heyn (1931). He postulated that there were three theoretical possibilities for the mechanism of cell enlargement:

(1) by active increase in wall material, enlargement of the wall being due to its active growth, independent of outer forces, as a result of deposition of new substances in the wall;

(2) by passive increase of wall material, deposition of new particles in the wall being only possible when there is elastic extension under turgor pressure. This extension becomes permanent as a result of the deposition (intussusception) of new particles;

(3) by plastic stretching of the wall under the influence of turgor pressure, the particles of the wall slipping along each other and becoming changed in their positions towards one another.

On the basis of his own work on Avena coleoptile Heyn came to the conclusion that the mechanism of surface enlargement proceeded according to (3) above. He measured the elastic behaviour of coleoptiles severed at their bases and placed in humid chambers. Cutting and application of hormone took place an hour and a half after decapitation. Subsequent bending under a weight showed increased plasticity of coleoptiles treated with growth substance as compared with untreated controls, permanent curvature resulting from bending treated plants only. This method of deriving "elastic constants" has been criticized on the grounds

that measuring extensibilities by this method is too drastic and therefore misleading (Audus, 1949). Heyn also placed severed coleoptiles in water, thus allowing the walls to be stretched by turgor pressure only, and found that plants provided with growth substances underwent considerable elongation compared with untreated plants. Contraction on plasmolysis was about the same for treated and untreated showing that the greater extension of treated plants was permanent. This permanent elongation of treated plants took place in water at 1°C at which temperature increase of cell wall substance could be assumed to be interrupted. This was taken to indicate that elongation is dependent on the plasticity of the wall as the primary factor. Recent work by Preston & Hepton (1958) has indicated that heteroauxin does not affect the wall directly, but rather indirectly through the metabolic activity of the cytoplasm. This is in line with recent chemical evidence.

It has been shown by Ordin, Cleland, and Bonner (1957) that esterification of pectin by methyl - derived carbon is an auxin - controlled reaction. This supports the hypothesis that methylation of carboxyl groups of adjacent pectin molecules under the influence of auxin may be involved in the splitting of anhydride or calcium bridges which contribute to the mechanical properties of the wall. The bond - splitting may require methyl esterification as a primary part of the reaction or may require it to stabilize the split once made. Bennett-Clark (1955) has summarised the effect on elastic properties of the

sort of mechanism discussed above as follows; "It will probably be agreed that the plastic and elastic extensibility of a poly-galacturonic acid, or in general, of an oxidized hemicellulose will be markedly controlled by the condition of the carboxyl groups. If these long chain molecules are associated with multivalent cations, minimum extensibility will be found owing to electrovalent binding together of adjacent molecules. If the carboxyls are free, hydrogen bonding will provide considerable tensile strength but much less than that formed in presence of cations and, finally, when, of if, they are converted to methly esters, there will be minimal tensile strength as hydrogen bonding will be replaced by Van der Waals forces and so extensibility will be maximal."

It has been shown (Tagawa & Bonner, 1957) that the physical properties of the Avena coleoptile are dependent on the nature of the inorganic ions exchangeably bound by the tissue (Calcium and Potassium ions). These ions are held by non-living as well as living tissues, possibly by the pectic materials of the wall. Evidence is presented that it is in fact the pectic substances which determine the mechanical deformation of the wall.

Glasiou & Inglis (1958) have proposed a more detailed theory of auxin action. They have postulated that auxin-controlled binding of pectin methylesterase (P.M.E.) controls the elastic properties of the primary wall by regulating the amount of P.M.E. absorbed.

Immobilization would reduce the activity of the enzyme and favour an increased degree of methylation of the pectic components of the wall. Ordin, Cleland and Bonner (1957) do not find any indication that P.M.E. activity is increased by auxin in the Avena coleoptile.

Some doubt has been thrown on this sort of hypothesis by recent work of Bishop, Bayley and Setterfield (1958) who have shown that the pectic substances amounted to only 0.3% of the dry weight of the primary wall of Avena coleoptiles. To quote, "- - - - it is difficult to imagine how the minute pectic dontent found here could have significant influence on the physical properties of the walls. Hexuronic acid residues, either as pectic substances or as components in non-cellulosic polysaccharide chains, could possibly have a disproportionate affect in plasticizing the wall by occupying strategic positions in the wall structure, but the degree of economy and order to achieve such a system would appear to make it improbable". They suggest that if the non-cellulosic portion of the primary wall is to be considered as an active agent in wall structure, then attention must be turned to the preponderant non-cellulosic polysaccharides (51% Dry weight).

There is, therefore, a good deal of evidence that an effect of auxin is to increase the plasticity of the cell wall. It remains to be seen whether this is the sole effect of auxin on the wall and whether it is the primary cause of growth in area. In this connection it might be pointed out that O'Kelley & Carr (1953) have found that the density of microfibrils appears unchanged in cotton fibres as they elongate suggesting that in this case synthesis keeps pace with elongation.

Again Ordin & Bonner in studying the inhibitory effect of galactose on Avena coleoptiles have found that galactose inhibits incorporation of radioactive glucose into cellulose. It is suggested that galactose specifically inhibits cell elongation by interfering with cellulose synthesis.

In the mosaic theory and its variations, growth is held to be primarily due to 'active' increase in wall materials and to the mechanism of their incorporation within the wall; the mechanical theories of growth hold that distrubance of molecular forces within the wall change its properties so that it is passively stretched.

If the second of these alternatives proves to be the primary mechanism, then an understanding of the effect of the molecular structure of the wall on its elastic properties is of prime importance in the complete understanding of the mechanism of growth in area. If it is the first of these alternatives which is the primary mechanism then the effect of the molecular structure on elastic properties will be of less direct importance, but may, nevertheless, make a significant contribution to our understanding of the various secondary processes accompanying growth.

In all of the work on the elastic and plastic extension of primary walls known to the author, the elastic properties of the growing wall have been inferred from experiments on complete tissues rather than from direct determinations on single cell walls.

Two main methods appear to have been used;

(a) The observation of the elastic and plastic component of

bending when the tissue is bent under the action of a small weight (tissue treated as a cantilever beam) e.g. Heyn (1931); Tagawa & Bonner (1957).

(b) The observation of plastic and elastic change in length when the tissues is subjected to a change in turgor pressure. e.g. Heyn (1931); Burström (1942); Czaja (1935); Lepeschin (1907); Diehl, Gorter, van Iterson Jr., and Kleinhoonte (1939). (Preston and Hepton (1958) did not use either of these methods but subjected the whole tissue to direct tension).

There are two principal objections to these methods;

(a) Because, in the whole tissue, the cells are held together by the pectic middle lamella, it is difficult to be sure whether it is the micro structure of the cell wall which is responsible for this or that elastic behaviour, or whether it is the middle lamella which is primarily responsible. An example which illustrates this point is given by the work of Wardrop (1951). He worked on the elastic properties of tangential longitudinal sections of conifer wood, and found that if the water content was increased the tensile strength of the whole tissue decreased. while that of the individual tracheids comprising the tissue increased. The difference between the behaviour of whole tissue and the behaviour of individual cells was attributed to the over-riding effect of the middle lamella in determining the behaviour of the whole tissue under conditions of varying moisture content.

(b) The second objection, which applies more particularly to the bending method, is that the stress is indeterminate. Even if the loading conditions are completely specified, calculation of the stress distribution in a complex tissue is a very difficult mathematical problem. Further, the 'apparent' tensile modulus of a tissue depends not only on the elastic constants of the wall, but also on the turgor pressure. Falk, Hertz and Virgin (1958) have found that there is an approximately linear relationship between turgor pressure and the tensile modulus of parenchyma tissue. This simple relation was also derived theoretically by Nilsson, Hertz and Falk (1958), and this treatment, although not concerned with this particular aspect of the problem, gives an insight into the difficulties of deriving the elastic constants of the wall from measurements on whole tissue. To solve even the limited problem of the elasticity of parenchyma tissue various simplifying assumptions had to be made; for example;

(a) The cell walls are homogeneous, isotropic, and elastic, and thin enough not to offer any resistance to pure bending.

(b) In the absence of external forces, all cell walls are free from internal stresses when the turgor pressure of the cell fluid is zero.

(c) All cells are of equal size and form, with three equal diameters. They are arranged in the form of a regular structure.

The form and arrangement of the cells had also to be stated.

This is not to say that valuable information cannot be obtained from measurements on whole tissue; particularly, for example, on the effects of auxin on cell wall growth. If the elastic properties of the wall itself are required, however, it is obviously better to work directly with strips of the wall, and it was decided to adopt this approach in the present investigation. This means, of course, that one cannot work with living cells, and the methods used here are intended to supplement the information obtained by other methods, rather than replace the methods using whole tissue.

PART II

WALL STRUCTURE OF NITELLA

INTRODUCTION

The choice of a suitable material for the investigation of the elastic properties of growing walls was limited by the decision to determine the constants of the cell wall directly, rather than to infer its properties from measurements made on a multi-cellular tissue.

A suitable material was sought which, as far as possible, would meet the following specifications;

(a) Single cells should be available as fresh material. Isolation of single cells from a tissue by maceration, or by any other technique which would alter the chemical or physical nature of the wall was 'ruled out'.

(b) The cells should be large enough to provide strips of wall material which could be handled in the extensometer.

(c) It would be an advantage to have material which could be cultured in the laboratory.

The number of plants which fulfil these requirements is not large. Among those considered were Valonia ventricosa, Cladophora prolifera and Nitella opaca.

Valonia ventricosa has large spherical or club shaped vesicles which sometimes attain a diameter of over 4 cm. From many points of view it is an ideal cell for this sort of investigation (not least of

which, is the fact that its wall structure has already been fully documented); but it has the disadvantage of not being available in this country. Further, it is not easy to culture.

Cladophora prolifera Kutz, which does occur in British waters, has filaments 300 - 400 microns in diameter and up to 8 mm. long at maturity (Newton, 1931). Although mature cells might be handled by the exercise of great care, immature cells would be rather too small and fragile for the sort of measurement planned. It has the additional disadvantage of being rather rare. Continuous supplies might therefore be difficult to obtain.

The third plant considered was Nitella opaca; and it does, in fact, meet the specification detailed above. It is available locally and it is not difficult to culture in the laboratory. It is able to exist with a small supply of oxygen (Fritsch, 1935) and a clone of Nitella can be grown quite successfully in a tank of pond water, on a sandy or muddy substratum. It has also been grown in a modified Chu No. 10 Blue-green algae medium (Green, 1954), and in an autoclaved medium of 2 per cent garden soil in distilled water (Green & Chapman, 1955).

The plant consists of successive nodes and internodes, and it is the large cylindrical internodes (single cells) which have been selected for this work. These cells frequently attain a length of 50 to 60 mm. (one, 100 mm. long, has been found locally), and a mature cell may have a diameter of 500 microns or more. There is no difficulty in cutting longitudinal test strips from such a wall and, although transverse strips are necessarily rather limited in length, with care, suitable strips can

also be obtained and mounted.

Besides meeting the requirements listed in the initial specification, its growth habit is such that it offers considerable advantages in the study of cell wall growth. Green (1958A) has calculated that the expansion of the internodal surface can be as large as 15,000 X, and the increase in cell wall volume may reach 30,000 X. Further, it is known (Green, 1954) that the internodes exhibit the phenomena of spiral growth. Preston (1948) and Preston and Middlebrook (1952) have already suggested that spiral growth in the sporangiophore of Phycomyces is mechanical in origin. Since the mechanical properties of the wall of Nitella were to be determined there would obviously be an opportunity of seeing whether a mechanical explanation of spiral growth also applied in this case.

From many points of view, therefore, Nitella seemed to be suitable, and most of the work which follows has been done on the cell wall of this plant. Dried Valonia vesicles have also been used to a limited extent, to throw light on some aspects of the problem, for which Nitella did not possess the requisite wall structure.

GROWTH HABIT OF NITELLA

The Nitella plant consists of successive nodes and internodes. The nodes are multi-cellular and contain the laterals of limited growth ("leaves") and of unlimited growth ("branches"). The internodes are large single cylindrical cells (Fig. 2).

The following description of the growth habit is taken largely from Fritsch (1935) and Groves and Bullock-Webster (1917). Growth is

effected by a dome-shaped apical cell (See Fig. 2, (1)) cutting off a single series of segments parallel to its flat base (2). Each segment divides into a biconcave upper and biconvex lower half (3), of which the former gives rise to a node and the latter to an internode. This primarily filamentous construction soon acquires the mature differentiation by considerable elongation of the internodal cell which forms part of the plant axis (4). At the same time the nodal cell divides longthwise into two, and this is followed by the successive appearance of a number of curved septa (6 - 9) cutting off a peripheral series of eight cells from the two central ones which latter subsequently undergo a few further longitudinal divisions. The peripheral cells become protruded to form the apical cells of laterals of limited growth (5) which are cut off successively from the marginal segments of the node. The laterals exhibit the same method of segmentation as the main axes, but the internodal cells commonly remain rather short. The first segment out off from the apical cell of a lateral becomes a nodal cell which divides in the way described to form a basal node. In Nitella opaca the lateral forms only one other node. It is from the basal node that branches of unlimited growth are produced.

The elongate cells have a single layer of small discoid chloroplasts of oval shape lodged in parietal cytoplasm. The chloroplasts do not completely line the walls, there being two neutral areas, appearing as narrow spiral bands, from which they are lacking (Fig. 3). The stationary chloroplasts are arranged in a well marked longitudinal series which are

parallel to the two colourless striations. In the living cell, streaming of the protoplasm can be clearly seen, and it is apparent that the direction of streaming is the same as that in which the files of chloroplasts are arranged. On one side of the cell the protoplasm is streaming upwards, and on the other side it is streaming downwards. The clear bands are the boundaries between the ascending and descending streams. According to Votava (1914), this clear striation, where the dense arrangement of chloroplasts is interrupted, is the site of an inwardly projecting ridge that serves to segregate the two streams. This observation has been critiqised by Green & Chapman (1955) on the ground that chloroplasts are occasionally present within the striations gap of living cells. These chloroplasts are in the same focal planeas those forming the sides of the gap. Similar observations, which support Green's conclusions, have been made during the present investigation.

According to Fritsch (1935), the orientation of the streams in the internodes is related to the position of the whorl of short laterals above, the ascending stream being situated below the oldest, the descending one below the youngest, member of the whorl. According to Sachs (quoted in Groves and Bullock-Webster), "the successive whorls of a stem alternate, in such a manner that the oldest branchlets of the whorl, ——— are arranged in a spiral line winding around the stem". As a consequence, the colourless striation is oblique and follows a steep spiral course around the axis, which in the internodes is always dextral. Fritsch states that in the cells of the nodes streaming of the cytoplasm is in the transverse sense.

Green & Chapman (1955) state that in very small cells no striations can be seen. In cells 0.3 mm. long, however, regular files of chloroplasts can be seen and these are parallel to the sides of the cylindrical wall.

According to Cazalas (1930) young cells do not exhibit 'rotation of cytoplasm'.

Green (1954) has carried out a careful study of the growth of the internode cell Nitella axillaris Braun from the time the cells were 0.3 mm. long until maturity (over 1 cm. long). By placing markers on the cell wall and measuring their displacement, he showed that growth was evenly distributed throughout the length of the cell. He also demonstrated by the same means that Nitella exhibits the phenomenon of spiral growth, young cells showing a right-handed rotational growth, while at a later stage (length about 1 cm) there is a reversal of the direction of rotation. Left-handed rotational growth ceased, however, while there was still a net right-hand rotation.

Green (1958 A) has also measured the 'optical' thickness of the walls in developing internodes using a Dyson interference microscope. The main conclusions of this work were that the wall optical thickness reaches a peak value where the cell is small, then drops suddenly as the cell attains its maximum relative rate of elongation, then gradually increases again as the cell elongates to its final length. This latter increase in optical thickness occurs during the bulk of the elongation (5 mm. to 50 mm.), and during this stage of growth the rate of deposition

of new wall material more than keeps pace with the increase of surface area.

Green (1958 B) has also attempted to determine the site of deposition of new wall material using a radioactive tracer technique. Tritium (H^3), when contained in the growth medium, was incorporated into the elongating cell walls. It was concluded that tritium - containing cell wall constituents were added to the growing wall at, or very near, the inner surface of the wall.

PREVIOUS WORK ON WALL STRUCTURE OF NITELLA

Prior to this investigation, there was not a very great deal known about the detailed wall structure of *Nitella*.

Correns (1893) and Votava (1914) had shown that the *Nitella* cell wall contains a substance giving the staining reactions of cellulose. Debski (1898) was not able to identify the skeletal substance. Fritsch (1935), referring to the work of Correns and Zacharias on the cell wall of the Characeae, writes; "The membrane, apart from a superficial layer of unknown composition, consists of cellulose." Green and Chapman (1955), and Green (1954, 1958 A, 1958 B) do not offer any evidence on the composition of the wall, and on the basis of the early work referred to above, assume that cellulose is a constituent of the wall. Nicolai & Preston (1952) have surveyed the fine structure of the walls in about sixty species of filamentous green algae, as revealed chiefly by the methods of X-ray analysis. They found, however, that the X-ray diagram of *Nitella* was much too poor in diffraction arcs to allow a decision to be made as to whether the skeletal substance was cellulose or not. No orientation was

observed in the X-ray diagram. This is understandable in the light of the present investigation. To anticipate work to be presented later, it may be noted that some pretreatment of the wall is necessary before a satisfactory X-ray diagram is obtained; and, further, it has been found desirable to carry out an infra-red analysis of the untreated wall to supplement the X-ray evidence.

The only information on the chemical nature of the wall, therefore, rested on the evidence of staining tests. In view of the unreliability of staining and solubility tests for native cellulose (Nicolai and Preston, 1952) it is desirable that the early work should be supported by evidence from more modern methods.

The optical properties of the wall have been investigated by Green and Chapman (1955) and by Green (1958 A) using the polarising microscope, and Green (1958 A) has also examined thin sections of the wall, and surface replicas of the inner surface of the wall, in the electron microscope. This work will be discussed in greater detail later. At this stage it is sufficient to note the main conclusions, viz.;

(a) On the basis of polarising microscope evidence, Green and Chapman concluded that the cellulose microfibrils were well oriented and ran strictly in the transverse direction in all portions of the wall and in all stages of its development. However, in a later work (1958 A), Green has found, by the method of surface replication, that the microfibrils on the inner face of the wall have different orientations at different stages of growth. Briefly, microfibrils are scattered at random in the

apical cell; in the most recently formed internode they are near parallel in the transverse direction; and in the older internodes they show considerable scatter about the transverse direction until, in the extreme case of a cell which has ceased to grow, they are present in randomly oriented fields of well oriented fibrils. Circular microfibrillar patterns and regions of radiating microfibrils are also observed in non-elongating cells.

(b) "Apart from the striations which are visible as linear regions of the wall which have different properties in polarized light from the rest of the wall, the cell is uniform in composition. Attempts to flake or tear the wall were in vain." (Green and Chapman, 1955).

If one leaves out of consideration Green's later work (1958), the amount of published information on wall structure when this investigation was started was rather meagre, and, even if one includes this later work, there is still a great deal to be known about the detailed structure before one is in a position to make an attempt to interpret the growth process of the wall. It is for this reason, that the work on wall structure was undertaken.

CHEMICAL ANALYSIS

The first step in the determination of cell wall structure was a chemical analysis in terms of the polysaccharides which correspond to the individual sugar residues. It is assumed that each sugar is produced by the hydrolysis of a single polysaccharide.

The method followed closely that of Cronshaw, Myers, and Preston (1958), which was a modified form of that described by Jermyn and Isherwood (1956). There is, therefore, no need to repeat their description in detail, and the following is merely a brief outline of the method.

The plants were frozen in liquid air, ground to a flour, and transferred to ethanol which was quickly brought to the boil. After extraction for half an hour, the washed and dried fragmented walls constituted the starting material.

The ethanol-insoluable algal material was first fractionated by extracting with boiling water for 12 hours, and then with 4N potassium hydroxide at 25°C for 12 hours. The material remaining was subjected to a mild chlorination to remove protein material, leaving the purified material designated α-cellulose.

The filtrate and washings from the above extractions were concentrated and after hydrolysis and precipitation of the sugars, the precipitate was dissolved in an appropriate volume of water to give 2% (W/V) solution.

The sugars were separated and identified by the method of descending paper chromatography using Whatman No. 1 Filter paper.

The developing solvents were:

(a) Pyridine: ethyl acetate: water 1:2:2.

(b) Acetic Acid: ethyl acetate: water 1:3:3.

The positions of the sugars, after separation, were revealed by spraying the dried chromatograms with one of the following reagents.

(a) 2% ammoniacal silver nitrate

(b) 3% p-anisidene hydrochloride in moist butanol.

The sugars and sugar derivatives were identified by comparison with the positions and colours of standard sugars run on the same chromatogram.

The results are given in Tables I and II.

TABLE I.

Analysis of Cell Wall of Nitella opaca

Water-soluble fraction (% Dry wt.)	- 29.9%
Alkali-soluble fraction (% Dry wt.)	- 34.8%
Chlorite-soluble fraction (% Dry wt.)	- 18.6%
α-cellulose (% Dry wt.)	- 16.7%

TABLE II

Sugars in the Hydrolysis Products

Water-soluble fraction	-	Galacturonic Acid	- M
		Galactose	- M
		Glucose	- S
		(Unidentified)	- V.W.
		Xylose	- trace
		Rhamnose	- trace
Alkali Extract	-	Galactose	- V.W.
		Glucose	- S
		Mannose	- V.W.
		Xylose	- M
		Rhamnose	- trace
α-cellulose	-	Galacturonic Acid	- trace
		Glucose	- S
		Mannose	- W
		Xylose	- W

S (strong); M (moderate); W (weak); V.W. (very weak) refer to the intensity of the spots on the chromatogram.

The following points might be noted:

(a) The water-soluble fraction of the wall amounts to about a third of the initial dry weight. Although this is a fairly large fraction of the total wall material, it is, nevertheless, small compared with that of most of the algae examined by Cronshaw, Myers and Preston (1958).

Comparable figures for the water-soluble fraction in that investigation were of the order of 50 to 70%. Only Cladophora, Enteromorpha, and Ptilota had water soluble fractions below 40%.

(b) Galacturonic acid in the water extract indicates the presence of pectic substances in the wall. There is X-ray evidence that the strong glucose spot is due partly to hydrolysis of starch.

(c) The α-cellulose fraction (17%) is not as high as that of Chadophora (28.5), Chaetomorpha (41%), and, particularly, Valonia (75%). It is, however, comparable with Enteromorpha, Ulva, Laminaria, Ptilota, and Griffithsia.

(d) The fact that sugars, other than glucose, occur in the hydrolysate of the α-cellulose, puts Nitella outside the unique group which yield only glucose. The galacturonic acid is only present as a minute trace and has probably been carried over from an earlier stage.

At each stage of the chemical treatment a small sample of the remaining material was taken for physical examination in the electron microscope and by the technique of X-ray diffraction. These results will be discussed in the appropriate sections.

POLARIZING MICROSCOPY

The polarizing microscope has been used for many years as an important tool in structural analysis. As early as 1858, Nageli was using it to interpret the structure of starch grains, and later of cell walls, in terms of the crystalline nature of the building material.

The method, which is complimentary to that of X-ray analysis, depends on the fact that, in general, the wall is optically anisotropic. Optically isotropic substances possess only one characteristic refractive index and light is transmitted through them with equal velocity irrespective of its direction of propagation. Where a ray of light passes through an anisotropic body, however, it does so in the form of two separate polarized rays - the extraordinary ray and the ordinary ray. The velocity of the ordinary ray is independent of the direction of propagation; but that of the extraordinary ray is not. In uniaxial crystals there is only one direction for which the velocities are equal. This is the optic axis of the crystal. The difference in velocity and, therefore, of refractive index, is greatest for propagation normal to the optic axis. In this case, if the refractive indices of the ordinary ray and extraordinary ray are denoted $n_{\parallel}$ and $n_{\perp}$, numerical difference between them, $n_{\parallel} - n_{\perp}$, is called the birefringence.

Cellulose can be considered, at least to a first approximation, to have only two principal refractive indices, and it therefore behaves

like an optically uniaxial crystal. Its birefringence is positive (cf., chitin, which is negative).

Birefringence, which depends on the anisotropy of individual structural units, is called "intrinsic" birefringence. There is, however, another type of birefringence, also due to morphological properties, which arises from quite another set of circumstances. If a body can be considered to be built up of long thin rods, or, of thin plates then, provided that the diameter of the rods (or thickness of plates) is small compared with the wavelength of light, the system will appear anisotropic whether the rods (or plates) are themselves anisotropic or not. This is called "form" birefringence (Wiener, 1912). In this case the degree of anisotropy depends on the refractive index of the continuous medium and this characteristic provides the means of distinguishing between "form" and "intrinsic" birefringence.

In general, "form" birefringence is of too small a magnitude to be of great importance in cell wall optical analysis; but it cannot be entirely disregarded. Preston (1931), and Bass Becking and Wayn Galliher (1931), have reported cases in which "form" birefringence has made the principal contribution. It does, in fact, make a small contribution to the total birefringence in the case of Nitella. Green and Chapman (1955) have attempted to distinguish between the two types of birefringence by immersing the wall in solutions of different refractive index. Solutions of refractive index 1.50 - 1.55 reduced the retardation (measured on a Berek Compensator) most of all. The reduction in retardation

using a concentrated solution of sucrose (R.I.1.50), amounted to 11-19 per cent of the retardation when the same walls were in water. There is therefore some evidence for "form" birefringence but its contribution is much smaller than that of the "intrinsic" birefringence.

To anticipate results not yet presented, it will be shown in the section on X-ray analysis that cellulose is present in the wall of Nitella, so that, provided there is a net preferred orientation of the cellulose chains, the wall will exhibit "intrinsic" birefringence. That this is indeed the case can be inferred from the work of Green & Chapman referred to above. The direction of the m.e.p.* is approximately transverse to the cell axis. This is consistant with the X-ray evidence, which also indicates transverse orientation of the cellulose component.

It was found, however, that the m.e.p. was not exactly transverse in Nitella opaca and that, in fact, its direction seemed to be correlated in some way with the age of the cell. One obvious feature of the cell which also varies with its age is the angle which the clear striation makes to the longitudinal axis (and, therefore, the angle which the streaming protoplasm makes to the longitudinal axis). This suggests that the two may be correlated.

It was, in fact, observed many years ago by Denham (1923) that the directions of protoplasmic streaming and of wall striations were often identical, and on this basis he suggested mechanisms whereby

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* The 'm.e.p.' is the major extinction position of the wall when viewed between crossed nicols. It corresponds to the direction of the major refractive index.

streaming might cause orientation in cell walls. Although this attracted considerable attention at the time and led to a good deal of speculation the subsequent observation of Martens (1932) that the striations concerned lay deep within the wall, led to the abandonment of the whole concept which was perhaps premature.

Since the direction of protoplasmic streaming is so well defined in Nitella, it seemed an ideal material on which to re-examine the possibility of a correlation between streaming and cellulose chain direction in the cell wall (Probine and Preston 1958). Before this was done, however, the angle between the files of chloroplasts and the cell axis (which is a fairly accurate measure of the direction of protoplasmic streaming), was measured for a number of internodal cells at various stages of growth, and it was found that there was a correlation between the streaming angle of the protoplasm and the dimensions of the cell. In Fig. 4 the length/diameter ratio (l/d) is plotted against cot θ, where θ is the angle between the files of chloroplasts and longitudinal axis of the cell (i.e. θ = streaming angle). The correlation between l/d and cot θ is highly significant. The correlation coefficient (R) is 0.91 (at the 0.001 level of significance, R = 0.617 for 23 degrees of freedom).

The regression equation is of the form,

$$l/d = a + b \cot \theta$$

where θ = streaming angle, l = length of cell, d = diameter of cell and a and b are constants.

There is, however, evidence which will be considered later that this linear relationship is only an approximation, and that the actual relationship is rather more complex.

Since the streaming angle is a function of cell dimensions (and, therefore of cell age) it is possible to obtain cells with a wide range of streaming angles by selecting cells varying in age from the very young to the mature. The appropriate observations have therefore been made on a series of cells of various ages. The streaming angle was measured on the living cell. The major extinction position (m.e.p.) of a single cell wall with respect to the cell axis was measured with a polarising microscope. The ends of the cell were cut off at an oblique angle to the cell axis, the protoplasm removed by stroking the cell gently under water, and the cell mounted on the slide in dilute glycerine. Because the cell was cut obliquely to the cell axis it was possible to mount it in such a way that when flattened by the cover slip a fairly large area of single cell wall was visible at each end of the cell (Fig. 5).

The measurement procedure was as follows:- With the specimen between crossed nicols and the red I plate in the system, the stage was turned until the wall was the same colour as the field. The stage angle was noted. The stage was then turned through 90°, a second colour match obtained, and the angular position again noted. The stage was then turned back to its original position and the whole ~~angle of~~ measurement repeated five times to give, finally, two mean positions. One of these corresponds to the major extinction position and the other to the minor. They were identified by observing whether wall showed 'addition' or

'subtraction' colour when the stage was displaced slightly clockwise from the colour - match position. The procedure of obtaining a colour match at two positions 90° apart is designed to remove any colour bias from the match. The analyser was then thrown out of the system, the stage turned until the edge of the wall was parallel to the cross wires, and the angle noted. This was repeated on the other side of the cell and the two angles meaned to give the 'zero' angle corresponding to the long axis of the cell (short cells are not always perfectly cylindrical, so that the sides of flattened cells are not necessarily parallel to the long axis). Finally, before it could be decided whether the m.e.p. made a right or a left-handed spiral around the wall, the single wall had to be identified as a "top" wall or a "bottom" wall. This was done by "through focusing".

It was found that the direction of the m.e.p. followed a flat helix around the cylindrical wall, and that the helix angle varied with the age of the cell and, therefore with the streaming angle and the dimensions of the cell. The results are shown graphically in Fig. 6. The correlation between streaming angle and angle of m.e.p. is highly significant. The correlation coefficient (R) is 0.85 (at the 0.001 level of significance, R = 0.617 for 23 degrees of freedom).

These latter results are in contradiction to those obtained by Green and Chapman (1955) for Nitella axillaris Braun. These workers state that "Structural investigations reveal that the cellulose microfibrils of the wall are well oriented and run strictly in the transverse direction. This orientation is found in all portions of the wall and in all stages

of its development." Unless there is a genuine difference between these two species there seems to be a conflict here. An apparent displacement of the m.e.p. from its true position can of course arise through, for instance, a misalignment of the cross wires in the microscope. Fig. 5 is designed to show that no such error is a contributing factor in the present case and fully to document the helical arrangement of the m.e.p. In Fig. 5a the double wall (on the left) is extinguished while the single wall (on the right) is bright; in Fig. 5b the single wall is extinguished while the double wall is bright. A moments' thought will satisfy the reader that the m.e.p. of the single wall must be tilted to the transverse.

It is, in fact, difficult to reconcile Green and Chapman's statement with the evidence of one of their own published photographs. In their Fig. 9 (1955), a single wall is shown which appears to be almost on the point of extinction, but which is, nevertheless, shown displaced 16° from the analyser direction. Taken on its own, the evidence of a single photograph can hardly be considered convincing, since film and printing paper characteristics are such that the wrong impression could easily be given. We have, however, the caption statement that the striation is "almost extinguished". In the authors' experience this must mean that the wall also is nearly extinguished. Although one cannot attach too much importance to this evidence, some doubts are raised. Further, although one does not wish to argue over small points, one would have thought that the use of the description "strictly transverse"

was hardly justified, in view of the very great difficulty in making really accurate measurements.

X-ray analysis of young cell walls was used to support the polarizing microscope evidence. A stack of young single cell walls (cells 5-6 mm long) was assembled by the method previously described. They were mounted in the camera with their longitudinal axes vertical, the vertical alignment being achieved by matching the edges of the cells against the vertical cross wire of a travelling microscope (previously checked against a fine plumb-line). A spirit level was used to set the base of the film holder (on which the film rested) at right angles to the cell axis. The X-ray diagram was in fact slightly tilted to the film axis in a direction consistant with the polarizing microscope evidence. The distribution of crystallites about the mean position was, however, such that the small angle could not be accurately measured.

The situation is, therefore, that a highly significant correlation between the direction of the m.e.p. and the streaming direction has been observed in the case of Nitella opaca, and concrete proof has been offered (Fig. 5) that the m.e.p. is not always transverse. This has been supported by X-ray evidence. There the matter must rest for the present, but it might be mentioned here, that corroborative evidence of a mechanical nature will be presented later in this thesis.

The correlation which has been shown to exist between streaming direction and the direction of the m.e.p., raises again the question whether these two phenomena are casually linked. It is not proposed to discuss this in detail until after the electron microscope results have

been presented. There is, however, a relevant observation made with the polarizing microscope which might be mentioned at this point. If protoplasmic streaming and wall deposition are related one might expect that, because of the discontinuity of flow which occurs there, the region of the wall within the area of the striation would be structurally different from the rest of the wall. This suggestion is, in fact, supported by the appearance of the wall when viewed between crossed nicols (Fig. 7).

Green & Chapman (1955) comment upon this as follows: "The striations are visible as linear regions of the wall which have different properties in polarized light from the rest of the wall."; and again, "The striations were visible as linear regions which appear to extinguish when their long axis, rather than that of the cell, was parallel to the vibration plane of either prism. Movement of the striations through the extinction position revealed changes in brightness which are somewhat difficult to interpret. Many striations showed a very narrow isotropic "line" down the centre. While their properties are difficult to determine, there is no question that the striations differ structurally from the rest of the wall."

The results of the present investigation are in general agreement with those of Green & Chapman, but there are some further observations which are relevant.

(1) The structural difference which occurs in the region of the striation is a difference in microfibril arrangement, i.e. it is a discontinuity of arrangement of the crystalline component, and not of the "so-called"

amorphous material (protoplasm, pectins, non-crystalline hemicelluloses, etc.). The basis of this statement is that after a wall has been chemically treated*, examination of "stripped" specimens in the electronmicroscope, reveals the presence of microfibrils with little or no amorphous material encrusting them. When cells which have been treated in this way are examined in the polarizing microscope, however, the "optically different" striations are still clearly visible.

(2) It appears that all striations are not structurally similar. Some show the very narrow isotropic line down the centre mentioned by Green and Chapman. The appearance of many striations between crossed nicols is, however, rather more like that shown in Fig. 7. In these cases, where it is viewed with the stage considerably displaced from the wall extinction positions, the striation shows up as a central bright "line" bounded on each side by a dark "line". The presence of these dark lines, which do not extinguish sharply, suggests that, in these localized areas, the structure differs from the rest of the wall in one or more of the following ways:

(a) The wall might be much thinner, or be less crystalline. The fact that the wall separates into layers which are continuous sheets, and which do not appear to fail preferentially in these regions, does not reinforce this view.

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* The cells were chemically treated with Hot 2% HCl for 30 mins, washed, treated with hot 4% KOH, and washed. This cycle was repeated three times. They were then given a chlorite treatment as described by Cronshaw, Myers, & Preston (1958).

(b) The microfibrils might be considerably disoriented about their mean preferred direction in these regions. This, of the two, seems to be the more likely explanation.

It is difficult to measure the retardation of these dark areas accurately, but such measurements as have been made indicate that the retardation is indeed considerably lower than in the rest of the wall.

This variation in birefringence across the striation is illustrated in Fig. 8, which is a composite photograph, of a striation and the wall on either side of it, built up in the following way. A piece of wall was set to extinction, and the stage then turned through 45° so that the wall appeared 'bright'. An elliptic compensator was inserted and the compensator plate rotated until extinction of wall was again obtained. A photograph of wall and striation was taken in this position, and further photographs at each 2° step on either side of the compensator extinction setting. The range covered was 10° either side of the extinction setting. The photographs of which Fig. 7 is an example, were printed and a narrow strip of the same portion of wall cut from each photograph, at right angles to the striation. These were then assembled side by side, in order, and rephotographed. The result is shown in Fig. 8.

The effect can best be seen by viewing the figure at a low angle to the plane of the page in the direction of the striation. It will be seen that the wall on either side of the striation extinguishes at a different compensator setting (shown on the side) to either the centre

of the striation or the bands on either side of the centre. These bands on either side appear to have a lower birefringence than the rest of the wall, and the centre a slightly higher birefringence. This piece of wall was chosen because the striation and wall appeared to extinguish together between crossed nicols so that this is not likely to be an artifact due to these regions having different m.e.p.'s.

The central bright area (crossed nicols; stage displaced from wall extinction position) often appears to be brighter than the rest of the wall (higher birefringence). No doubt in some cases it merely appears brighter because it is viewed incontrast to the two dark lines on either side. In many cases, however, the retardation measured with an elliptic compensator is higher than that of the rest of the wall - see above. With the Red I plate in position it is yellow (or blue) when the rest of the wall is yellow (or blue), indicating that the birefringence is of the same sign as the rest of the wall (negative). When the stage is rotated through the extinction position the bright region does not, in general, extinguish uniformly. In some cases a dark band sweeps across from one side to the other, indicating a continuous change in m.e.p. across the striation, and that the structure on one side of the centre line is a mirror image of that on the other. In other instances, as the stage is rotated, two dark bands appear to close in towards each other from the sides until they meet at the middle, indicating that the direction of the m.e.p. on each side of the centre line is the same at equal distances from the centre line. Sometimes the whole striation cannot be distinguished from the rest of the wall, at extinction.

One further observation might be made regarding these dark areas. It has been noticed on a few cells, but not all, that if the high power objective (X95) is focussed on the inner wall surface and is then racked back a small amount (3 microns), two narrow parallel ridges are visible, one in the centre of each dark area (Fig. 9). It was at first thought that these represented the edges of the rib, said by Voltava to separate the two streams of protoplasm. After careful inspection, however, it is now thought that these are tiny folds in the wall at this point. The fact that they occur in such perfect parallel lines in these rather special locations on the surface cannot of course be due to pure chance, and is further evidence of a structural discontinuity along these dark lines. The alternative view that they are areas of local thickening is not entirely ruled out. However, the fact that they are not continuous, but exhibit the "broken-ridge" effect of a fold, and, further, that they do not appear to be present on all cells, makes this explanation rather less likely.

The nature of the structural discontinuity which occurs in the region of the striation is somewhat variable, and, therefore, difficult to define with precision. This topic will be discussed further after the E.M. evidence has been presented. At this stage it is merely noted that a definite structural discontinuity occurs at the boundary of the two streams, and that the fact that it does occur is consistant with the suggestion that direction of streaming and microfibril direction might be linked. It does not, of course, prove that they are so linked.

~~It does not, of course, prove that they are so linked.~~ One further point might be made, however, before leaving this topic. It has been tacitly assumed in the foregoing discussion that the flow boundaries are more or less fixed in relation to the cell surface. Evidence that they do indeed occupy the same region of the cell surface throughout most of the life of the cell, will be presented in the section on spiral growth.

When a beam of polarized light is incident normally on a section of anistropic crystal, it is resolved into two components, which travel through the section with different wave velocities. One wave will arrive at the other side of the section later than the other and is said to have been retarded. The two waves are, therefore, separated in air by a "path difference" which is the distance the leading wave has travelled in air ahead of the retarded wave. Expressed in other terms, we have,

$p = (n_{/\!/} - n_{\perp}) d$ where p = path difference

$n_{/\!/}$ = refractive index parallel to the m.e.p.

$n_{\perp}$ = refractive index at right angles to the m.e.p.

d = thickness of wall in some units as, p.

The path difference (or, retardation) has been measured with an elliptic compensator (Hallimond, 1953), using a sodium lamp as a source of illumination. The retardation varied between 9 and 17 millimicrons for cells ranging in length from 8 to 50 mm. These values tend to be a little higher than those published by Green (1958A), whose values for comparable cells were between 6 to 10 millimicrons. However, having in

mind that they were measured on a different species grown in different conditions, the agreement is quite good.

The birefringence of the wall was defined earlier as $(n_{\parallel} - n_{\perp})$, so that the birefringence may be calculated from the path difference if the thickness is known. The birefringence is of the order of 0.003. The significance of this rather low value will be discussed at the end of this 'Part'.

X-RAY DIFFRACTION

The technique of X-ray analysis is now well established as a method of elucidating the detailed structure of plant cell walls (see, for example; Preston, 1952). This technique allows deductions to be made about the way in which the crystalline component is organised within the wall. In the present investigation it has been used, with support from other methods, to identify the crystal substance and the form in which it crystallized, to determine whether any particular chemical treatment has modified the form in which the material is crystallized, to determine the orientation of the crystallites with respect to the longitudinal axis of the cell, and to determine whether any particular crystal plane is oriented with respect to the face of the wall. It has also given a qualitative indication of the amount of crystalline and non-crystalline material present in the sample.

Where possible X-ray evidence has been supported by information obtained using other methods. The importance of this safe-guard has been pointed out by Cronshaw, Myers and Preston (1958), who have drawn attention to instances of chemically differing carbohydrates giving the same X-ray diagram.

The crystalline component of most plant cell walls is cellulose (the alga, Porphyra, is an example of an exception in which the building unit of the microfibrils is mannose - Cronshaw, Myers, and Preston; 1958). In the strict chemical sense, cellulose is an insoluble polysaccharide, in which only β -glucose residues occur built into long unbranched molecular chains. Recent work has shown, however, that most plant

celluloses do not come within this strict definition. Cronshaw, Myers and Preston (1958) have found that most of the purified algal celluloses they examined have yielded considerable quantities of sugars in addition to the expected glucose. This has also been found to be true of a number of higher plant celluloses. Celluloses free, or almost free, of non-glucose sugars, have, however, been found in Valonia, Cladophora and Chaetomorpha.

Myers and Preston (1959) have proposed that "true" cellulose, yielding only glucose on hydrolysis, should be termed eucellulose. The term cellulose should, however, continue to be used in the following rather loose sense; "A group of high molecular weight polysaccharides yielding β-glucose and possibly other sugars and sugar derivatives on hydrolysis. The material stains blue with iodine and 70% sulphuric acid; is insoluble in 4N-KOH, is soluble in 70% sulphuric acid, in zinc chloride solution and in cuprammonium. All celluloses yield X-ray diagrams with more or less diffuse arcs."

Chemical analysis of the wall of Nitella has shown that mannose, and xylose are present, as well as glucose, in the hydrolysate of the α-cellulose fraction. The microfibrillar component of the Nitella wall is, therefore, not eucellulose.

The crystal structure of what we would now term eucellulose I, has been worked out in some detail and the unit cell proposed by Meyer and Mark (1930) has been generally accepted. The parameters of the cell are;

$a = 8.34$ Å

$c = 7.9$ Å

$b = 10.3$ Å

$\beta = 84^{\circ}$

A diagrammatic representation of two unit cells with the cellulose units in position is shown in FIG. 10. Successive β -glucose residues are rotated through 180° giving a two-fold screw axis as an element of symmetry.

In the discussion of the X-ray analysis results which follow, the diffraction diagrams will be interpreted in terms of the Meyer and Mark cell. The fact that the crystalline component of Nitella is not eucellulose means that this is not, strictly, a correct procedure. However since little is known as yet, about the way in which the non-glucose sugars are built into the crystal lattice, it might well be that the highly crystalline central core of the microfibrils can be considered, in a limited "X-ray crystallographic" sense, to be eucellulose.

To cover all situations, however, when discussing the crystal structure of Nitella cellulose, in this thesis, nothing more is implied than that its unit cell is "something like" the unit cell of eucellulose I (or, II, as the case may be).

(a) Analysis of powder diagrams.

Following each stage of the chemical treatment, a small amount of the powdered material was removed from the parent sample and an X-ray

diagram obtained using a flat film at either 3 or 4 cm. from the specimen. The CuK_{α} X-ray beam was collimated to 0.5 mm. diameter The specimen /film distance was calculated from the diameter and known spacing of the diffraction arcs of a silver diagram.

In table 3 the principal spacings are listed in order of intensity. Where scattering from amorphous material is rather strong it is sometimes difficult to decide between the relative intensities of two arcs of widely different diameter. The intensity order in the table is therefore approximately only, and is given as a guide to identification and not as a criterion of identification.

The following is a step-by-step interpretation of the data in table 3.

(a) It is already known from the chemical analysis that the α-cellulose yields glucose on hydrolysis, with mannose and xylose also present. The material is, therefore, not eucellulose.

(b) The X-ray spacings of the α-cellulose component (post chlorite) are similar to those of cellulose II, both in numerical value, and in the relative intensity of the various reflections (Fig.11). This close agreement, taken with the fact that glucose was the principal sugar in (a) above, undoubtedly means that the α-cellulose component is cellulose in the crystal form of cellulose II (or, something very like it). The spacing at 9.6 Å, however, is longer than any of the accepted cellulose spacings. Anomalous spacings of this order have previously been reported in cellulosic bodies. Sen and Wood (1948) have reported anomalous long spacings in jute; Preston and Singh (1950) have done so for bamboo fibres;

TABLE 3.

Principal Spacings in Order of Intensity Calculated from Powder Diagrams

Post-Alcohol	Starch-B	Post-Water	Cellulose I	Miller Index Cellulose I	Post-Alkali	Post-Chlorite	Cellulose II	Miller Index Cellulose II
5.18	5.17 s	3.90	3.93	002; 301	4.41* - 4.46*	4.39	4.42; 4.38	002; 021
16.00	15.6 s	4.25	4.34	021	4.03	4.01	4.03	002
4.00	4.00 m	8.94	-	-	7.35	7.23	7.35	101
8.33	-	5.25	5.39	$10\bar{1}$	-	3.10	3.14	031;131;130
6.11	6.29m;5.89m	2.58	2.58;2.59	040;212;311	2.58	2.58	2.58	040
4.43	4.51 m	2.14	5.97	101	2.13	2.13	2.21	202; 103
2.58	-	6.10	1.95;2.16	004;240,042	-	9.64	-	-
3.69	3.68 m				-	5.15	5.19	020

Notes:- (1) The spacings marked * are very diffuse

(2) The 4.42 and 4.38 spacings of cellulose II are not resolved and have been bracketed.

(3) s, strong; m moderate.

(4) The order of intensity is approximate only and is intended to serve as a guide to identification, and not as a criterion.

Myers, Preston and Ripley (1956) found an anomalous spacing at 10.8 Å in Griffithsia and Cronshaw, Myers and Preston (1958) report long spacings in several marine algae. Sen and Wood considered that the long spacings in jute (14.3 Å and 9.7 Å) were associated with the presence of lignin, but that there was also some evidence that the smaller spacing was associated with the presence of xylan. There is, therefore, a good deal of evidence for the presence of anomalous long spacings, but no really satisfactory explanation of their presence.

(c) The diffraction diagram obtained from the material after alkali treatment shows much more fogging due to background scatter than was present on the 'post chlorite' diagram indicating that, at this stage of the chemical treatment, amorphous material is still present. There is, however, little doubt that at this stage the crystalline material is cellulose in the crystal form of cellulose II.

(d) The question now to be answered is whether the cellulose was originally present as cellulose II, or whether it has been converted from another crystalline form of cellulose to cellulose II by treatment with 4N-KOH.

After extraction of the water - soluble material (post water), the spacings compare very favourably with the principal spacings of cellulose I (with the exception of the long spacing at 8.9 Å, to which the remarks on long spacings in (b) above, also apply). There is no evidence of a spacing of 7.35 Å. Further, there is evidence in the diagram which show orientation (see over) that the spacing listed at 5.25 Å is an (h01) spacing, which is consistant with it being

the $10\bar{1}$ spacing of cellulose I. The nearest principal spacing of cellulose II (5.19Å) has a Miller index of (020).

The indications are, therefore, that the crystalline substance present after removal of the water soluble material and before the alkali treatment, is something like cellulose I. Treatment with 4N-KOH converts this to something very like cellulose II.

(e) Finally, reference must be made to the X-ray powder diagram of the material after the alcohol extraction. In this case the order of the spacings listed with regard to intensity of the diffracted beam is different from that in the 'Post Water' column, and there is a long spacing at approximately 16 Å (Fig.12). If the spacings are compared with those, in column (2), for Starch type B it is found that there is fairly close agreement. The Starch figures are taken from Bear and French (1941). Katz and Derksen (1933) found that native starches yield a variety of similar but nevertheless distinct, X-ray powder diagrams. There are two chief types, type A (cereal starches) and type B (tuber and potato starches), both exhibiting a fairly high degree of crystallinity. It seems likely, therefore, that in the present case the cellulose I diagram has been partially masked by a starch diagram of type B. The starch diagram is absent after boiling in water for 12 hours and glucose is present in the hydrolysate of the water extract. It might be mentioned here that Kooiman (1957) has found that the cell walls of many seeds contain amyloid as indicated by the blue stain of

an iodine in potassium iodide solution. Threads of Tarmarindus indica amyloid when stretched and dried produced an X-ray diagram showing diffuse equitorial interference arcs at 16.5 Å and 4.5 Å and has diffuse meridonal interference arcs at 9.7, 5.1, 3.3 and 2.54 Å. (Kooiman and Kreger, 1957). The "post alcohol" diagram of Nitella shows rather similar spacings to the amyloid spacings listed by Kooiman and Kreger, but the wall does not take up the indicating stain, and, further, the sharpness of the arcs is rather typical of starch granules.

Diagrams which do not show a strong starch diagram have been obtained by carefully removing the cell contents from individual cells by stroking the cells with a loop of hair. It seems likely, therefore, that in the case discussed above, starch granules have been carried over from the alcohol extraction (the material for chemical analysis was prepared by freezing the material in liquid air and grinding it to a flour).

The general conclusion reached from inspection of table 3, is, therefore, that the crystalline component of the wall is Cellulose I. The spacings listed in the table were, however, calculated from diagrams which were generally badly fogged due to scattering from amorphous material and on which the interference arcs were not very sharp. Rather more conclusive evidence that the crystalline component was cellulose I was therefore obtained by an alternative method.

The X-ray diagram of Nitella was compared directly with the X-ray diagram of eucellulose I, (purified Cladophora cellulose). A mask of

brass was placed over the film in such a way that only two opposite quadrants in a circular aperture were exposed at one time. The X-ray diagrams of two specimens (Nitella and Cladophora) were therefore photographed on one film and processed together. Before this could be done in a really satisfactory manner, however, background fogging had to be reduced. It was found that boiling material in 2% H_2SO_4 for 30 mins. removed a good deal of the amorphous material and the diagram was markedly improved. Comparison of diagrams before and after this treatment revealed no evidence of a change in the crystal structure due to the treatment. It was also found that the diagrams from these poorly crystalline walls can be very much improved by reducing air and collimator scattering. A lead stop was introduced between film and collimator to intercept the primary beam soon after it passed through the specimen, but far enough from the collimator to avoid interference with the lowest-angle diffracted beam. For poorly crystalline materials it produces a marked improvement. Fig. 13 shows a Nitella diagram in which one quadrant was exposed in the normal manner while in the other an extra stop had been fitted as described (same exposure time for both quadrants).

Fig. 14a shows an X-ray diagram of Nitella (treated in dilute acid), compared with a Cladophora diagram. Although there is a very close similarity between the two diagrams, there appear to be some very slight differences. The relative intensity of reflections from the various crystal planes is different. In particular, the difference between the intensity of the reflection from the 101 plane (inner ring), and the intensity of that from the 002 plane (fourth ring from centre)

appears to be greater in Cladophora than in Nitella. (Compare 14b) Reflection from the 230 plane is stronger (relative to the intensity of that from the 040 plane) in Cladophora than Nitella; but, the combined reflection from the 130,031, and 131 planes is stronger (relative to that from the combined 221, and 122 planes) in Nitella. Where one of a pair of rings, which are close together, does not show up on the print, it appears that there is a small difference in crystal spacing. On the original negative where the apparently missing rings are present, it is clear that there is complete correspondence of spacings within the limits of resolution of the photograph. The reason for the difference in relative intensity of the rings is not clear. It may be due to a difference in molecular packing in the crystal, or to a difference in degree of crystal perfection. Whatever the cause it is not due to a major difference in crystal structure.

One other point should be mentioned. The interference arcs are much more diffuse in the Nitella diagram than in the Cladophora diagram. This, again, might be due to a less perfect crystal structure in Nitella, or, to the presence of much smaller crystallites. The very great deal of amorphous scattering indicates that the wall is not highly crystalline.

All the evidence in this section points to the fact that the crystalline component in the wall of Nitella is something very like Cellulose I. There is some very slight evidence of minor differences in the detail of the X-ray diagrams of eucellulose and Nitella cellulose.

and for this reason the X-ray evidence has been supported by an infra-red analysis. This will be discussed in a later section.

(b) Organisation of the Crystalline Component within the Wall

So far attention has been confined to the use of the X-ray diffraction technique as a diagnostic method to determine the nature and crystal form of the crystalline fraction of the wall. It has also been used to obtain information about the way in which this crystalline component is organised within the wall.

Because information obtained by the use of this technique will later be used in attempts to interpret the growth process of the Nitella cell wall, it is desirable to point out that the wall structure of Nitella, as revealed by X-ray methods, has been determined on air dry material. From some points of view (for example; to guard against artifacts being produced by the drying process - Berkley and Kerr, 1946; Sisson, 1936), it might be considered desirable to use fresh material. A difficutly arises, however, because the cell wall of Nitella contains a large proportion of amorphous material and interpretation of X-ray diagrams of even dry material is fraught with some difficulty. Additional fogging would make it well-nigh impossible to work with fresh material.

However it has been demonstrated by Preston, Wardrop and Nicolai (1948) that the use of dried material is reasonably safe. They examined the X-ray diffraction diagrams of fresh and dried material (cell wall material from the alga Rhizoclonium and also from the cambial tissue of Pinus sylvestris). The conclusion reached was that, while it was not

certain that the size of the crystallites was the same in fresh material as in dried tissue, it was certain that regions do exist sufficiently extensive to give rise to X-ray diagrams. It was therefore considered reasonably safe to interpret the growth processes of cells in terms of a wall structure substantially similar to that described for dried material. That this is so, is implied in all that follows.

The orientation of the crystallites in the wall should preferably be determined on a single piece of wall oriented in the camera with respect to some morphological axis of the cell. Unfortunately, the cell wall of Nitella is not highly crystalline and impracticably long exposures are required to obtain a diffraction diagram of a single cell wall using a normal camera (CuK_{α} beam, collimated to 0.5 mm. dia; specimen/plate distance, 3 cm.). A micro-camera can be used for exploration or qualitative work, but it is not really suitable for quantitative measurements. In this case, therefore, an accurately aligned and stacked bundle of single cell walls, cut from the same cell, have been used in a normal camera. The mounting procedure is detailed below.

The cell contents were removed from Nitella internodes by cutting the ends off the cylindrical cell and gently stroking it under water with a loop of hair mounted on a glass rod (Green, 1958A)

The cells were then dried flat on a polished sheet of perspex and one edge of the flattened cylinder cut off with a sharp razor blade. The cell was then rewet and opened out flat to make a single sheet of wall. It was then cut into equal lengths (2mm. approximately). The top right hand corner of each length was cut off at 45° to avoid any confusion about the orientation of the wall section at a later stage. After the lengths of cell wall had again dried down onto the perspex, one of them was rewet and slid over the top of one of the dry walls. With care the two portions of cell could be aligned accurately relative to one another, before the bottom one became wet and failed to adhere to the perspex. These two cell fragments were then allowed to dry down and a third wet section was placed on top of them and aligned by the same procedure. This was repeated until there was a stack of cell walls all presenting the same face uppermost and all aligned accurately relative to one another. Since this stack was usually much wider than the diameter of the X-ray beam, the dry stack could be cut longitudinally down the centre, one half eased up the perspex surface, and placed on top of the other half-stack. A small drop of water on the dry interface lubricated the interface sufficiently for the two stacks to be accurately aligned and the two stacks adhered together when dry. In this way, up to 24 cell walls were stacked on top of one another and accurately aligned.

An X-ray diagram of a Nitella wall (stacked as described) is

reproduced in Fig. 15. The beam was directed normal to the plane of the wall with the longitudinal axis of the cell vertical (parallel to longitudinal axis of page in the figure). The material had been treated with dilute acid to remove amorphous material and the front beam-stop was used to reduce background scatter.

The diagram shows that there is some preferential orientation of the crystalline component. The strong arcs on the meridian have been indexed (002), ($10\bar{1}$) and (101). The fact that they occur on the meridian means that the b axis of the crystallites is preferentially oriented in the transverse direction. There is, however, a considerable distribution about this mean direction. The angular distribution of the crystallites about the mean direction has been determined by measuring the variation of optical density around the 002 arc. Optical density has been related to X-ray intensity by a method similar to that described by Meredith (1951). The X-ray intensity distribution function around the 002 arc can be taken to represent the angular distribution of crystallites provided that they are not preferentially oriented about their b axis. It will be shown later that in fact the 101 planes are preferentially oriented in the plane of the wall. There is, however, a considerable angular dispersion about this mean position, so that the curve reproduced in Fig. 16 is probably a reasonable approximation to angular distribution of crystallites.

The wall has also been X-rayed with the beam parallel to the face of the wall, (a) at right angles to the longitudinal axis.

and (b) parallel to the longitudinal axis.

The results are shown in Fig. 17 and Fig. 18 respectively. Unfortunately the diagrams do not reproduce at all well and some of the detail is lost. There is, in fact, some difficulty in seeing detail on the original films. When the X-ray beam is parallel to the plane of the wall and at right angles to the longitudinal axis the $10\bar{1}$ plane is represented by an equitorial arc. The $10\bar{1}$ plane is represented by a very faint meridional arc. This indicates that the $10\bar{1}$ plane is oriented parallel to the plane of the wall. The $10\bar{1}$ plane occurs only by virtue of the fact that there is a wide angular distribution of crystallites in the plane of the wall (see previous paragraph). When the wall is X-rayed parallel to the plane of the wall with the longitudinal axis parallel to the beam, the position of the arcs is the same but the distribution of optical density around the arcs is quite different.

In Fig. 15 with the beam normal to the wall one would not expect to see the 101 plane if it was oriented parallel to the plane of the wall. The fact that a short arc does appear is interpreted to mean that the preferential orientation of crystallites about the b axis has a large angular distribution.

INFRA-RED ANALYSIS

In the previous section it was shown that, after the wall had been treated with boiling 2% H_2SO_4 for thirty minutes, the remaining crystalline component in the wall was something very like cellulose in the crystal form of cellulose I. Comparison of the X-ray powder diagrams before and after this treatment seemed to indicate that the spacings of the main arcs had not been changed by the treatment. The X-ray diagrams of the untreated material on which this conclusion was based were so poor, however, that it was thought desirable to support the X-ray evidence on untreated material with an infra-red analysis. Chemically untreated cells, from which the protoplasm had been removed by the stroking technique described earlier were fragmented in a blender using a Teflon pestle. The material was reduced to a fine suspension in water, which was then smeared onto a microscope slide and allowed to dry. The quantities of water and wall material were so adjusted that the mean density of the dry film was 3 micro-grams /mm^2. This film was found to be quite satisfactory for infra-red analysis although a slightly higher concentration could have been used. After the film had been dried it was removed from the slide with a sharp razor blade, and was ready for examination.

The infra-red measurements were very kindly made by Dr. J. Mann in the laboratories of the British Rayon Research Association (Manchester)

at the request of Professor Preston. The instrument usedwas a Grubb-Parsons double beam spectrometer equipped with a lithium fluoride prism. The techniques have been described by Marrinan and Mann (1954, 1956A, 1956B).

The principle of the method is briefly outlined below. In the study of cellulose by infra-red spectroscopy, the absorption that occurs in the frequency range 3600 to 3000 cm^{-1} can be identified with the stretching of the OH groups. These groups are responsible for the hydrogen bonding in cellulose. A difficulty arises, however, because the absorptions of the crystalline and amorphous regions of cellulose overlap to a marked extent. This difficulty is overcome by converting OH groups in the amorphous regions into OD groups by exchange with heavy water. Absorption bands due to stretching OD groups in the amophous regions and OH groups in the crystalline regions are then well separated.

Four different crystalline modifications of cellulose have been studied by this method (Marrinan and Mann, 1956A). The main conclusions as they affect the present discussions, are;

(a) Two types of spectra were obtained from cellulose I. Bacterial cellulose and <u>valonia ventricosa</u> gave a type A spectrum, while cotton, ramie, linen, etc., gave a type B spectrum.

(b) All samples of cellulose II studies showed similar spectra.

The results for the untreated sample of Nitella are shown in Fig. 19. ~~A and B.~~ After a vapour deuteration the material gives a Cellulose I, Type B spectrum. The prominent band at 3242 cm^{-1}, typical of a Type A spectrum is absent. There is no evidence of any cellulose II. Dr. Mann has added a note that the amount of material deuterated indicates a high proportion of amorphous material (rough estimate 70%). This is consistant with qualitative X-ray evidence.

The identity of the skeletal substances of the wall of Nitella opaca is thus confirmed.

One further point might be made before leaving this topic. Marrinan and Mann (1956A) consider that the differences between the two types of spectra imply a difference in the molecular packing in the crystals of the two types of material. They go on to say; "This could be due to a different crystal structure in the two cases or to a difference in degree of perfection of crystals having the same basic molecular arrangement". The work of Marrinan and Mann on the infra-red spectrum of cellulose, therefore, lends support to the view of Myers and Preston (1959) that the highly crystalline eucellulose, e.g., Valonia, yielding only glucose on hydrolysis, should be distinguished from the less highly crystalline and less rigorously defined celluloses. There is not enough evidence on which to base a conclusion, but one cannot help wondering whether a type A spectrum is typical of eucellulose and type B of the other celluloses.

SWELLING

Anisotropy of swelling has been used in the past to indicate anisotropy of structure of botanical material (Schwendener, 1891); and, more recently attempts have been made to relate anisotropy of swelling to orientation in man-made fibres, e.g. see Hermans, 1949.

In the present investigation measurements of the degree of swelling in the radial, transverse and longitudinal directions in the cell wall of Nitella, have yielded very valuable information on the structure of the wall.

The increase in thickness of the wall (radial direction) due to wetting has been measured in two ways. In the first method a Philips displacement transducer (GM 5537) was used as a thickness gauge. In this instrument linear displacement of a lightly loaded measuring stylus is converted into an electrical signal, and the magnitude of the displacement is indicated directly on the output meter of a direct reading measuring bridge (GM 5536). The following ranges can be switch selected; 0 to $\pm$ 1 mm., 0 to $\pm$ 300 microns, 0 to $\pm$ 100 microns, 0 to $\pm$ 30 microns, 0 to $\pm$ 10 microns, and 0 to $\pm$ 3 microns. There is provision for resetting the meter to "zero" over a wide range of displacement.

For this application the transducer was rigidly held vertically above the surface of a good precision ground and lapped surface plate. A cell, from which the contents had been removed, was opened out flat and dried down on to a glass plate which was rigidly fixed to a precision Ground Vee block. The Vee block could be moved about over the surface

of the surface plate in such a way that the stylus rested either on the glass reference surface or on the specimen. The difference in the readings obtained, in these two positions, represented the dry thickness of the specimen. In practice, a reading was taken in the middle of the specimen width and on the glass reference surface on either side of the specimen, these two latter readings being meaned to give a "zero" reading. The procedure was repeated at several points along the length of the specimen.

To measure increase in thickness due to wetting, the transducer was set up with the stylus resting on the dry specimen and the meter was set to zero. A drop of water was placed on the specimen and, after swelling had ceased, the bridge output meter indicated the increase in thickness directly.

The results obtained from a number of specimens are recorded in table. 4.

TABLE 4.

Dry Thickness	Increase due to Wetting	Increase in Thickness
1.9 microns	1.8 microns	95%
3.2 microns	2.0 microns	63%
4.3 microns	4.6 microns	107%
3.6 microns	2.0 microns	56%
4.4 microns	4.0 microns	91%
4.2 microns	3.5 microns	83%
3.2 microns	4.2 microns	130%
3.5 microns	4.1 microns	118%
	Mean	93%

Extreme accuracy can not be claimed for the "percentage increase in thickness" figures, as in order to obtain an accuracy of only ± 10%, the thickness measurements would need to be accurate to better than ± 0.00,01 inch (to use the English system of measurement). Measurements to this sort of accuracy normally require the facilities of a metrology laboratory, so that, bearing in mind the limitations of the equipment and laboratory conditions, the results are surprisingly good and certainly can be taken to indicate the correct order of the increase in thickness.

The dry and wet thickness of a strip of wall was also measured directly by an optical method. This measurement was much more difficult and inconvenient and served only as a check on the transducer measurements. A piece of wall was dried down across a narrow gap cut in the edge of a thin piece of perspex sheet. It could then be viewed edge on under a microscope and its thickness measured with an eyepiece micrometer before and after wetting. The increase in thickness was again of the order of 100%.

The percentage increase in length in the longitudinal and transverse directions was also measured optically using the microscope and eyepiece micrometer. In this case the dimensional changes on wetting were very small. In the transverse direction the increase in length was of the order of 4%, and, in the longitudinal direction, 6%.

The large percentage increase in the thickness of the wall on wetting, suggests that there is little reinforcement of the wall in the

radial direction, such as would occur, for instance, if the microfibrils were interwoven and entangled throughout the thickness of the wall. The observed behaviour of the wall is rather more consistant with the idea that the microfibrils are arranged in discrete layers with pectic substances (galacturonic acid is present in the water extract) providing an amorphous matrix between these microfibril-reinforced laminations, or, at very least, that the microfibrils lie strictly in the plane of the wall. That the first of these models is substantially correct is shown by the fact that when the wall is treated with hot 0.5% Ammonium Oxalate for 30 minutes to remove pectic substances, it can be separated into thin lamellae using a needle manipulator and fine forceps. In Fig. 20 a photograph is reproduced of a piece of wall which had been torn across; andhad failed in such a way that five separate wall layers are visible. The photograph was taken between crossed-nicols so that differences in optical thickness show up clearly and reveal the boundaries of the laminations. This is a new observation for Nitella and is in fact contrary to a conclusion reached by Green (1955) who makes the following statement (for Nitella axillaris Braun); "In cross section the wall appears non-lamellar when viewed in normal light and reveals a uniform ability to polarise light. Attempts to flake or tear the wall into thin lamellae were in vain. The striations are visible as linear regions of the wall which have different properties in polarized light from

the rest of the wall. These regions are the only apparent exceptions to the general conclusion that the wall is uniform in composition." He also publishes electron microscope sections without drawing any conclusion with regard to lamellation, although he does draw attention to the presence of a fibrillar structure, well oriented in the transverse plane.

Apart from its structural importance and its bearing on the elastic symmetry of the wall (to be discussed in Part III), the fact that the wall can now be separated into thin lamellae, opens up new possibilities for more precise structural examination in the electron microscope. This will be further discussed in a later section.

One further point needs to be made, however, and that is that the anisotropy of swelling in the longitudinal and transverse directions is not as marked as one might thus at first suppose it should be. It was pointed out in the section on X-ray analysis that the crystalline component is not highly oriented, so that there will be some reinforcement of the wall in all directions in the plane of the wall, and lateral expansion might be limited. This will be further discussed at the end of Part I when the structural evidence is complete.

ELECTRON-MICROSCOPIC EXAMINATION

The electron microscope has also been used in the determination of Nitella wall structure. At each stage of the chemical analysis a small sample of the remaining material was taken for electron microscopic examination. The principle reason for doing this was to discover whether the physical nature of the material had been changed by the chemical treatment. The material was fragmented in a blender using a Teflon pestle, and reduced to a fine suspension in water. A drop of a suitably diluted sample was dried down onto a formvar film supported on a metal grid. It was shadowed with Pd-Au.

Since the material is mechanically fragmented in the above process nothing can be said about the wall organisation, other than that a microfibrillar component and an amorphous component are present initially, and that, after chemical treatment, the remaining material is almost completely microfibrillar. There is no obvious indication of microfibrils being broken up as in Porphyra (Cronshaw, Myers & Preston, 1958).

A portion of wall as it appears after the alcohol extraction is shown in Fig. 21. Amorphous material can be seen binding the microfibrils. In Fig. 22 a fragment of material, after alkali treatment shows only microfibrils. It is known from the X-ray diagram, however, that a small amount of amorphous material is still present and that the crystalline form is now that of cellulose II.

It was mentioned in the section on swelling that it had not previously been found possible to strip the wall of Nitella into thin layers for examination in the electron microscope, as had been done in

the case of Valonia, Cladophora, etc. (Preston, 1952). However, after treatment of the wall with 0.5% Ammonium oxalate at 80°C for 30 minutes to remove pectic substances, it was found possible to 'strip' the wall, under water, using a needle manipulator and fine forceps. An electron micrograph of a thin piece of wall, so treated, is reproduced in Fig. 23. Microfibrils are clearly visible buried deep in an amorphous matrix, and this supports the suggestion, made earlier in this thesis, that the microfibrils are arranged in discrete layers with pectic substances providing an amorphous matrix between microfibril reinforced laminations.

Apart from the structural significance of these laminations, the ability to strip the wall opens up a new method of examining the detailed organisation of the microfibrils within the wall. Further chemical treatment is necessary, however, before the wall layers are sufficiently clear of amorphous material to enable a detailed study to be made. The following treatment, which is a slight modification of one used by Kreger (1957), has been found satisfactory. The wall is treated with hot 2% HCl for 30 minutes, washed, treated with hot 4% KOH and washed. This cycle is repeated three times. The wall is then given a chlorite treatment as described by Cronshaw, Myers & Preston (1958). After this treatment the wall is easily stripped, under water, and is free of amorphous material when examined in the electron microscope. If the chlorite treatment is omitted the wall is not perfectly 'clean', due, possibly, to protoplasmic debris and hemicellulones remaining. X-ray analysis confirmed that this treatment did not mercerize the cellulose.

An electron micrograph of a piece of wall, so treated, is reproduced in Fig. 24, at a magnification of 120,000. In spite of the high magnification, the microfibrils show up quite clearly and cleanly. One thing which is most noticeable is that in spite of the very high magnification, the microfibrils appear very small. In fig. 25 an electron micrograph of Valonia microfibrils is reproduced for comparison. The comparison is most dramatic. The Valonia microfibrils are very much larger and have a more obviously crystalline appearance. The microfibrils of Nitella are very small and have a rather less 'rigid' appearance. It is very hard to judge the size of the smallest microfibrils of Nitella from such a photograph, but it can be said that they are of the order of 50 Å wide. Valonia microfibrils are of the order of 200 Å wide by 100 Å thick (Preston and Cronshaw, 1958). Cladophora rupestris has microfibrils 300 - 250 Å wide and the alga with the smallest microfibrils in the group of algae listed by Cronshaw, Myers & Preston (1958), was Ulva lactuca (180-90 Å). Microfibrils 50-100 Å wide have been recorded for conifer tracheids by Hodge & Wardrop (1950). Ranby (1952) lists widths of 73-80 Å, 87-90 Å and 108-116 Å for wood cellulose, cotton cellulose, and tunicin respectively. Nitella microfibrils appear, therefore, to be on the lower limits of microfibril size. Small size of the crystallites was one of the reasons advanced for the diffuse nature of the X-ray diagram of Nitella, and this electron microscope evidence is qualitative confirmation of this suggestion.

Examination of 'stripped' material also enables some estimate of the fibril orientation to be made. The mean direction of the microfibrils

with respect to the longitudinal axis cannot be derived with any very great accuracy by this means (within $\pm$ 20°, say), as it has not always been possible to pick up the 'stripped' piece of wall on the grid so that it accurately oriented with respect to the grid slot (Philips grids were used in all this work). It is possible, however, to make a reasonably reliable estimate of the distribution of microfibrils about the mean fibril direction - subject to the proviso that the act of stripping has not caused misalignment at the interface as the strip was pulled away.

Again, since electron micrographs with magnifications (on the print) of 20,000 and 40,000 times, only cover a wall area of about 8.5 microns X 7 microns, and 4.25 microns by 3.5 microns, respectively, a very large number of examples must be looked at to ensure that one has a truly representative 'picture' of the wall. Although, from this point of view, it has not been possible to examine as many micrographs as one might wish, some very interesting wall features have been observed, which are given more as examples of the power of the method than as the results of a completed investigation. Most of the work has been carried out on fairly mature cells (length 30 mm.) but some fairly young cells (length about 10 mm.) have also been examined.

An example of the appearance of an inner layer stripped from a young cell is shown in Fig. 26. The approximate direction of the longitudinal axis is shown. It is apparent that the bulk of the microfibrils are lying at right angles to the longitudinal axis (i.e. there is a transverse orientation which agrees with the X-ray and

polarising microscope evidence).

There are, however , a fairly large number of microfibrils lying roughly at right angles to the main direction. It is almost as if this is a very imperfect example of a crossed-fibrillar structure (cf., Valonia, etc.). The angular frequency distribution of the microfibrils has been obtained in the following way. The central area of the micrograph was enlarged (two-fold with respect to the print shown), and the enlarged print was pricked with a pin all over at random. The direction of the microfibril nearest the pin point was measured and a histogram constructed showing the number of microfibrils having directions within the angular ranges, 0-10°, 10-20°, 20-30°, etc., relative to some fixed direction. The angular frequency distribution obtained in this way is shown on the diagram traced on transparent tissue overlying the photograph. It is evident that there are two main directions.

It is not suggested that the amplitude of the frequency distribution can be taken to represent the relative number of microfibrils lying in each of these two main directions. The method used will tend to cause a bias in favour of the top layer of fibrils. In this case the bias will be in favour of the 'longitudinal' fibrils which appear, in general, to be overlying the 'transverse' fibrils.

The possibility has been considered, that the 'longitudinal' microfibrils are artifacts due to fibril displacement in the stripping process. This is unlikely for two reasons. Firstly, if one looks

closely there appear to be fibrils with 'longitudinal' orientation lying deep within the 'mat' with 'transverse' fibrils overlying them. Secondly, Green (1958A) has published replicas of the wall which show microfibrils considerably displaced from the transverse direction - particularly in his plate 247, Fig. 6. His replica technique was not likely to cause displacement of the microfibrils from the transverse direction, so that it must be concluded that, in some circumstances, the microfibrils can assume this peculiar orientation. He notes that this particular micrograph is of an internode approaching the end of its elongation. He also states that, in young internodes, the microfibrils are nearly parallel and that the angular distribution about this mean direction increases as the cell becomes more mature. This is not an example of multinet growth as all of Green's observations are based on surface replicas of the inside wall of the cell.

Another electron micrograph is shown in Fig. 27, also with its superimposed frequency distribution. Here again there is a suggestion of a cross-fibrillar pattern, but a χ^2 test shows that the black curve is not significantly different from the green curve, which runs through the mean of all the points ignoring the fluctuation (curve fitted by eye).

Fig. 28 which is from an unknown location in the wall, also shows evidence of a crossed-fibrillar pattern, but with considerable disorientation about both directions.

This evidence of a crossed-fibrillar structure does not agree with the X-ray evidence which, although it indicates that transverse component is not well oriented, does not show the characteristic 'crossed-fibrillar'

diffraction diagram. Further, the photometric trace around the 002 arc (Fig. 16) does not indicate the presence of a large proportion of longitudinal fibrils although there are some fibrils lying in this direction. The evidence on the point is, therefore, rather confusing, but it seems likely that, if the crossed pattern does indeed exist, the 'longitudinal' series occurs very much less frequently than the transverse - perhaps as an 'error'; cf., the 'third' orientation of Valonia, etc. Again, Green's evidence is that large departures of microfibrils from the mean transverse direction occur only in internodes approaching the end of the elongation. Additional evidence that mature internodes have a rather peculiar wall structure, will be presented later in this section. This evidence of a rather poor crossed-fibrillar structure is, however, very interesting indeed.

In Fig. 29 an electron micrograph is reproduced of the extreme outside layer of an immature cell. This layer was notable in that, after the wall had been prepared for stripping, there was very little evidence of bonding between it and the rest of the wall, and it was separated with very little manipulation indeed. In this case the microfibrils are aligned longitudinally and this was the case for all grids examined on which this particular layer was mounted. This agrees very well with what one would expect on the basis of the multinet theory, although of course it does not constitute a proof. Other outer layers showed this tendency to longitudinal orientation (see Fig. 30), but this particular layer provided the most notable example.

The region of the wall in the neighbourhood of the 'striations' was shown to have different optical properties in polarized light to the rest of the wall (Figs. 7, 8 & 20). It is of some considerable interest, therefore, to see if this region of the wall can be recognised in the electron microscope, and, if it can be recognised, to see if additional evidence can be obtained on the nature of the discontinuity which occurs there.

Fairly mature cells (i.e. length > 30 mm.) were chosen because optical studies suggested that the striation might be more easily recognised in these cells. The protoplasm was removed as previously described and each cell was cut into short lengths (about 2 mm. long). These were chemically treated for stripping and each cell was then cut and opened out to form a flat sheet. In the first instance, a thin layer of wall was removed from the inside surface of the cell, and picked up on a Philips grid by bringing the grid up below it as it floated in the water. The Philips grid is in the form of a single slot (approximately 1 mm. X 0.25 mm.). A great deal of care was taken to ensure that, when the specimen was picked up in this way, it was aligned so that the length of the cell was, as nearly as possible, at right-angles to the grid slot, and centrally placed on it. Since the circumference of the cell and the length of the slot were very nearly equal, at least one (possibly both) of the striations should cross the slot. It was not possible to align the grid and specimen length exactly at right-angles, but with care the departure was not more than $\pm$ 15° from the desired position.

It was found that, in general, each striation showed up as two electron dense bands close together and easily recognisable (see for example, Fig. 31a and the folded photograph in the back pocket). Identification of these bands with the striation region was based on the following evidence;

(a) The bands were always at right angles to the grid slot within the limits given (for cells oriented transversely to the grid slot).

(b) Where two pairs of such bands were visible they were parallel to each other and separated by a distance of the same order as half of the circumference.

(c) On the one occasion when grid slot and longitudinal cell axis were parallel, a pair of electron dense bands, present by chance, ran the whole length of the specimen.

(d) The bands did not have the appearance of folds which are easily distinguished.

(e) The width of the electron dense bands agreed very well with the width of the high birefringence areas at the centre of the striation which were visible in the polarizing microscope.

(f) The appearance of 'disturbed' areas on the wall of mature cells will be described later. In those cases where these were present in the wall they were not present in this 'striation' region.

There are, therefore good reasons for identifying these electron dense bands with the region of the wall in the neighbourhood of the striation, and for these bands being regions of special structure.

The difficulty has been not so much in identifying this electron-dense region with the striation region, but in deciding in what way this region is different from the rest of the wall (other than being electron dense). That these regions are generally electron dense is not in doubt. Electron dense regions have been observed on far more grids than have actually been photographed. (More often than not, stripped layers are too thick, so that, although these regions can be observed, they cannot be photographed because of tearing of the specimen in the beam).

The best example of a striation region is provided by Fig. 31a. It is an electron micrograph of the inner surface of a cell whose length was greater than 30 mm. The folded composite photograph, Fig. 31b, is of the same region taken at a higher microscope magnification. Here the two electron dense bands are clearly defined and separated. On either side of the striation region there is a relatively 'undisturbed' region of the wall, and the remainder of the wall has a very 'disturbed' appearance (Note: a very much larger area was examined than appears in this photograph).

In the electron dense bands there appear to be a number of fibrils running parallel to the striation, but this does not appear to be typical of the whole striation length. In the relatively undisturbed region on either side of the 'striation' area, and some distance from it, the topmost fibrils seem to be roughly at right angles to the striation, but the area is too limited to draw any very definite conclusions.

In Figs. 32, 33 & 34 electron micrographs are reproduced from

another grid, showing both 'striation' regions and a region of wall between the two striations for a cell about 10 mm. long. The electron dense regions Figs. 32 & 33, are not clearly visible on the prints, but they were easily distinguishable from the rest of the wall in the electron microscope. In both cases, although the underlying fibrils are greatly disoriented, approximately transverse fibrils can be seen crossing over the striation region. The region of wall between the striations (Fig. 34) shows what might be a 'disturbed' wall in its early stages. Again there is the apparently 'crossed striation' arrangement of fibrils, with 'transverse' microfibrils overlying 'longitudinal' microfibrils.

An electron micrograph of an outside layer, from a cell of length greater than 30 mm., is reproduced in Fig. 35. This seems to indicate that this disturbed area extends right through the wall (this is the only case of its kind to occur in all of the grids examined). Here again the striation region (just off the picture) is 'undisturbed'. A high power electron micrograph of one of these local areas is reproduced in Fig. 36.

The situation might be summed up by saying that it has not been possible to draw any very definite conclusion about the nature of the discontinuity in the region of the striation, other than that there is very strong evidence that a discontinuity of wall structure does exist there. Provided that the wall is not thinner in the striation region (and there is no reason to think that it is) then the low birefringence at the edges of the striation could be due to a greater disorder of molecular chains in the plane of the wall. If one is correct in identifying the electron

dense region with the centre of the striation, then this greater density of packing might account for the higher birefringence at the centre, relative to the rest of the striation.

With regard to the 'disturbed' areas, Green (1958A) makes the following comment based on his study of the replicas of non-elongating cells*; "Examination of the inner surface shows a clear change in microfibrillar pattern. The fibrils are present in fields of well ordered microfibrils, ... The direction of fibrils of a given field may bear any relation to the cell axis. The fields may partially overlap one another through a gradual curving of fibrils. The superposition of fields of microfibrils of different orientation thus leads to the deposition of an essentially isotropic wall after elongation. The overlapping fields may be the basis for a faint cross-striation pattern noticed in Nitella walls in 1893 by Correns ..... Various unusual structures were observed in these non-elongating walls. Circular microfibrillar patterns .....were found as well as regions of radiating microfibrils. These latter were found on very old walls ..... and not on all of these ...."

The present electron microscope investigation has not been as carefully related to cell age as was Green's investigation - mainly because the technique of stripping was discovered too late in the investigation for it to be fully exploited. The most that can be said is that the results, in so far as they relate to the change in wall

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* It is not possible to say at what rate the cells in this investigation were elongating - merely that the 'stripped' layers were from cells of the length stated.

structure with advancing age, are generally in harmony with Green's conclusions.

The technique of ultra-thin sectioning was used to examine the wall in transverse section. Short cylindrical lengths of cell wall were fixed in buffered osmic acid (pH, 6.91), washed in dilute buffer solution, taken up through the alcohols, and finally into a mixture of butyl and methyl methacrylate in the ratio of 9:1, containing 2% Luperco. The short cylinders were oriented, at the bottom of the geletin capsules, by standing the cylinders on their ends. Polymerization was carried out at 48°C.

Sections were cut on a Philips microtome using Pyrex glass knives. An electron micrograph of a transverse section of wall is shown in Fig. 37 and a portion of such a section in Fig. 38. Three micrographs of a similar section, which had been gold shadowed after removal of the methacrylate, are shown in Figs. 39, 40 & 41.

It is difficult to deduce lamination from such a compact mass of shadowed material, although the general appearance of the wall is consistant with transversely oriented fibrils. In the unshadowed sections there is evidence of lamination, particularly in Fig. 38 where the layers are, for some reason, further apart.

DEPOSITION OF WALL MATERIAL DURING GROWTH.

At a later stage of this thesis, the mechanism of extension growth will be discussed in terms of the mechanical properties of the wall. In their simplest form, mechanical theories of growth suggest that, to some degree, extension growth is brought about by a plastic stretching of the wall due to turgor pressure. That this does not completely describe the growth process is obvious from the fact that the wall thickness does not generally decrease at the rate to be expected if growth was a mere plastic extension at constant volume. In other words, deposition of new material is continuing during the process of extension. It is, therefore, relevant to later discussion to know something of this process in the case of Nitella.

The work of Green (1958,A) on the variation of the 'optical thickness' of the wall of Nitella during growth, has already been referred to in the introduction. Briefly, he used the technique of interference microscopy to estimate the optical thickness of the cell wall, and found that it reached a peak when the wall was small, then dropped suddenly as the cell attained its maximum relative rate of elongation, and then gradually increased again as the cell elongated to its final length. This final phase extended over the bulk of the elongation (5mm. to 60mm.).

Other workers have studied other materials. Overbeck (1934) found that during the rapid elongation of the setae of the moss Pellia epiphylla the length increased as much as 3100% over a period of a few days, whereas the corresponding increase in cell wall volume was only 500%.

Diehl, Gorter, van Iterson (Jr.) and Kleinhoonte (1939) found that, after the application of heteroauxin to Helianthus hypocotyls, the wall thickness at first decreased but, after this initial stage of extension, the wall either continued to enlarge without further decrease in thickness, or actually increased in thickness during elongation. Preston and Clark (1944) working with Avena coleoptiles found that the wall material for coleoptile increased steadily during elongation, but that wall material per unit length decreased continuously over the growth phase. The rate of deposition of new material was, therefore, not keeping pace with the increase in area. It seems, therefore, that, while a decrease in wall thickness often accompanies a rapid increase in elongation, there can equally well be an increase in wall thickness during the process of elongation.

During the present investigation measurements have been made which support Green's conclusion that there is an increase of wall material per unit area over the length range 5mm. to 60mm. (approx.). The measurements were actually made to provide information which could be used in the reduction of the mechanical test results, and, therefore, do not cover as complete a range of cell development as that covered by Green's work. They are, however, interesting as a confirmation of his observation that wall deposition can increase at a greater rate than elongation.

The mass per unit area was determined directly by weighing a section of dry wall (dried over calcium chloride) whose area had been

measured previously. The results are set out in Table 5.

It will be seen from the table that, if one limits oneself to consideration of the same plant, and plant axis, then the weight per unit area increases as area or length of the internode increases.

Discussion

The reasons forreaching this or that conclusion on the nature of any particular structural feature have been given in the appropriate section, In this section the evidence obtained by each separate technique will be examined in relation to the evidence provided by the other techniques.

(1) X-ray diffraction, chemical analysis, and infra-red analysis, have all been used to confirm that cellulose is present in the wall of Nitella. The chemical analysis indicated that the α-cellulose component, comprising about 17% of the dry weight of the wall, contained both xylose and manose, and from this it was concluded that the α-cellulose component was not eucellulose. Confirmation of this view was providedby X-ray evidence that treatment with 4N-KOH, mercerized the cellulose, and that there were minor differences between the eucellulose X-ray diagram and the Nitella diagram before treatment. Infra-red measurements and X-ray measurements indicated that before chemical treatment, the wall contained cellulose I. Infra-red evidence that

TABLE 5.

Plant	Cell	Plant axis	Diameter mm.	Length mm.	Area $_2$ mm	Weight/unit area gms/mm^2
26	A	1	0.293	3.3	3.1	1.19
"	B	1	0.421	20.3	26.1	1.41
"	C	1	0.498	41.2	64.4	1.65
"	D	1	0.486	45.5	69.4	2.20
"	E	2	0.289	3.7	3.4	1.26
"	F	2	0.404	19.2	24.4	1.24
"	G	2	0.453	44.1	62.8	1.72
14	A	1	0.381	9.7	11.6	2.40
"	B	1	0.482	14.2	62.3	3.24
"	C	1	0.442	50.5	112.0	3.47
"	D	2	-	14.2	-	2.42
"	E	2	0.396	42.0	52.3	2.75
"	F	3	0.364	26.0	29.7	2.22
16	A	1	0.388	15.0	18.3	2.17
"	B	1	0.469	45.5	67.0	2.44
22	A	1	0.292	6.0	5.5	2.03
"	B	1	0.395	30.5	37.8	2.11
21	A	1	0.350	11.75	12.9	1.92
"	B	1	0.447	36.5	52.2	2.11

Note: (a) Cells from the same plant have the same number, e.g. 26.

(b) Individual internodes are lettered A, B, C, ———. etc.

(c) Not all Cells from the same plant were from the same plant axis. The axis has, therefore, been distinguished by the number 1, 2, or 3.

this was of the type B variety supported the view that it was not eucellulose. Infra-red analysis, X-ray analysis and chemical analysis all indicated a high proportion of amorphous material. Electron micrographs of untreated material also showed amorphous material.

(2) The suggestion that a possible cause of the diffuse X-ray diagrams was due to the presence of small crystallites, was supported by the fact that the microfibrils revealed in the electron microscope were of the order of 50 Å wide.

(3) Swelling measurements indicated that the wall might be laminated and this was confirmed by devising chemical treatments which allowed the wall to be stripped into discrete layers. That pectic substances might be contributing to the binding of these layers was suggested by the high degree of radial swelling, the presence of Uronic acid in the water extract, and the fact that treatment with Ammonium oxalate prepared the wall for stripping. Electron micrographs of thin sections of wall, while not revealing obvious lamination, were quite consistent with this form of structure.

(4) The rather poor orientation revealed in the X-ray diagrams, and the electron micrographs of stripped walls, were consistent with the low birefringence of the wall so measured in the polarizing microscope.

(5) Preferential removal of amorphous material from the wall by chemical treatment was indicated by the X-ray diagrams and the electron micrographs.

(6) Inclination of the map in young walls was qualitatively

confirmed by X-ray analysis, and by mechanical evidence to be presented later.

(7) There is some evidence from electron micrographs of thin layers stripped from cell walls that under some circumstances microfibrils can be arranged in what might best be described as a rather poor crossed-fibrillar arrangement. This evidence needs to be confirmed; but it is supported by one of Green's (1958A) electron micrographs of the inner wall of an internode approaching the end of its elongation, which shows a similar arrangement to that observed in this investigation. Green used a replica method so that in his case the question of artifacts caused by the act of stripping do not arise.

The presence of a crossed-fibrillar structure is not confirmed by the X-ray measurements although there is evidence of fibrils in directions far removed from the transverse including the longitudinal direction. It seems, therefore, that this 'second' orientation if it is a genuine wall feature, is not one which occurs at all frequently; but is more in the nature of an orientation which 'may' occur (cf., Valonia). It is going well beyond what is justified on the basis of available evidence, but, if one might venture a speculation, demonstration of a 'second' orientation would mean, perhaps, that the difference between the wall structure of Nitella, and the wall structure of Valonia, Cladophora, etc., might be one of perfection rather than one of structural principle.

(8) It has been established that there is a correlation between direction of protoplasmic streaming and the direction of the m.e.p. It was pointed out that Denham (1923) observed that the directions of protoplasmic streaming and of wall striations were often identical, and on this basis he suggested mechanisms whereby streaming might cause orientation in cell walls. Martens (1932) pointed out, however, that the striations concerned lay deep within the wall and not on the inner face against the cytoplasm and the idea was abandoned. More recently Necesany (1956) has suggested that orientation of microfibrils in the secondary walls of fibre cells in the secondary xylem depends on speed of protoplasmic streaming. The mechanism is obscure.

The correlation observed here is not quite of the type suggested by Denham, since streaming direction and the m.e.p. tend to lie at right angles to each other. Again it is not of the same form as the correlation between cell dimensions and angle of the m.e.p., which has been shown to exist for the secondary wall of some other types of plant cell, namely, conifer tracheids, bamboo fibres, and sisal fibres (Preston, 1952). In the latter cases, the longer the cell, the smaller the angle of the m.e.p. to the longitudinal direction. In Nitella (a growing wall) the m.e.p. is nearly transverse in long cells.

Since the chloroplasts are stationary it does not seem likely that the cytoplasm near the wall is in movement. It could be, however,

that the proteins of this surface layer are oriented by the streaming of the inner regions of the cytoplasm in which movement does occur, and these in turn are responsible for wall orientation. The present observations offer no evidence on this point. Myers and Preston (1956) have suggested that the site of wall formation is on the cytoplasm side of the wall / cytoplasm interface. The suggestion that cytoplasm and wall interpenetrate is supported by the observation of microfibrils twisted around each other, and, in the case of Nitella, by the electrical behaviour of the surface (Bennett and Rideal, 1954). That the mechanism responsible for orientation resides not in the wall but in the cytoplasm is not a new idea. A possible mechanism was suggested by Preston in 1941.

If protoplasmic streaming and wall deposition are linked, one might expect that because of the discontinuity of flow which occurs there, the region of the wall within the area of this striation would be structurally different from the rest of the wall. The evidence of the polarizing microscope and the electron microscope is that such a discontinuity does exist. It is also worth remembering, that the flow boundary is not the only special feature of this region - there are no chloroplasts within it.

PART III

MECHANICAL PROPERTIES OF THE CELL WALL

INTRODUCTION

As a result of the work on cell wall structure of Nitella reported in Part II, it is possible to make some general observations about the nature of the material which is to be submitted to mechanical test. It is proposed, therefore, to begin by considering some of the more significant structural features, from the point of view of their probable influence on the mechanical properties of the wall. Having done this, it should then be possible to decide on the sort of measurements which are most appropriate, and what are the likely limitations of such measurements.

The relevant structural features of the wall might be summarized as follows:

(a) There is a crystalline fraction which is present in the form of long microfibrils. The α-cellulose fraction (17%), which is almost entirely crystalline, consists of cellulose I, type B. It is not known whether the hemicelluloses are also present in the form of microfibrils, but it is known that at least a fraction of the hemicelluloses are in the amorphous state.

(b) The microfibrils are imbedded in an amorphous matrix consisting mainly of pectic substances, hemicelluloses, and (probably) protein material.

(c) There is a net preferred orientation of the crystalline component, the mean direction of which, referred to the cell axis,

varies with the age of the cell. In mature walls the mean orientation is almost transverse to the cell axis; but, in very young cells, it is inclined at about 10° to the transverse direction.

(d) The microfibrils are arranged in descrete layers with pectic substances, and, probably, other non-crystalline materials providing an amorphous matrix between successive microfibrillar - reinforced laminations.

The influence of this type of structure on the number of coefficients necessary to describe completely the elastic behaviour of the wall will be considered first. In order to do this, it is necessary to make some simplifying assumptions. These are set out below:

(1) The material is homogeneous; i.e., it has identical physical properties at all locations (in a given direction).

(2) The material is perfectly elastic.

(3) Each of the components of stress at every point is a linear function of the corresponding component of strain at that point.

(4) The strain is infinitesimal.

(5) The material possesses three mutually perpendicular axes of elastic symmetry; i.e., it has the same elastic symmetry as an orthorhombic crystal.

The last assumption, (5), requires some elaboration. The fact that the crystalline component has a preferred orientation will probably mean that the elastic properties in the directions of the major and minor extinction positions will be different (To anticipate results to be presented later, it will be shown that this is indeed the case).

Further, it has shown that the wall is laminated and that, on wetting, there is a very much larger dimensional change in the radial direction, than in either of the other two principal directions. It is, therefore, to be expected that the wall will have different elastic properties in the radial direction to those it possesses in the longitudinal and transverse directions. For these reasons orthorhombic elastic symmetry has been assumed, i.e., three mutually perpendicular axes of elastic symmetry.

The most general expression for Hooke's Law relating strains in an elastic solid to stresses is;

$$x_x = S_{11}X_x + S_{21}Y_y + S_{31}Z_z + S_{41}Y_z + S_{51}Z_x + S_{61}X_y$$

$$y_y = S_{12}X_x + S_{22}Y_y + S_{32}Z_z + S_{42}Y_z + S_{52}Z_x + S_{62}X_y$$

$$z_z = S_{13}X_x + S_{23}Y_y + S_{33}Z_z + S_{43}Y_z + S_{53}Z_x + S_{63}X_y \quad \ldots\ldots\ldots\ldots (1).$$

$$Y_z = S_{14}X_x + S_{24}Y_y + S_{34}Z_z + S_{44}Y_z + S_{54}Z_x + S_{64}X_y$$

$$z_x = S_{15}X_x + S_{25}Y_y + S_{35}Z_z + S_{45}Y_z + S_{55}Z_x + S_{65}X_y$$

$$x_y = S_{16}X_x + S_{26}Y_y + S_{36}Z_z + S_{46}Y_z + S_{56}Z_x + S_{66}X_y$$

In these equations x, y, z are three perpendicular co-ordinate axes; x_x, y_y, and z_z are extensional strains; y_z, z_x, and x_y are shear strains; X_x, Y_y, Z_z are extensional stresses; Y_z, Z_x, and X_y are shear stresses; the coefficients S_{ik} where i and k may take any values from 1 to 6 are constants of the material. The general properties of S_{ik} may be summed up as follows:-

(a) $S_{ik} = S_{ki}$ ..... (2).

(b) If i = k = 1, 2, or 3; S_{ik} relates an extensional

strain to an extensional stress in the same direction and

$$S_{ik} = 1 / E_k$$

where E_k is Youngs modulus for the direction k.

(c) If i = 1, 2, or 3, k = 1, 2, or 3 ($i \neq k$); S_{ik} relates an extensional strain in one direction to an extensional stress in a perpendicular direction and

$$S_{ik} = -\sigma_{ik} / E_i = -\sigma_{ki} / E_k \quad \text{(by equation, 2).}$$

where σ_{ik} = Poisson's Ratio = $\frac{\text{contraction in k direction}}{\text{extension in i direction}}$ for a tensile stress in i direction.

(d) If i = k = 4, 5, or 6; S_{ik} relates a shearing strain to a shearing stress in the same plane and

$$S_{ik} = 1 / G$$

where G is a rigidity modulus.

If i = k = 4, $S_{ik} = 1 / G_{yz}$

i = k = 5, $S_{ik} = 1 / G_{zx}$

i = k = 6, $S_{ik} = 1 / G_{xy}$

where the suffixes to the G's specify the direction of shear stress.

(e) If i = 4, 5, or 6, k = 1, 2, or 3; S_{ik} relates an extensional strain to a shear stress and visa versa.

(f) If i = 4, 5, or 6, k = 4, 5, or 6 ($i \neq k$); S_{ik} relates a shear strain in one plane to shear stress in a perpendicular plane.

Because $S_{ik} = S_{ki}$ the number of independent coefficients is reduced from 36 to 21. Further, if the material is symmetrical in structure the number of independent coefficients is reduced still

further.

It has been assumed that the symmetry of the Nitella wall appraximates to that of an orthorhombic crystal. The number of independent non zero coefficients is, therefore, reduced to nine. They are;

$$S_{11},\ S_{21},\ S_{31},\ S_{22},\ S_{32},\ S_{33},\ S_{55},\ S_{66}$$

If the longitudinal (L) direction (parallel to axis of cell) is identified with the x axis, the transverse (T) direction with the y axis, and the radial (R) direction with the z axis, then;

$$S_{11} = \frac{1}{E_L} \quad ; \quad S_{21} = -\frac{\sigma_{TL}}{E^T} = -\frac{\sigma_{LT}}{E_L} \quad ; \quad S_{44} = \frac{1}{G_{RT}}$$

$$S_{22} = \frac{1}{E_T} \quad ; \quad S_{32} = -\frac{\sigma_{RT}}{E_R} = -\frac{\sigma_{TR}}{E_T} \quad ; \quad S_{55} = \frac{1}{G_{RL}} \qquad \ldots\ (3).$$

$$S_{33} = \frac{1}{E_R} \quad ; \quad S_{31} = -\frac{\sigma_{RL}}{E_R} = -\frac{\sigma_{LR}}{E_L} \quad ; \quad S_{66} = \frac{1}{G_{LT}}$$

where, for example;

E_L = Youngs modulus in L direction

$RT = \frac{\text{contraction in T direction}}{\text{extension in R direction}}$ for tension in R direction

G_{RT} = rigidity moldulus for sheer stresses in R and T directions.

This presentationis quite standard and is similar to that given by Hearmon (1948).

The above treatment applies to the special case in which the axis of the reference directions relate to the symmetry of the material

It has been shown that, in general, the m.e.p. is not quite transverse in Nitella and the effect of a rotation from the principal axes on the elastic coefficients S_{ik} should be considered. For the purposes of the present discussion, however, it will be assumed that the m.e.p. is strictly transverse. The effect of a rotation from the principal axes will be considered in the section on spiral growth.

This is not the first time that orthorhombic elastic symmetry has been assumed for a material of biological origin. For example; the theory of wood elasticity is based on the assumption that the bulk material possesses three mutually perpendicular axes of elastic symmetry (Savant, 1830; Price, 1929; Hönig, 1931; Hearmon, 1948; etc.). In this case, however, origin of the elastic anisotropy is rather different. Price (1929), in a theoretical paper, has examined the behaviour of a model composed of equal isotropic cylindrical cells, and after due allowance is made for medullary rays, he found that such a model could satisfactorily account for the elastic behaviour of wood. Barkas (1941) extended Price's work to take into account the difference in cell wall properties between the longitudinal direction and the transverse direction; but it is still true to say that the elastic behaviour of wood can largely be explained in terms of its histological structure without making any special assumptions about the nature of the wall material.

Again, Makinson (1954) has determined the elastic constants of keratinous solids (ram's horn, rhinoceros horn, baleen, and porcupine

quill) and found that the type of elastic symmetry was transverse isotropy about a morphological axis, i.e., symmetry such that all directions in any plane perpendicular to the morphological axis are elastically equivalent (5 independent, non-zero constants). Her main conclusion was that in the region of small strains, the elastic anisotropy depends as much on histological as on molecular structure.

So far as the author is aware this present case is the first time that orthorhombic elastic symmetry has been proposed for a plant cell wall. This suggestion is made on the basis of its submicroscopic structure.

The methods used to determine the elastic coefficients of anisotropic materials are similar, in principle, to those used for isotropic materials, but as the complexity of the symmetry system increases, so does the number of specimens required to determine all the principal coefficients. The measurement of the elastic coefficients of anisotropic materials, has been reviewed by Hearmon (1946). He points out that in the orthorhombic system at least six specimens are needed. To quote:- "These would most conveniently consist of three specimens cut with their lengths in the three perpendicular directions characterising the system and three with their lengths in the principal planes at an angle of 45^{o} with the two principal directions contained in the plane. Bending and torsion measurements on the first three specimens yield S_{11}, S_{22}, S_{33}, S_{44}, S_{55}, S_{66}, directly, while bending or torsion measurements on the remaining specimens enable S_{12}, S_{23}, S_{13} to be calculated from the appropriate equations ....."

Unfortunately, it is not possible to follow this procedure in the case of the Nitella wall. Firstly, since the wall is only about 8 microns thick (wet), it is not possible to cut an adequate test specimen with its length in the radial direction. For the same reason, it is not possible to cut two of the required 45° specimens. Again, the wet wall of Nitella is not a suitable subject for bending measurements and Youngs modulus must be determined by direct tension. It seems, therefore, that the elastic coefficients, which can be measured, are limited to S_{11}, S_{22}, S_{12} and S_{66}.

Fortunately, these latter coefficients, are the ones which are most likely to be of significance from the point of view of mechanical theories of extension growth. Although, from a purely academic point of view, the present series of measurements are incomplete, in that all of the elastic coefficients of the wall have not been determined, the coefficients which have been determined are sufficient to provide interesting and relevant information on the nature of the growing wall, and, indeed, go further than previous investigations in the determination of the elastic properties of a single wall.

Before leaving this topic, it is convenient to consider the limitations, imposed on testing, by the anisotropic nature of the material. In the general expression for Hooke's Law, elastic coefficients occur of the type, S_{ik}, where i = 1, 2, or 3, and k = 4, 5, or 6. These coefficients, called coupling coefficients, relate an extensional stress to a shear strain and vice versa, so that an anisotropic substance,

subjected to an extensional stress, undergoes both extensional and shear strain. In the case of material with orthorhombic symmetry, these coupling coefficients vanish if the principal axes of stress relate to the symmetry of the material.

In the present investigation, cells longer than 5 or 6mm. have been used, for which the m.e.p. departs from the transverse by not more than 4 or 5°. It has, therefore, been possible to cut test strips, for direct tension tests, parallel to the longitudinal and transverse axes of the cell without risk of serious error.

It has been pointed out by Hearmon (1946), that for an accurate measurement of rigidity modulus on anisotropic specimens of rectangular cross section, the restrictions on orientation are very stringent. Such measurements are only possible if (a) the specimen length lies in a fourfold or six-fold symmetry axis, or (b) if two of the specimen edges lie in symmetry axes. For this reason rectangular test pieces are usually avoided.

It is possible that too much attention could be fixed to the restrictions outlined above, since it is unlikely that a very high degree of accuracy can be obtained when using biological material which is generally not very uniform in its physical properties. It is, however, important to be aware of these restrictions if systematic errors are to be avoided.

Discussion on the anisotropy of the material was based on a

number of simplifying assumptions. The validity of these assumptions will now be examined.

The first assumption, viz., that the wall is homogeneous, is, of course, not born out by the structural determinations. For example, it is known that there is a crystalline component in the wall consisting of long crystalline microfibrils. A cellulose crystallite can be elongated only by stretching primary valence bonds and by opening valence angles. Meyer (1942) has estimated the modulus of elasticity of cellulose from the known strength of the C–C, and the C–O–C, linkages, and the known deformability of valence angles. The value of 12,000 kgm./ $mm.^2$ so obtained, is in good agreement with the highest value determined experimentally on well oriented native fibres. The microfibrils can, therefore, be considered as regions with high elastic modulii, not readily subject to flow. In the amorphous regions, however, the individual molecular chains are not so well oriented and a wide range of shapes and configurations are possible. Secondary valence bonds (intermolecular forces) will play a more important part in the cohesion of the amorphous material and the modulii of elasticity in these regions will be much lower than in the crystalline regions. Further, the possibility of rotation about primary valence bonds, allowing long chain molecules to uncurl and unlink, will probably mean that these regions will exhibit 'time' effects. In general, high polymers do exhibit time effects and it is to be expected that the wall of Nitella (which was growing until shortly before the test specimens used in this investigation were cut)

will be no exception. Because of different elastic properties in different localized areas of the wall, there will, therefore, be a greater or lesser inhomongeneity of local stress, even where the macroscopic stress is completely uniform.

The assumption of homogeneity is, therefore, only an approximation. In the present work, however, elastic constants will be calculated from the deformations as though the material is homogeneous. Indeed, the is no choice in the matter. There are precedents for this procedure. Wood is a non-homogeneous substance, but, in test methods, the bulk material is treated as though it were homogeneous. The same is true of crystalline high polymers, native fibres etc. The justification for this procedure is that it leads to useful results. The fact of the inhomogeneity of the material needs to be born in mind, however; particularly in theoretical studies of the mechanical behaviour of such materials.

Another of the assumptions which needs further consideration is the assumption that the material has orthorhombic elastic symmetry. In Part II, in the section on the Polarizing Microscope, it was assumed that the wall behaved optically as a uniaxial crystal. Since an orthorhombic crystal is optically biaxial there is a contradiction here. This contradiction is, however, more apparent than real. Orthorhombic elastic symmetry has been assumed on the basis of the morphological structure of the wall, i.e., on the basis of the preferred orientation

of the crystallites and radial lamination. Although radial lamination is likely to have a marked effect on the elastic properties (it certainly has a marked effect on anisotrophy of swelling) such lamination will give rise only to "form" birefringence, which is generally much smaller than the intrinsic birefringence (Frey-Wyssling, 1953). The assumption that the wall is optically uniaxial is, therefore, only an approximation, but because of the small magnitude of the "form" birefringence it is a quite reasonable one, and one which is normally made in cell wall optical studies (Preston, 1952; Frey-Wyssling, 1953).

Finally, it is necessary to consider the assumption that the material is perfectly elastic. It has already been mentioned that the nature of the amorphous component in the wall is such that the wall as a whole is likely to exhibit "time" effects. Here again the simplifying assumption is only an approximation. Whether it is appropriate to assume that the material is perfectly elastic, or not, will depend on the nature of the "time" behaviour and the time scale of the experiment.

From the point of view of its suitability for the precise determination of elastic properties, the Nitella cell wall is, therefore, a very poor material indeed. It is inhomogeneous and anisotropic; it probably exhibits time effects; and the size and shape of the test specimens are severly limited. A less promising set of attributes is difficult to imagine. In these circumstances, therefore, the aim is not so much to obtain an accurate determination of a set of physical constants,

as to obtain evidence as to the physical state of the wall in different circumstances.

Alfrey (1948) has attempted to set out the general requirements for tests on complex materials. In abbreviated form, they are:-

(1) Complex time behaviour in the material gives rise to the demand for simple time sequences in the mechanical test.

(2) Non-linearity of elastic or flow elements gives rise to the demand for spatial homogeneity of stress and strain in the sample under test.

(3) A material exhibiting thermomechanical effects demands a testing method in which the temperature is held constant.

(4) If a phase change accompanies deformation, it is advisable to augment the purely mechanical test with some independant means of following the phase change.

(5) Since no analysis of complex time behaviour has yet been couched in terms of definitions of the strain components which are applicable for large as well as small deformations, there exists no phenomenological framework into which can be fitted data involving both large strains and complex time effects. This mathematical combination may be realizable, but until it is made, there can be no unequivocable reduction to fundamental quantities of experimental data involving plastoelastic materials at large deformations. This does not mean that a fundamental mechanical test should not involve large deformations,

but it does mean that the mathematical complications connected with large deformations are unavoidable.

Since the wall of Nitella is a complex material, the test methods have, in general, been based on the principles laid down by Alfrey and stated above. Static tests have been used rather than dynamic tests because they are more likely to yield information, which can be made on wet material.

THE TENSILE TEST SPECIMEN

The length of a transverse test specimen which can be cut from a Nitella cell is limited by the diameter of the cell (say, 0.3 mm.). The circumference is, therefore, of the order of 0.9 mm. Since some allowance must be made for clamping, the 'Free' length of the test strip cannot be more than about 0.5 mm. (about 0.020", or, a little over 1/64"). It is desirable to have as large a length/width ratio as possible to reduce 'end' effects. The mean width in the present investigations was about 0.12 mm. (0.005") giving a length/width ratio of about four. This is lower than is desirable, but the difficulties of cutting and handling strips thinner than five thousandths of an inch, in effect determined the width (in point of fact, thinner strips could probably be used - but this is only after many months of practise with the present width). The thickness of the strips is about 4 microns (dry).

A more favourable length/width ratio could have been obtained for 'longitudinal' strips, but it was decided to standardize on one size to avoid systematic differences due to 'shape' effects.

Mechanical clamps for so small and delicate an object were not practicable with the available facilities, and the test pieces were held with a waterproof cement - "Ames" copper dental cement. The mounting procedure will be described later.

Before considering the test procedure in detail it is desirable to decide on the 'stress limits' of the proposed test. Plasmolysis

measurements indicate that the turgor pressure is of the order of 8 or 9 atmospheres in Nitella. It is, therefore, possible to make a rough calculation of the wall stess by assuming the cell to be a thin-walled cylinder under hydraulic pressure. If the wall thickness is small compared to the diameter of the cell (which it is), then;

Stess in the longitudinal direction; $\sigma_L = \frac{Pr}{2t}$

Stress in the transverse direction; $\sigma_T = \frac{Pr}{t}$

where P = pressure, r = radius, t = thickness.

Load on the wall per unit width of strip, $w = t\sigma$

$$w_T = Pr$$

$$w_L = Pr/2$$

If in a cell of diameter 0.3 mm., the internal pressure is 8.0 atmospheres (8.1×10^4 dynes/mm^2), then;

$w_T = 8.1 \times 10^4 \times 0.15 = 12.2 \times 10^3$ dynes/ mm.

$w_l = 8.1 \times 10^4 \times 0.15/2 = 6.1 \times 10^3$ dynes/ mm.

These figures indicate the order of magnitude of the stress which should be applied to simulate conditions in the plant cell wall.

EXPERIMENTAL METHODS

Two methods of mechanical testing have been used which will be referred to as the 'Constant Load Rate' method (C.L.R.) and the 'Dead Weight' method.

The C.L.R. method was developed by Spark, Darnborough, and Preston (1958) for testing single cells of Sisal. The principle of the testing machine may be understood by reference to Fig. 42. A long arm is fixed to a pivoted vertical shaft (A) and is free to rotate in the horizontal plane. A flat spiral (spiral is used in its correct sense) has its inner end fixed to the shaft and the outer end is driven, about (A) as axis, by a synchronous motor operating through a worm drive. As the free end of the spring is driven from its rest position, the torque on shaft (A) increases at a constant rate, and tends to cause a displacement of the arm. The test sample, which is mounted between a fixed pillar at (C) and the machine arm at point (B), prevents movement of the arm, and the constant increase in torque, therefore, results in the specimen being loaded at a constant rate.

As the test piece is strained the arm is displaced through a very small angle, and this movement is detected by using a Proximity Meter to indicate the change in capacity between a fixed condenser plate (D) and a plate (E) attached to the end of the arm. The signal from the proximity meter is fed to an Elliot Recorder, the pen displacement being proportional to the displacement of the arm. The recorder chart is driven by a synchronous motor so that, if the chart-drive motor and the spring-drive motor, are started simultaneously, the distance travelled by the chart is proportional to load. In this way it is possible to record a load/deflection curve directly.

'Rate of loading' and 'range' are determined by the motor speed

and the spring rate. The load scale is calibrated by balancing the arm against a known force (using calibrated light helical springs). The deflection scale was calibrated using the Philips displacement transducer and Direct-reading bridge used for the swelling measurements. The stylus rested against the back of the arm, in line with the test specimen, and the displacements corresponding to various recorder deflections were read directly off the bridge meter. During actual tests the transducer was racked back clear of the arm to avoid having to take into account the spring rate of the stylus.

Handling and mounting the tiny test specimens required considerable care. Spark, Darnborough and Preston (1958) mounted a single cell of sisal over a narrow slot in a small square piece of strong paper using dilute seccotine as adhesive. The paper was clamped to the arm and the fixed pillar. The paper was then cut with a razor blade, leaving only the fibre connecting the arm and fixed pillar. The arm was then set free under zero load and the test commenced.

The procedure, in the hands of the author, resulted in rather too many test pieces being broken during the "paper cutting" stage, and an alternative method of mounting was devised. It is illustrated in Fig. 43. The specimen mount consists of two pieces of stainless steel (A & B) which are held together by a circlip (C). The specimen (D) is cemented across one or other of three adjoining slots (three slots give a range of specimen length) using "Ames" copper dental cement. The whole assembly is then transferred to the testing machine and, with the machine arm

clamped, the mount is screwed to the arm at one end and the fixed pillar at the other using the lugs (E & F). The circlip is then removed, using circlip pliers; the machine arm is unclamped, and the test commenced.

The following details are worthy of mention;

(a) The copper cement tended to break free from the smooth metal surface. A small hole was therefore drilled behind each slot and filled with copper cement. The fresh cement adhered to the dry cement in the hole and no further trouble was experienced.

(b) The cement is made up by adding a powder to a liquid component. The speed of setting can be controlled by varying the ratio of liquid to powder. In practice a fairly "thin" mix was made up first and smeared over the mount up to the edge of the slot. A dry test piece was picked up using fine tweezers and placed across the gap. If the mix was 'thin' enough, the specimen was 'wetted' up to the edge of the slot. Another 'thicker' mix was made (cream-like consistancy) and the top of the specimen covered up to the edge of the slot. The cement was left for 15 minutes to set hard. The specimen was then wetted with a drop of water and after mounting in the extensometer as already described, the test was commenced.

(c) The mount was designed so that, with the circlip holding the two halves together, it could be used as a specimen holder for the X-ray microcamera, thus allowing specimens to be X-rayed before and after test. In fact, it turned out that Nitella had much too poor an

X-ray diagram for it to be used in this way but it could be so used for other cells.

Although the machine described above is extremely good for the job for which it was designed, it has some disadvantages when used for testing Nitella wall strips;

(a) If there is any 'slip' of the specimen in the cement during a test, it is not possible to distinguish between movement of the arm due to this cause, and movement due to strain. The "initial" length is in doubt (or, varies during the test) and the strain is wrongly registered. Successive tests on the same specimen showed that after the first stretch, there was no further slip; but a doubt remained as to whether there had been any slip during the first test. This doubt about the possibility of slip is particularly serious in creep testing, where the problem arises as to whether a continuing deflection is due to creep or to 'slip' of the material in the cement. (It has now been shown by another method that, although slip does occur sometimes, in general, errors due to this cause are small).

(b) Occasionally, when strips are being cut with a blade which is not quite sharp, the edge is damaged (or, is not quite 'clean'). Small tears are not always visible until after the load is applied. It is therefore, an advantage to be able to observe the test specimen during the test. This could not be done with the present C.L.R. machine, without embarking on a complete redesign.

(c) Finally, there is a more fundamental objection. The

testing principles suggested by Alfrey and quoted earlier, state that complex time behaviour in the material gives rise to the demand for simple time sequences in the mechanical test. Although the C.L.R. test is quite simple in principle, the fact that a rate of loading (or, a range of loading rates) must be fixed, is a departure from simplicity which should be avoided on an unknown material if possible.

This is not to say that the C.L.R. method has not proved very useful; indeed, all the exploratory work was doen using this method. A rather more simple method, which avoids some of the objections to the C.L.R. method, has been devised however - the 'dead weight' method. Most of the later results have been obtained using this method.

The principle of the 'dead'weight' method is very simple. The test strip is mounted vertically with a known load suspended from its lower end. The deflection which occurs under the ihfluence of this load is measured. The apparatus is illustrated in Fig. 44.

A perspex frame (A) is clamped in the vertical position to the side of a parallel sided perspex tank (not shown). The test strip is mounted across the gap (B) as described in the C.L.R. method. The upper support (C) - referred to as the upper grip - is also of perspex and is cemented permanently to the frame. The lower grip (D) is completely free of the frame and during a test is prevented from falling by the test strip. During the mounting procedure, however, it is held in place by a phosphor-bronze leaf-spring (E). The load (F) is suspended from the wire frame (G), and there is provision for raising the load clear

of the lower grip by a stirrup (H).

The mounting procedure is as follows. The frame is laid horizontally on the bench with the clamping spring holding the lower grip in position. The test specimen is cemented in position across the gap, and, after the desired load has been suspended from the wire frame, the whole assembly is clamped to the wall of the tank. After the tank has been filled with water, the stirrup is raised so that the load is no longer suspended from the lower grip, and the leaf-spring is released, thus freeing the lower grip. This is called "zero" load. At the beginning of the test the stirrup is carefully lowered and the load is taken on the specimen via the lower grip. To release the load the stirrup is raised.

At "zero" load the specimen is in fact supporting the weight of the grip, but this is small (in water) compared with that of the lowest load used. The frame is not quite vertical but is tilted forward 2 or 3° to eliminate friction (which in any case would be very small) between the lower grip and the frame. Twisting about the vertical axis is prevented by the lugs on the side of the grip.

The whole apparatus is fixed to the vertical microscope stage (microscope in the horizontal position) of the cine apparatus designed by Belford (1958). The test specimen is photographed on 16 mm. cine film (Ilford, Pan F - a fine grain film) and the processed film is projected as a series of "stills". There are always small surface

details which can be used as reference marks, so that the intial length, and the length under any subsequent load regime, can be measured. There is provision for continous running at 8 frames / sec, 16 frames / sec, and 32 frames / sec, and there is also provision for 'time-lapse' photography at various intervals up to 1 frame per 10 minutes. During creep tests the apparatus can be left largely unattended - except for very occasional focus adjustment.

The dead weight method has a number of advantages;

(a) Because of the extreme simplicity of its working principle, it is possible to devise very simple test sequences.

(b) There is a permanent record of the state of the specimen throughout its test history.

(c) Any flaws in the test sample are visible - particularly those not apparent before commencement of test.

(d) Because measurements are made directly off reference marks on the specimen, minor "slip" in the cement is no longer a serious objection.

Its principal disadvantage is that records take very much longer to analyse than the very elegant automatic recording of the C.L.R. method.

MECHANICAL CONDITIONING

The simplest type of test is a load / deformation test, in which the load is applied according to some definite pattern with time and the corresponding deformation observed. For example, the load may

be applied and removed after a period of time; or, it may be applied a uniform rate and then removed at the same rate, etc.

If a test sample of Nitella wall is loaded on the C.L.R. testing machine to some point below the yield point and the load is then removed at the same rate, the sample does not regain its original length. There is a small 'set'. If after the load is reapplied at the same rate up to the same maximum load, it is found that the tensile modulus has increased substantially. If the maximum load applied during the first cycle was fairly high (close to the yield point) there is very little further change in the tensile modulus during subsequent cycles. If the maximum load applied during the first cycle is small, the increase in tensile modulus is not as great, but after the third or fourth cycle at the lower load it reaches a maximum value and there is no further change. This process will be referred to as "mechanical conditioning". An example of this behaviour is shown in Fig. 45.

If after mechanical conditioning in this manner, the strain is relieved and the sample is left unstrained (wet) for a period, there seems to be a small drop in the tensile modulus which is apparent in the first cycle after testing is resumed; but the former value is restored for subsequent cycles. Even if the sample is left for quite long periods (24 hours) the initial low value for the original test cycle is not regained.

In all cases this change in tensile modulus is accompanied by

a small irreversible change in length, and the two observations seem to be different manifestations of the same phenomenon since the reaching of a constant tensile modulus coincides with the reaching of a stable value for the 'set'.

This phenomenon has also been observed by the "dead weight" method. The full load was applied at "zero" time and the deformation observed over a period of 1 minute by means of the cine camera operating at 8 frames / second. The load was then removed for 1 minute, and at the end of this time the whole cycle repeated. The deformation during two successive "on" cycles is shown in Fig. 46.

There are several observations which may be made;

(1) When the load is removed after the first load application the test strip does not regain its original length, and there is a further smaller 'set' during the second cycle. On successive cycles there is very little further change. This is consistent with the observations made using the C.L.R. method.

(2) At the beginning of the first cycle, the final deflection is not reached instantaneously, but a deflection very close to the final value is reached within about 10 seconds. The time constant on subsequent load changes, both 'on' and 'off', is very much smaller - of the order of 1 second or less.

(3) Over the period for which the load is applied there is a small but observable change in deformation, indicating that the material is probably exhibiting the phenomenon of "creep".

(4) The "Instantaneous"* deformation which occurs when the load is applied is different in both cases, but the "instantaneous" recovery is the same. As has been pointed out by Freudnethal, the potential (strain) energy alone is the source of stress; the portion of the applied energy directly dissipated as heat is associated with irrecoverable deformation; it does not affect the condition of stress. There is, therefore, a one valued relation between stress and that component of strain which is instantaneously recovered on unloading.

The phenomenon of "mechanical conditioning" probably has its origin in the fact that the sample is mounted dry and is then rewetted. It is known from swelling measurements that there is a dimensional increase on wetting (length about 6%; width about 4%; and thickness about 100%). It is possible that during the process of drying and rewetting, that molecules (of the amorphous component) take up more

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* The term "instantaneous" as used above needs to be specified. It appears that response to stress (ignoring long term creep), after the sample has been mechanically conditioned, might consist of an instantaneous component and a retarded elastic component which has a very short retardation time (of the order of one second, or less). Since the load is applied slowly and gently and because this phenomenon, if it exists, is on the observable limit with the present equipment, its magnitude (and even its existence) is in doubt. For this reason, no attempt is made to separate it from the true instantaneous elasticity. This is equivalent to saying that the time scale of the experiment is large compared with the retardation time of one second. (This is also true for the C.L.R. method - at the loading rate used, the time taken to load a sample from zero load to full load varied between about 30 and 45 seconds, depending on the sample).

This definition of 'instantaneous' is used in all the work which follows on the measurement of the tensile elastic modulus. Shortening the time scale could only be conveniently achieved by employing a dynamic method, and for wet specimens this would involve some rather special problems.

random configurations than they occupy in the living wall. During the first stretching, cross-links between 'kinked' molecules are broken as the stress is taken up unevenly and the molecules are straightened. While in the strained state new cross-links are formed some of which are not subsequently broken when the stress is relieved. At the beginning of the second cycle and subsequent cycles the alignment of the molecules is better and the number of 'unkinked' molecular units sharing the load is greater. The tensile modulus is, therefore, greater than in the first case. This explanation is no more than a suggestion, but the observed magnitude of the 'set' and the mechanical behaviour support this view. 'Mechanical conditioning is observed in both transverse and longitudinal strips.

It seems likely that the mechanically conditioned state corresponds more nearly to the condition in the living wall. Unless otherwise stated, all figures for the tensile modulus will refer to mechanically conditioned strips.

All tests were carried out at a temperature of 23°C.

TENSILE MODULUS

The tensile modulus was measured by both the C.L.R. method, and the 'Dead weight' method. An example of a load/extension curve, measured on the C.L.R. machine, was shown in Fig. 45. Measurement of the tensile modulus by the dead weight method was simple in principle, but not as convenient as the C.L.R. method in practice. However, because it was less susceptable to systematic error, this was the method which was adopted for most of the later measurements. Unless otherwise stated all results were obtained with the 'Dead weight' method.

The procedure used is as follows. The sample was mounted as previously described and after the frame had been clamped in the tank, and the sampleimmersed, the load was raised clear of the lower grip, the spring clamp released, and the test strip photographed at zero load. The load was then applied and three or four seconds later the test strip photographed. The load was removed, and after a similar interval the sample rephotographed with the load off. The lower grip was then clamped, the load changed, and the lower grip released again at zero load. The loading and unloading cycle was then repeated. The whole procedure was repeated at a number of suitable loads. An example of the load/extension curve of a longitudinal strip and of a transverse strip, is shown in Fig. 47.

In all cases the test strip was mechanically conditioned by applying a load of about 5×10^3 dynes per millimeter width of strip.

The tensile modulus, E, is defined as;

$$E = \frac{\text{Stress}}{\text{Strain}}$$

$$= \frac{W}{A} \cdot \frac{\Delta l}{l} \quad \text{where} \quad W = \text{load}$$

A = cross sectional area of test strip.

l = length

Δl = change in length.

Unfortunately, it is very difficult to measure the cross sectional area (A) of the strip to the required accuracy because the thickness, t, is very small (8 microns, wet). For this reason it was decided to measure the mass/unit area (m), and this has been done.

If the mass/unit area = m

then, thickness, $t = \frac{m}{\rho}$ where ρ = density.

$$E = \frac{W}{A} \cdot \frac{\Delta l}{l}$$

$$= \frac{W}{b.t} \cdot \frac{\Delta l}{l} \quad \text{where } b = \text{width}$$

$$= \rho \cdot \frac{w}{m} \cdot \frac{\Delta l}{l} \quad \text{where } w = \text{load/unit width.}$$

$$\text{or, } E' = \frac{w}{m} \cdot \frac{\Delta l}{l} \quad \text{where } E' = E/\rho \text{ or } E/k$$

k is defined below.

Results will be expressed in terms of E', and it will be assumed that ρ is constant for all samples. In order to bring the units more into line with usual practise ρ will be made equal to $k\rho'$, where $\rho' = 1$ gm./cc^3, and k is dimensionless constant.

It will sometimes be convenient, where tests are being run on the same sample (e.g., in creep testing), to express the load in terms of the load per unit width of test strip.

Since the investigation is directly concerned with the effect of the mechanical properties of the plant cell wall on the growth of the cell, it is desirable to know something of the state of growth of the cells selected for test. In all cases the following parameters have been measured; length, diameter, mass/unit area, and the Growth Rate. The Growth rate (or, Growth Index) is defined as the per cent increase in length in the 24hours preceding collection. After collection, the protoplasm was removed, the cells were dried in Acetone and stored in a desiccator over fused $CaCl_2$ until required.

The growth rate of each cell used in the investigation, has been plotted against its corresponding length and the results are shown in Fig. 48. The correlation coefficient (r) is - 0.683 (for 21 degrees of freedom, r = 0.641 at the 0.001 level of significance). The correlation is, therefore, highly significant.

It is not surprising that as the length of the cell increases, the Growth Rate (as defined above) diminishes. The information is, however, relevant to discussion to come later and it is well to establish the fact. There is considerable scatter of points on the diagram, and although the growth rate of long cells is generally lower than that of short cells, there are cases in which long cells have

high rates of growth and short cells, low rates.

After the tensile modulus of a number of cells had been determined for strips cut in the longitudinal and transverse directions it became obvious that the modulus was considerably higher in the transverse than in the longitudinal direction. In some cases the ratio E_T/E_L was of the order of 2, while in others it was as high as 5.

In Fig. 49 the ratio E_T/E_L is plotted against Growth Rate. For cells with a high growth rate the ratio E_T/E_L, which is a measure of the anisotropy of the wall, is generally high, while it is low for slow growing cells. The correlation coefficient, r, is 0.793 (For 10 degrees of freedom, r = 0.708 at the 0.01 level of significance, and 0.823 at the 0.001 level). The correlation is, therefore, highly significant.*

If the correlation between cell length and the ratio E_T/E_L is considered, the correlation coefficient is found to be 0.487, which is only significant at the 0.1 level. This seems to indicate that the anistropy of the wall is more closely related to factors affecting growth rate, than it is to cell length. (Note: Of the two quantities the length is more accurately known than the growth rate, which depends on the measurement of two lengths).

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* In the early stages of the investigation, while measuring techniques were being developed, test strips were cut exclusively from mature cells, because, being large they provided plenty of material. There is, therefore, rather more evidence than has been presented for low values of E_T/E_L for mature cells, but it has not been included because the growth rate was not verified.

The question arises as to whether the change in the ratio E_T/E_L as the growth rate changes is due to a change in the value of E_T, or, E_L, or, a combination of both. In Fig. 50 E_L' is shown plotted against Growth Rate, and in Fig. 51 E_T' is shown plotted against Growth Rate. E_T' and Growth Rate are not significantly correlated. There is an increase in E_L' as the growth rate falls off. The correlation coefficient (r) is-0.561, which for 10 degrees of freedom, is significant at the 0.05 level. (i.e., probably significant).

It would seem, therefore, that it is the anisotropy of the wall, as indicated by the ratio E_T/E_L, which is more closely related to factors affecting growth rate, rather than the longitudinal or transverse tensile modulus considered separately. The anisotropy expected from structural considerations is confirmed. As the cell reaches maturity the value $E_T/E_L \rightarrow 2$, which is the ratio of the principal stresses in a thin-walled pressurized cylinder. This may be just a coincidence, but it is interesting none the less.

As the stress is increased a limit is reached, beyond which, an irrecoverable extension occurs, i.e., a 'permanent set'. In table 6 the approximate stress (σ^{PS}) at which this 'set' is first observed, is listed for both longitudinal and transverse strips. In every case σ_T^{PS} is very much greater than σ_L^{PS}. This is a further indication of the difference in elastic properties in these two directions (Note: the figures for σ^{PS} are only approximate as in the 'dead weight' tester the

load is increased in finite steps, and it is, therefore, not possible to be sure of the exact point at which this 'set' occurs).

The data discussed in this section are set out in table 6.

In general only the longitudinal and transverse elastic moduli were determined. In two cases, however, the tensile modulus was measured for a number of strips cut at various angles to the longitudinal direction. The result of one of these series of measurements is shown in Fig. 52. For a material having orthorhombic elastic symmetry;

$$\frac{1}{E_\theta} = \frac{1}{E_L} \cdot \cos^4\theta + \left(\frac{1}{G_{LT}} - \frac{2\sigma_{LT}}{E_L}\right)\cos^2\theta \sin^2\theta + \frac{1}{E_T} \cdot \sin^4\theta$$

where the terms are defined as before.

If the tensile modulus is measured in the L & T direction, and at 45° (say), then if the value of G_{LT} is known, σ_{LT} can be calculated. Failing a reliable value of G_{LT}, however, no attempt has been made to do this.

TABLE 6.

Cell No.	Length mm.	Growth Rate	10^8 E_T' dynes/cm^2	10^8 E_L' dynes/cm^2	E_T/E_L	Mass/unit area gms/mm^2.	$10^8 \sigma^T_{PS}$ (approx.) dynes/cm^2	$10^8 \sigma^L_{PS}$ (approx.) dynes/cm^2
14_C	50.5	1.0	226	92	2.5	3.47	> 4.9	2.7
14_E	42.0	2.4	287	79	3.6	2.75	> 7.6	2.7
22_B	30.5	10.9	346	84	4.1	2.20	9.4	-
21_A	11.7	11.9	263	70	3.8	1.92	-	2.7
16_B	45.5	9.6	323	68	4.8	2.44	-	-
16_A	15.0	22.5	291	54	5.3	2.17	> 8.8	0.8
20_C	49.5	1.0	375	207	1.8	2.10	> 10.9	6.5
14_F	26.0	8.3	269	77	3.5	2.22	7.7	2.6
14_B	41.2	16.2	237	45	5.2	3.24	5.2	1.9
20_A	10.0	8.1	354	80	4.4	1.80	> 7.6	1.7
15_C	47.2	6.2	244	102	2.4	3.12	5.7	1.6
20_B	36.5	6.6	262	60	4.4	2.34	8.5	2.8

CREEP

It was suggested earlier that the nature of the wall was such that it would probably exhibit 'time' effects. This suggestion was tested in the following way. A test strip was mounted in the 'dead weight' tester as previously described, and after the strip had been photographed at 'zero' load, the load was applied. A photograph of the test strip was taken at the following times after the application of the load; 10, 20, 30, 40, 50 and 60 seconds; 2, 4, 6, 8 and 10 minutes; and every 10 minutes thereafter.

Initially, tests were run for about 1000 minutes (17 hours). Another test strip, cut from the same cell wall, was then mounted and another test begun at a different load. An example of a test series of this type is shown in Fig. 53 for a longitudinal strip cut from cell 17C. It will be noticed that the extension at one minute, for even very light loads, is of the order of 10% and that the curves are not in their correct order with respect to magnitude of load, in every case. This is, to some extent, due to the 'initial set' (see section on mechanical conditioning) which is somewhat variable. The effect of increasing the load on the time behaviour can be seen more clearly in Fig. 54, in which the extension has been arbitrarily adjusted to zero at 1 minute. It is now clear that as the load increases the rate of extension increases, and there is no suggestion, for the loads and extensions shown, that a limiting extension has been reached. The linear rate of extension is, however, falling off as the test proceeds.

This method of presentation is convenient if one is interested in the 'time' behaviour of a single cell wall, but, in order to compare the 'time' behaviour of a number of cell walls, it would be more convenient if the family of curves for each cell could be reduced to a single curve. In Fig. 55 the same set of data is presented in another way. In this figure, the per cent extensions occurring between the first minute after load application, and the tenth, hundredth, and thousandth minute, are shown plotted against the load. All three curves convey much the same sort of information, viz., for loads below about 4×10^3 dynes / mm., the creep rate is small, but at higher loads, the creep rate increases rapidly as the load increases.

This method of expressing the results of creep tests has been adopted and the time interval, 1 to 100 minutes has been adopted as the creep criterion. A ten minute interval was considered to be too short, and a thousand minutes, unnecessarily long. Tests were run for periods of between 500 and 1000 minutes on a number of samples, but as soon as the general pattern of behaviour became clear, tests were concluded after 100 minutes (except in the case of test strips used for creep recovery measurements).

Leaderman (1943) suggests that reversible delayed elastic effects, together with the associated relaxation behaviour, be referred to as "primary creep phenomena". The non-recoverable creep which is superimposed upon the primary creep under load, is called "secondary creep". No

attempt is made in the present investigation to separate these effects. That the material exhibits both "primary" and "secondary" creep is clear from Fig. 56. In each case the load was applied for 100 minutes and then removed. The rate of creep recovery is much less than the rate of creep under load, and the initial length is not regained (within 24 hours).

Creep tests were run as described, on a number of cells and it soon became clear that, for 'transverse' strips, the rate of creep was so small as to be within the limits of error of the measurements, for the time intervals considered and for loads well beyond the yield point of 'longitudinal' strips. This was true even for cells for which the rate of creep of 'longitudinal strips was very high. For example; a 'longitudinal' strip cut from the wall of cell 16B, increased in length by about 12% between one minute and 100 minutes at a load of 8×10^3 dynes/millimeter width of strip; whereas a transverse strip showed negligible creep at a load of 16.2×10^3 dynes /mm. If the load of transverse strips was increased, they broke suddenly at a load a little beyond the 'yield' point.

This difference between the creep behaviour of 'longitudinal' strips and 'transverse' strips is further evidence of the difference in mechanical properties in these two directions.

The creep behaviour of longitudinal strips was found to be extremely variable. It has already been shown that the anisotropy of the tensile modulus varies with the growth rate, and the creep of longitudinal strips was, therefore, examined to see if there was a

correlation between creep rate and growth rate. The result of a series of tests on longitudinal strips cut from a number of cells, for which the growth rate was known, is shown in Fig. 57. It will be observed that, in general, strips cut from cells with high growth rates exhibit high rates of creep, and vice versa. This is particularly true of cells taken from the same plant, viz., 17B and 17C, and 14C, D and E. Taken over all, however, there are cases in which the curves are not in the correct order with respect to growth rate, and this raises the question as to whether this is the best method of expressing the data.

As it is expressed in Fig. 57., the data relates to the "time" behaviour of the wall material itself when subject to increasing stress. No account is taken of the magnitude of the stress to which it is likely to be subjected in the wall of the particular cell from which the test strip is taken. The data in Fig. 57 has, therefore, been replotted expressing the stress condition as the ratio of the stress applied in the creep test, to that which the wall would be subjected when the turgor pressure is at some 'standard' value (say, 8 atmospheres).

If the cell is considered as a thin-walled cylinder under the influence of an internal hydrostatic pressure, then;

Stress in longitudinal direction, $\sigma_L = \frac{Pr}{2t}$ (symbols as before)

If the turgor pressure is 8 atmospheres: $P = 8.10 \times 10^6$ dynes/cm^2.

The wall stress in the longitudinal direction when the pressure is 8 atmospheres is, therefore,

'Standard' Stress, σ_L' = $4.05\frac{r}{t} \times 10^6$ dynes/cm^2

Since the wall stress depends on the ratio r/t, it will be different for each cell, since each cell has different dimensions. The wall stress in each cell has, therefore, been calculated for the 'standard' pressure, using the known radius and substituting the mass/unit area for t as previously described. The ratio, Test Stress/'Standard' wall stress, has been calculated for each cell and the data in Fig. 57 is replotted in Fig. 58 using the stress ratio scale.

All of the curves now fall in their correct order with respect to growth rate. This is not surprising since, if the growth mechanism does depend to some extent on mechanical stretching of the wall under the action of turgor pressure, it is reasonable to expect that deformation will depend not only on the physical state of the material but also on the stress to which it is subjected in the wall. This method of plotting the results takes both factors into account.

No evidence is offered as to whether the turgor pressure was the same in all these cells during the period over which the growth was measured. However, although the turgor pressure was not accurately measured during the plasmolysis experiments, which were conducted to test the spiral growth hypothesis (see Part IV), these measurements indicated that the turgor pressure did not vary over a very wide range. The ratio, r/t, varies over a two-fold range.

One apparently puzzling feature of the results is that the stresses at which very high creep rates are observed in growing walls, seems to be rather low compared with the 'standard' wall stress. It should be noted, in this connection, however, that in these tests the load has been applied uniaxially, whereas the living cell is subjected to a multiaxial stress. These results should not, therefore, be accepted as literal statements of the behaviour of the material in situ. All that is implied, as a result of these tests, is that the physical properties of a growing wall are rather different from those of a non-growing wall.

This latter point is quite clearly made in Fig. 59 and Fig. 60. In Fig. 59 a longitudinal strip cut from cell 20G (Growth Rate, 1% / 24 hours) is shown before load application, and just before failure under load. In Fig. 60 a similar strip from cell 20A is also shown unloaded, and just before failure. There is a notable difference in the appearance of the specimens when stretched, and in the magnitude of the deformation up to the breaking point.

Evidence is accumulating which suggests that the physical properties of a growing wall are rather different from those of a non-growing wall. It is, therefore, of some interest to see if some indication can be obtained as to the nature of the difference between growing and non-growing walls. It is known from the work of Green (1958, A) that the birefringence of the wall decreases with the age

of the cell and measurements made during this investigation confirm this trend. The electron microscope evidence in Green's paper is such as to suggest that for internodes approaching the end of their elongation, microfibrils are being laid down "in directions quite removed from the transverse", while in young cells microfibrils are laid down "mostly in the transverse direction". In the case of cells which have ceased elongating, the evidence is that "an isotropic, or nearly isotropic, wall is being deposited". As was pointed out earlier, the electron microscope observations in the present investigation are not sufficient to confirm this, but they are in harmony with these general conclusions.

It is to be expected that, if a greater proportion of fibrils occupy directions in the longitudinal (or near longitudinal) direction as the cell approaches the end of its elongation, they will tend to reinforce the wall in this direction. Such a view is supported by the fact that the transverse modulus, E_T, is much higher than the longitudinal modulus in young cells and that the E_T/E_L ratio falls as the growth rate falls. Again, creep in the transverse direction is negligible, presumably because the fibrils, which are not readily subject to flow, are oriented in this direction. Fall in the creep rate in the longitudinal direction is consistent with the behaviour expected if a greater proportion of fibrils are longitudinally oriented, in cells nearing the end of their elongation. It would seem reasonable, therefore, to ascribe at least some of the change in the mechanical properties of

the wall to this increasing disorientation of the microfibrils.

It has been suggested by several workers (Van Overbeek, 1939; Kerr, 1951; Bennet-Clark, 1955; etc.) that pectic substances and related compounds, rather than ~~cell~~ cellulose, strengthen the young growing wall in the longitudinal direction and that increased plasticity of the cell wall during growth is brought about by disturbing the intramolecular lattice of the young wall.

In the chemical analysis (see Part II) the analysis was carried out on bulk material, and no attempt was made to separate cells of different age and do separate analyses on each age group. However, in Fig. 61 the per cent water-soluble materials (dry weight basis) in the cell wall of eight cells is shown plotted against growth rate. The correlation coefficient (r) is 0.789 which is significant at the 0.02 level for 6 degrees of freedom. The evidence of the measurements is, therefore, that it seems likely that the proportion of water-soluble substances present in the wall decreases as the growth rate decreases.

Attempts to define the role of the water-soluble substances in strengthening the wall and conferring plasticity on the wall have not been completely successful, but there are some indications that they play a role in determining the 'time' behaviour of the wall. Such measurements as have been made are described in the following paragraphs.

It has been shown by Tagawa and Bonner (1957) that the physical properties of the Avena coleoptile are dependant upon the nature of the inorganic ions exchangeably bound by the tissue. Briefly, they cut 20 mm.

sections from selected coleoptiles, 3 mm. from the tip and floated the sections on the surface of the test solutions for incubation periods ranging from 0 - 120 minutes. The concentration of calcium and potassium ions (both given as the chlorides) were 0.02 N. This concentration of calcium was known, from earlier work, to inhibit completely the auxin induced growth of coleoptile sections. The same concentration of potassium, on the other hand, has no marked effect on the growth rate of coleoptile sections, but does antidote the growth inhibiting effect of 0.02 N calcium solution. Indoleacetic acid (I.A.A.) was used as the auxin throughout and given at a concentration of 2×10^{-5} M.

It was found that wall plasticity was greatly influenced by the nature if the cations to which the tissue had been subjected. The effective ions appeared to be exchangeably bound by the tissue. Thus calcium ions, which reduced wall plasticity, did so in such a way that they were not subsequently lost to water but were lost to solutions of, for example, K ions. It seemed that the material which bound the calcium ions need not be directly dependent on the metabolic activity or structure of the cell since it was found that dead coleoptile tissue, killed by 5 hours incubation under argon, possessed 80 to 90 per cent of the calcium exchange capacity of living tissue. It was suggested that it might be the cell wall itself which contained the cationic binding groups. Further, a model was suggested to explain the results,

in which pectic chains in the coleoptile cell wall were considered to be cross-linked at intervals by ionic bonds, in which calcium ions bound carboxylate ions of adjacent chains. Replacement of calcium ions by potassium ions weakened ionic interaction between pectic chains and yielded the more plastic coleoptile characteristic of potassium equilibrated tissue.

The suggestion thatthe wall itself contains cationic binding groups has been verified for Nitella in the following way. About 100 milligrams of wall material (dry) was separated into three equal parts. One part was soaked in 0.1 N $CaCl_2$ solution for 18 hours at 5°C, and one of the remaining parts was soaked in 0.1 N KCl solution for a similar period. One part was left untreated. The treated samples were carefully washed on the filter several times with distilled water, once with acetone, and dried in a vacuum desiccator. All these samples were then checked for the presence of calcium and potassium, using a Philips all vacuum X-ray fluorescent spectrograph using a flow counter and pulse height discrimination (the measurements were kindly made by Dr. D.S. Belford of Hickson's Timber Impregnation Co. Ltd., Castleford, Yorkshire).

The results of the analysis are shown in Fig. 62. The height of each peak in the 'calcium' section is proportional to the calcium present in the sample, and the height of each peak in the 'potassium' section is proportional to the quantity of potassium present. The scales of the 'calcium' and 'potassium' sections are, however, not the

same so that they cannot be intercompared. The determination is purely qualitative.

The graphs show that there is some calcium and potassium present in the untreated wall. Treatment with $CaCl_2$ has increased the calcium content and reduced the potassium content to zero (or, a negligible quantity). Treatment with KCl has reduced the calcium to about half the original amount, and increased the potassium about four-fold. These measurements, therefore, support Tagawa and Bonner's suggestion that the cell wall contains cationic binding groups.

The question now arises as to whether the 'time' behaviour of strips cut from the $CaCl_2$ and KCl treated walls is different. Sections of wall from the same cell were soaked in 0.1N solutions as previously described and longitudinal strips cut for creep tests. An example of the change in the plasticity of the wall of cell 21B, brought about by the treatment described, is shown in Figs. 63, 64, and 65. Fig. 63 shows a series of creep curves for a $CaCl_2$ - treated wall; Fig. 64 shows a similar set of curves for a KCl - treated wall; and Fig. 65 shows the creep behaviour compared on the same diagram, using the 1 to 100 minute criterion of 'time' behaviour. There is a notable difference in the creep rates at the higher loads.

Fig. 66 shows a similar pair of curves for cell 22B, but, in this case, the difference is less marked.

In Fig. 67 untreated test strips are compared with $CaCl_2$ - treated strips, cut from cell 17C.

The procedure outlined above is open to the objection that the strips used in each test are different. It might be argued that it is better to observe the effect on the same strip. This has been done for cell 17C. A section of wall was treated with CaCl2 and creep tests carried out at two different loads using $CaCl_2$ - treated strips. Two further creep tests were started on the same material, but at 70 minutes from the start in one case, and at 650 minutes from the start in the other, sufficient N.KCl solution was added to the water to bring the solution to 0.1N strength. In both cases the addition of potassium ions caused the creep rate to increase (see Fig. 68).

The evidence obtained from measurements on these three cell walls is that the 'time' behaviour of strips of cell wall can be influenced by the nature of the cations to which it has been subjected. Unfortunately, the matter does not seem to be as simple as was first thought, since the wall of the remaining cell tested (19B) did not show such a difference after it had been subjected to $CaCl_2$ and KCl treatment.

A possible reason for the fact that some cells show the effect while other show it to a lesser extent, or not at all, is that the magnitude of the effect depends on the condition of the carboxyl groups, as has been suggested by Bennet-Clark (1955); e.g. on the degree of esterification of the carboxyl groups. It would be interesting to compare the reaction to $CaCl_2$ and KCl treatments of walls before and after treatment with pectin methyl-esterase. Glasiou and Inglis (1958) have proposed a detailed theory of auxin action, in which they have

postulated the auxin-controlled binding of Pectin methylesterase controls the elastic properties of the primary wall by regulating the amount of P.M.E. absorbed. Immobilization would reduce the activity of the enzyme and favour an increased degree of methylation of the pectic components of the wall. Unfortunately, there has not been time to check on the behaviour of wall strips before and after Pectic methylesterase treatment.

It has been suggested that the role of water-soluble substances in the wall (pectins and related compounds) play an important part in strengthening the cell wall during growth. One method of determining their role, is to selectively remove them from the cell wall and to observe the effect of their absence. The results of such a procedure need to be examined rather critically, since the remaining wall components are likely to take up different configurations relative to one another and it is possible that entirely misleading results could be obtained.

Creep tests were carried out on longitudinal strips cut from the cell walls of three cells (17B, 17C, and 20C). 17B and 20C were examples of cells having extreme ~~creep~~ growth rates (16% and 1% respectively) and extreme creep rates (see Fig. 54). In neither case did removal of water-soluble materials make any significant difference to the creep curves. In the case of 17C, which had an intermediate growth rate (9.5%) and creep rate, the effect was very marked indeed (Fig. 69).

Such behaviour could be accounted for if, in the two 'extreme' examples, the water-soluble component was not the primary factor in determining the rate of creep, but, in the 'intermediate' example, it was important in determining the creep rate. If the low creep rate of 20C was due primarily to disorientation of the microfibrillar component, and if the high creep rate of 17B was due to an almost complete esterification of the carboxyl groups, then in neither case would the removal of water-soluble materials be expected to affect the creep rate appreciably. Such an explanation is, however, only tentative as the amount of experimental data on which to base a conclusion is too small.

The tensile modulus in both the longitudinal and transverse direction was determined on strips of wall cut from cells 16B, 21A, 22B, and 14E, before and after removal of water-soluble substances. The proportion of these substances in the wall on a dry weight basis, was 11.5%, 26.3%, 19.2% and 15.7%, respectively. There was no significant difference between the load deformation curves determined before and after treatment. The load at which permanent set of longitudinal strips occurred was, however, considerably lower in every case.

As was pointed out earlier, the interpretation of this sort of experiment is rather difficult and this method of approach was not continued.

EFFECT OF STRETCHING ON OPTICAL PROPERTIES

Balashov, Preston, Ripley and Spark (1957) investigated the relation between the extensibility and fine structure of sisal fibres, and found that the spiral (Helix) angle of the microfibrils changed with extension, in a way analogous to the steepening of the helix angle of a helical spring.

During the present series of measurements the opportunity was taken to observe the change in 'path difference' (defined in Part II, Polarizing microscope Section) which occurs when the wall is subjected to a uniaxial stretch in the longitudinal direction. (It would also have been desirable to observe the reorientation of the crystalline fraction by the technique of X-ray diffraction. Unfortunately the diffraction diagram of untreated Nitella is too poor for such a measurement to be made.) The 'path difference' was measured (cell wall dry) on a number of longitudinal strips. Each strip was then stretched a measured amount, and dried in the stretched condition. The path difference was then redetermined. The results are shown in Fig. 81. As the strain approaches 20% the path difference falls to zero and at larger strains the path difference is positive.

These measurements highlight a difficulty in the way of accepting mechanical theories of growth; viz., the fact that the tendency towards axial orientation, which one would expect to occur during elongation appears to be of rather small magnitude. For example,

in the case of the Nitella cells considered in Figs. 4 and 6, the length/diameter ratio covers a 40-fold range, whereas the m.e.p. is approximately transverse for all cells measured (indeed, the departure from the transverse was less for the more mature cells, than for the very young cells). Further, using the figures given by Green (1958A) it appears that, over the same sort of length range, the birefringence of the wall decreases only about 40%. If the observation that the disorientation of fibrils on the inner wall increases with cell age is correct, then not all of the decrease in birefringence may be due to disorientation due to elongation.

It is rather difficult, therefore, to reconcile this constancy of the m.e.p. and of the birefringence with the idea of passive reorientation of the microfibrils as the wall elongates.

TENSILE MODULUS OF VALONIA

All of the data presented so far has been obtained from measurements made on wall strips cut from Nitella. The wall of Nitella is an example of one particular type of structure. The wall of Valonia is an example of a very different type of wall organisation. Its structure has been described by Cronshaw and Preston(1957). Briefly, the microfibrils (highly crystalline eucellulose) are present in three orientations, in separate lamellae. The third orientation corresponds to microfibrils which are much less abundant than those of the other two orientations both because the lamellae with this orientation are less frequent and because the microfibrils are more loosely packed in each lamella. The two major directions lie on an average at rather less than at right-angles to each other; the third direction forms a bisector of this angle.

The question arises as to whether in a cell wall having such a structure the extensibility of the wall is likely to be the same along any line in the wall surface (i.e., whether the wall is effectively isotropic). The question has an added interest because it has recently been discovered (Preston and Frei; private communication) that Cladophora and Chaetomorpha, which have a rather similar type of wall structure, exhibit the phenomena of spiral growth. From the point of view of mechanical theories of spiral growth it is therefore, of some interest to know if the wall is elastically isotropic.

The tensile modulus of wet strips cut at various angles to the chain direction was determined by the procedure previously described for Nitella. The angle of the strips relative to the chain directions was determined by X-raying each specimen, and measuring the inclination of the principal arcs relative to the longitudinal axis of the specimen. The results are shown in Fig. 70 and Fig. 71. They apply to two separate pieces of wall. The figures are not as complete as one would wish, but the main point is clear; in the direction of the main chains the modulus is higher than in the other directions. The wall is, therefore, not elastically isotropic.

Discussion

Discussion of points arising from the work presented in this part will be postponed until after Part IV has been presented.

PART IV

SPIRAL GROWTH

INTRODUCTION

In Part III, evidence was presented which suggested that growth may not be entirely unrelated to the mechanical properties of the wall. The question which might now be asked, is whether there are any cases in which one may be reasonably sure that purely mechanical forces control or modify growth. The phenomenon of spiral growth, exhibited, for example, by the sporangiophore of Phycomyces, could well be a case in point.

The sporangiophore grows in the form of a thin-walled cylinder, the tapering tip of which carries the developing sporangium as an inflated ball. Growth occurs only in the apical 2 mm. or so. It has been demonstrated by Oort (1931), Oort and Roelofsen (1932), and Castle (1937, 1942) that markers placed in the zone of growth not only move upwards, but at the same time are displaced transversely indicating that there is a rotational growth of the cell about its own axis. Before the sporangium has begun to appear, the cell twists in a left-handed direction (i.e. in the direction of left-handed screw). During the swelling of the sporangium the cell ceases to elongate and to rotate. Once the sporangium is fully swollen the structure rests for a time and then begins to elongate and twist in the opposite sense from the original direction (i.e., there is now a right-handed

rotation). This continues for a period, but the rate of twisting gradually diminishes, falls to zero, and then develops in the left-handed sense. Preston (1952) comments as follows: "Since the sporangiophore is simply a hollow tube of chitin filled with liquid, or semi-liquid, cytoplasm containing a vacuole, the conclusion can hardly be escaped that these peculiar effects of growth must be due to some feature of the wall".

Various theories have been put forward to account for this phenomenon.

Castle (1936) suggested that twisting might be due to the anisotropic response of such a wall to the hydrostatic pressure within the cell due to turgor. Although this suggestion is probably on the right lines it is rather too vague a statement to be accepted as an explanation.

Heyn (1935) suggested that, during elongation, plastic stretching of the wall occurs by failure of the wall along planes of greatest weakness in the crystallites. Since these planes are at an angle to the cell axis, spiral growth results. It is difficult, however, on this model to account for the reversal of spiralling which is known to occur. Heyn (1939) also proposed an alternative theory. He suggested that chitin molecules are originally deposited in the direction of the long axis parallel to the direction of protoplasmic streaming. As they arrange themselves into a crystal lattice, the forces of crystallization cause an oblique orientation of the chitin chains and the combination of "rotating forces and of extending forces of elongation then results in spiral growth". Again, there is the

difficulty of accounting for the reversal of spiral growth.

Preston (1948) and Preston and Middlebrook (1952) have interpreted the behaviour of the wall in terms of its submicroscopic structure. They made careful X-ray and polarising microscope measurements and concluded that chitin chains were oriented at about 14° to the transverse direction. A model of the wall, in the growth zone, was therefore proposed in which the wall was thought of as being composed of a number of spiral springs (chitin chains) which are extended during the growth process. Now it can be shown that when a flat spiral spring (helical is more correct) is axially extended by an amount, ΔL , there is a rotation of the free end which is given by,

$$\frac{\Delta \phi}{\Delta L} = \frac{\cos\alpha \sin\alpha \left(1 - {}^{2n}/_{q}\right)}{a\left[\cos^2\alpha + \left({}^{2n}/_{q}\right)\sin^2\alpha\right]}$$

where q = Youngs modulus of the spring material

n = its torsional rigidity

α = angle of spring winding to the transverse direction

a = radius of spring.

Extension of the spirally arranged long chain molecules of chitin by the hydrostatic pressure within the cell would, therefore, on this model cause a rotation of the free end of the cell.

The theory was successful in accounting for the fact that elongation and rotation always go together; from the known value of $\left(\frac{\Delta L}{\Delta \phi}\right)$ and the dimensions of the cell, a value of $\left({}^{n}/_{q}\right)$ could be calculated which agreed

very well with the expected value for chitin and the externally applied torque required to just stop rotation was shown to vary as (a^3), as is predicted by the spring model. To account for the reversal of spiral growth, it was assumed that, at this stage of growth (e.g. transition from an almost parallel texture with low angular dispersion of microfibrils to a bigger angular dispersion) q might be lowered and n raised. If this change was sufficient to make $2n/q$ greater than unity, the direction of rotation of the end would be reversed, and it was postulated that a change of this nature accounted for the reversal of spiralling.

Frey-Wyssling (1952) has criticised this theory on the grounds that the spiral spring model is not consistent with the structure of the primary wall of Phycomyces as revealed in the electron-microscope, viz., la mellae of crossed interwoven microfibrils with two distinct main directions. He suggests that growth is by intussusception and that the small area where the primary wall is loosened and, into which new fibrils are woven, moves around the wall. He goes on to say: "If this movement is a rotation the result must be spiral growth as elongation takes place at the same time." Leaving aside for the moment all question of the validity of the argument for this special kind of mosaic growth, it is not immediately obvious, in the absence of a more complete description of the chitin chain arrangement, that such a growth mechanism would lead to rotational growth. It is possible to imagine circumstances in which such a process would lead only to a circular movement of the top of the

sporangiophore, and not to rotational growth of the cell about its own longitudinal axis. While maintaining that it is the fundamental phenomenon of weakening the wall prior to intussusception which provokes elongation and rotation by the action of turgor pressure, Frey-Wyssling proposes a rather different mechanism to account for reversal of spiralling. To quote: "As long as turgor pressure is constant no reversal of the rotational sense is possible. But if this pressure changes its value, e.g., in raising or lowering the stress in the primary wall, its elasticity may cause a reversal of the rotation due to the two crossed spiral systems within every one of the primary lamellae. To my mind therefore, the complicated spiral growth of the sporangiophore of Phycomyces is due to wandering local changes of the rheological properties of the primary cell wall (growth by intussusception) in the growth zone and variations of the turgor pressure". Here again the mechanical details are left rather vague. Further, so far as the author is aware, there is no evidence for this special kind of mosaic growth, other than the phenomenon of spiral growth which it is invoked to explain.

The phenomenon of spiral growth is also exhibited by the internodes of Nitella and, as has already been mentioned, the most complete description of the phenomena has been furnished by Green (1954). The wall structure was investigated by Green and Chapman (1955) and their conclusions have already been discussed in Part II. Briefly, however, their principal finding was that "the cellulose microfibrils of the wall are well oriented

and run strictly in the transverse direction. This orientation is found in all portions of the wall and in all stages of growth. Thus the twisting or spiral growth of the wall is not related to a helical arrangement of wall elements but to a lateral shifting of the transverse structural members". And again: "Because the orientation of microfibirls remains transverse while growth of all portions of the wall follows a helical course (the pitch of the helix varying with development .....) no theory relating spiral growth to a spiral (helical) orientation of wall elements can apply. It is apparent, however, that the simple increase in transverse wall elements in an evenly growing cylindrical wall would lead to simple longitudinal extension and increase in diameter. The helical nature of growth must, therefore, result from a torque which changes the major direction of elongation from the longitudinal to a diagonal direction corresponding to the direction of spiral growth at that time. Since the action of this torque preserves the transverse orientation of structure, it must involve the lateral shifting of structural elements in the growing area. If the torque changes the direction of extension more than the amount needed to maintain the pre-existing pitch of spiral growth, the growth spiral will flatten and the wall will become more highly twisted. It the torque becomes less and less, the growth spiral will steepen and the wall will become less twisted. It is our opinion that the torque probably arises during the attainment of the stable "nesting" position by all the transverse elements. This most stable position (and hence the twist of the

wall) may vary reversably during wall development. The tendency to shift might be influenced by the closeness of packing of wall elements, the extending force of vacuole pressure, and the relative ability of the cell interior to elongate."

This "most stable meeting position" theory proposed by Green and Chapmanis rather reminiscent of the "forces of crystallization" theory proposed by Heyn for Phycomyces. There is, however, an important difference between them. While Heyn's theory was proposed to suit a wall structure in which the wall elements were known to be inclined to the transverse, the theory of Green and Chapman must not only account for spiral growth with structurally transverse elements, but must also account for a reversal of spiralling without any (known) change in wall structure. If spiral growth is to be interpreted in terms of strictly transverse wall elements, it is difficult to imagine just how the mechanism is to operate. That Green and Chapman are not entirely happy about the situation and consider that the last word has not been said on the wall structure of Nitella is clear from the following extract from their paper: "Future research will attempt to find structural peculiarities correlated with the twisted state of the wall. From such research the mechanism of spiral growth in Nitella may become clear".

N.B. Evidence was presented in Part II of this thesis which suggested that Green and Chapman's conclusion of transverse orientation for the microfibrils was not correct, and that crystalline component was, in general, inclined to the transverse direction at a small angle.

Although all of these theories of spiral growth propose rather different mechanisms, they have this in common; they all ascribe the mechanism of spiral growth to some sort of mechanical force (arising within the wall structure) which causes a lateral displacement of the wall elements. It seems to be generally agreed that, in the phenomenon of spiral growth, we have one case at least where mechanical forces control or modify growth.

The remainder of this section of the thesis will be devoted to a consideration of the spiral growth in Nitella, in terms of the wall structure derived in Part II and the mechanical properties determined in Part III.

A THEORY OF SPIRAL GROWTH IN NITELLA

In part III it was suggested that the wall of Nitella could be regarded as possessing three mutually perpendicular axes of elastic symmetry, i.e., that its symmetry approximates to that of an orthomombic crystal.

The question now to be discussed is the significance of this in relation to the growth of Nitella and the suggestion will be put forward that spiral growth is a direct consequence of the coupling between shear and extension which can occur under some circumstances in anisotropic materials.

The usual crystallographic representation of the elastic constants of an anisotropic solid were given earlier, the three co-ordinate axes, x, y, and z being arbitrarily identified with the longitudinal direction, the tangential direction and the radial direction of the cell respectively (See Fig. 72). If the reference axes relate to the elastic symmetry of the substance then, for a material possessing orthorhombic elastic symmetry, nine independent elastic coefficients+, S_{11}, S_{21}, S_{31}, S_{22}, S_{32}, S_{33}, S_{44}, S_{55}, S_{66}, will completely specify the elastic behaviour of the material (see equation 1, Part III).

It has been shown, however, that the m.e.p. of Nitella opaca is not strictly transverse, but is inclined to the transverse direction at an angle which varies with the streaming direction and, therefore, with the dimensions of the cell. If we identify the axes of elastic symmetry of the wall material with the directions of the major and minor extinction

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+ Sometimes known as moduli of compliance.

positions, displacement of the m.e.p. from the transverse direction means that the effect on the elastic coefficients of a rotation from the principal axes must be considered.

The general formulae for the effect of rotation from the principal axes on the elastic coefficients of an orthorhombic crystal were given by Hearmon (1948). For this purpose they will not be given in full. It is sufficient to note that, for a rotation θ in the x y plane, from x towards y, about an axis z, 13 coefficients are now required. Four new coefficients have appeared, viz., S_{61}', S_{62}', S_{63}', and S_{54}', where the dashes indicate elastic coefficients in the new orientation. They are not independent coefficients, but are expressible in terms of the nine fundamental coefficients listed above, and the angle of rotation. To quote Hearmon: "The existence of S_{61}', S_{62}', and S_{63}', means that shear stresses in the x and y directions produce pure extensions in the x, y, and z, directions and, conversely; the existence of S_{54}' means that shear stresses in the z and x directions produce shear strain in the zy plane and conversely".

The coefficients S_{16}', S_{26}', and S_{54}', change sign as θ changes. For example:

$$S_{16}' = -\tfrac{1}{2}(S_{11} - S_{22}) \sin 2\theta + \tfrac{1}{4}(S_{11} + S_{22} - 2S_{12} - S_{66}) \sin 4\theta$$

$$= -\tfrac{1}{2}\left(\frac{1}{E_L} - \frac{1}{E_T}\right) \sin 2\theta + \tfrac{1}{4}\left(\frac{1}{E_L} + \frac{1}{E_T} + \frac{2\sigma_{TL}}{E_T} - \frac{1}{G_{LT}}\right) \sin 4\theta$$

where E_L = Youngs modulus in L direction

E_T = " " " T "

G_{LT} = rigidity modulus for shear stress in L and T direction.

$$\sigma_{TL} = \frac{\text{contraction in L direction}}{\text{extension in T direction}}$$

These coupling coefficients are zero, when $\theta = n.\frac{\pi}{2}$ (where $n = 0$, or any integer).

The conditions for isotropy include $S_{11} = S_{22}$ and $S_{11} - S_{12} - \frac{1}{2}S_{66} = 0$.

It is this coupling between shear and extension which is thought to be the basic mechanism in spiral growth.

So far discussion has been limited to the general analytical expression for Hooke's Law relating strains, in an orthorhombic crystal, to stresses. This does not, directly, solve the problem of the behaviour of the cell wall of Nitella when subjected to turgor pressure. Ideally, one should now proceed to compute the stress distribution in a thin-walled pressurised cylinder, which is built of a material possessing orthorhombic elastic symmetry,* the axes of elastic symmetry being inclined to the principal axes. Given such an analytical expression, one could insert the values of the elastic constants and for known values of θ (angle of m.e.p.

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* Note:- For completeness full three-dimensional orthorhombic (or monoclinic) structure has been attributed to the wall for the reasons given above. It has been pointed out by Mr. M.J.P. Musgrave, however, that insofar as the cell wall approximates to a thin-walled tube, the meaningful observable quantities are the stresses and strains which can occur in the two-dimensional structure which may conveniently be developed on to a plane by a cut along a generator of the cylindrical cell wall. There are three stresses and three strains related by the six moduli S_{11}, S_{22}, S_{12}, S_{16}, S_{26}, S_{66}. Unless the cell is considered to have a thick wall, the introduction of further moduli can serve no useful purpose.

to transverse) test the validity of the proposed theory by comparing the observed and computed direction of twist at various stages of growth, in much the same way as was done by Preston and Middlebrook (1952).

Unfortunately, this is a very difficult theoretical problem, for which there is no (known) published solution. Attempts to solve the problem as part of the present investigation have not been successful.* There are, however, some general remarks which can be made on the basis of the analytical expressions for Hooke's Law given above. The existence of coupling between shear and extension suggests that a cylinder of the type described would, when pressurised, not only deform longitudinally, tangentially, and radially, but would also exhibit a torsional rotation about its own axis. Further, since it is only the coupling coefficients which change sign as θ changes sign, it seems likely that the direction of twist would change sign as the m.e.p. changes from a left-hand to a right-hand spiral (provided one is correct in identifying the m.e.p. with an axis of elastic symmetry). When the m.e.p. is strictly transverse, the coupling coefficients are zero and there would be no tendency to twist in this case. Further, it seems likely the magnitude of the effect (and even its sign) depends on the relative magnitude of the elastic coefficients.

Such coupling effects are well known in the piezoelectric crystals which are used in communication engineering in filters, oscillators, and electromechanical transducers. Various modes of motion (flexural, extensional and shear) can be excited in crystals by cutting them at

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* The assistance of Messrs. M.J.P. Musgrave and H.L. Cox of the National Physical Laboratory has been sought and through their co-operation a solution may, in fact, be found.

specified orientations and by applying a suitable driving force. Coupling between modes is often reduced to a minimum by choosing a crystal orientation for which the elastic coefficient governing coupling is a minimum.

Again, a thin strip of rotary cut timber (rectangular cross section) under the action of a bending moment, will both bend and twist if it is cut at an angle to the grain (Hearmon, 1943). This is another example of the effect of coupling between shear and extension, which arises when S_{16} differs from zero.

It is reasonably certain, therefore, that coupling between shear and extension would tend to make wall of Nitella exhibit the sort of motion which has been described; and it is suggested that this could well be the mechanism by which wall elements are laterally displaced relative to one another, i.e., this is the basic mechanism of Spiral Growth. In the absence of a general analytical expression, however, it is not possible to predict either the sign or the magnitude of the effect.

This deficiency has been rectified to some extent by demonstrating experimentally that the cell wall of Nitella does exhibit a torsional rotation about its own axis, when the internal pressure is increased, i.e. that there is, in fact, a coupling between shear and extension. The experimental method and the results will be discussed in a later section. It is sufficient to say at this stage that the effect is measurable and that the sign of the rotation is consistent with it being connected with spiral growth in Nitella.

It is, of course, not sufficient merely to demonstrate the presence

of a measurable torsional strain when the cylindrical wall is pressurised. It is still necessary to make some assumptions about the nature of the growth process in order to understand the connection between a static strain, and the continuous rotation exhibited during growth. If extension growth is in some way linked to a plastic stretching of the wall under turgor (as is supposed in mechanical theories of extension growth), there seems to be no great obstacle to extending the concept to include rotational growth provided that a mechanism for producing lateral displacements of the wall elements is known to exist. Coupling between shear and extension is thought to be this mechanism.

It has been assumed in the foregoing discussion, involving the general equations for Hooke's Law, that the wall of Nitella can be regarded as a perfectly elastic homogeneous material and that the strains are infinitesimal. It has already been shown that material exhibits time effects and is not homogeneous at the submicroscopic level. Since the aim of the discussion is to show merely the sort of mechanism which may be operating, the assumption of an ideal material is probably quite safe.

SPIRAL GROWTH OF NITELLA OPACA AG.

As had already been mentioned, a study of the growth habit of Nitella axillaris Braun was made by Green (1954). Briefly, he found:-

(a) Growth was evenly distributed, throughout the length of the internodal cell, and

(b) that internodal cells exhibited the phenomenon of spiral growth.

Young cells exhibited a right-handed rotational growth, while at a later stage (length about 1 cm.) there was a reversal of direction of rotation. Left-handed rotational growth ceased, however, while there was still a net righthand rotation.

Although it seemed very likely that N. opaca Ag. would behave in much the same way as N. axillaris Braun, it was thought advisable to verify this behaviour. Particularly so, in view of the fact that there is some disagreement about wall organisation.

Green measured spiral growth by placing markers on the cell wall and observing their relative movement. This is a difficult and time-consuming method, and an alternative method was therefore sought. If it is assumed that there is no "slip" of the striation relative to the ends of the cell, then rotational movement of the cell during growth can be deduced from the dimensions of the cell, and the angle which the striations make to the cell axis.

$$n = \frac{l}{\pi d \cot \alpha}$$

, where n = number of turns which the striation makes about the cylindrical wall.

l = length of cell

d = diameter of cell

α = angle of striation to axis of cell.

By measuring , l, and d, it is possible to calculate, n, at various stages of growth and to relate this directly to the spiral growth of the cell.

There is also an alternative method suitable for more mature cells. If both "front" and "back" striations can be observed and the distance between points measured, then n can be calculated as follows (see Fig. 73):

$n = \frac{l}{l'}$; or, $n = \frac{l}{2l''}$ where l = length of cylinder

l' = "pitch" of striation

l'' = "½ pitch", or, distance between two successive "cross-overs".

These methods do not give as much information as Green's method (e.g., they give no information about growth distribution), but they are quite suitable for verifying that rotational growth is occurring.

The "striation" method is based, however, on the assumption that there is no "slip". According to Fritsch (1935), "The orientation of the streams in the internodes of the long axes is related to the position of the whorl of short laterals above, the ascending stream being situated below the oldest, the descending one below the youngest member of the whorl". Green (1954) states: "Because the total θ (rotation - m.e.p.) of a cell, computed from the configurations of the striations, is greater than the observed θ of the highest mark by a fairly constant number of degrees

through time, the assumption that the striations are representative portions of the wall is justified".

The assumption of no "slip" seemed, therefore, to be confirmed by this earlier work. In spite of this, however, it was decided to check this point independently. This was done because it is not only important to establish that the striation is fixed in relation to the wall surface in order to use the "striation" method of inferring spiral growth, but it is also important with regard to the interpretation of the discontinuity of wall structure which occurs in the region of the striation (see "Polarizing Microscope", Part II).

A photographic method was used in which the top and bottom of an internode were photographed at intervals, and the rotation occurring during the intervals was calculated from the relative positions of surface details, leaf bases etc. at the beginning and end of each interval. To calculate rotation, d', x', d'', and x'' (refer Fig. 74) were measured off the photographs, and the values inserted in the following:

$$\theta' = \sin^{-1}\frac{2x'}{d'} \quad ; \quad \theta'' = \sin^{-1}\frac{2x''}{d''}$$

$\theta'' - \theta'$ = rotation of point, A, during the time interval $(t'' - t')$.

The figures were finally adjusted to give the rotation of the top of the internode relative to its base.

At the same time as the photographic method was being used,

measurements of the rotation were made by the "striation" method outlined earlier. The rotations obtained from the "photographic" method and the "striation" method, are plotted against time in Fig. 75. With a small allowance for experimental error, the two methods give the same total rotation. The errors amount to no more than ± 0.1 in the value of n. Small errors can arise in the caluclation of "n" by the striation method by virtue of the fact that the cell is assumed to be a perfect cylinder and the striation angle constant, whereas both these assumptions, although very good, approximations are not exact (see Table 7 which is for the set of data referred to above). When these sources of error are taken into consideration, the agreement between the two methods is very good, and suggests that the striation does not "slip" during growth.

TABLE 7

Time (hrs)	0 11.12.58	5	26	46	71	95	120	143	167	190	216	240	288	384
Angle A		-	-	-	-	-	-	-	7.4					
Angle B	17.1	15.8	12.8	12.2	10.4	9.6	8.7	7.3	7.1	Angle not measured -				
Angle C	16.7	16.2	13.8	12.4	11.2	11.0	9.2	7.8	7.0	crossed striations used				
Angle D	17.5	16.4	14.2	13.1	11.6	11.6	9.8	8.3	7.9					
Mean Angle	17.1	16.1	13.6	12.6	11.1	10.7	9.2	8.1	7.35	-	-	-	-	-
Diameter														
A	-	-	-	-	-	-	-	0.322	0.325	0.332	-	0.347	-	-
B	0.281	-	0.285	0.296	0.299	0.303	0.314	0.322	0.330	0.336	-	0.344	-	-
C	0.272	-	0.285	0.296	0.299	0.312	0.312	0.319	0.329	0.336	-	0.344	-	-
D	0.283	-	0.286	0.297	0.301	0.306	0.315	0.329	0.331	0.335	-	0.340		
Mean Diameter	0.280	0.278	0.284	0.296	0.300	0.304	0.314	0.323	0.329	0.335	-	0.344	-	-
Length	4.5	4.8	6.2	7.6	8.6	9.7	11.9	14.6	17.8	21.7	25.4	26.7	27.3	28.3
"n"	1.57	1.59	1.68	1.82	1.80	1.92	1.96	1.98	2.22	2.42	2.62	2.63	2.68	2.60
Rotation Angle	0	7	40	90	83	126	140	148	234	306	378	381	400	370

N.B. Points, A, B, C, and D are points at which the striation is visible on 'front' of cell; or, to put it another way, where striations "cross" - See Fig.

An additional check on the position of the striation was obtained directly from the photographs. Whenever a striation was visible on successive photographs and could be followed through a substantial rotation, it always terminated at the same location relative to the bases of the "leaves" at the internodes. It is concluded, therefore, on the basis of this work, and on the basis of the earlier work referred to above, that the position of the striation is fixed in relation to the surface of the wall (it is important to remember that Green has established that the whole wall is taking part in the growth process, and extension and rotation are uniform over the wall surface).

It has already been shown in Part II ("Polarizing Microscope"), that there is acorrelation between streaming direction and cell dimensions. The data obtained to establish this relationship, contained the necessary parameters (length, diameter, angle of striation) to calculate n, as defined above. This has been done, and in fig. 76, n is shown plotted against cell length. These data were obtained from measurements on separate individual cells and do not, therefore, reflect the behaviour of an individual cell during growth. It is clear, however, that n varies with the age of the cell and, that as the cell elongates up to a length of 10 mm. (or a little over), n increases. Further, since the striation makes a right-hand spiral around the wall, an increase in n implies that the cell is exhibiting right-handed rotational growth. For cells longer than this rather arbitrarily selected length, there is considerable scatter in the value of n, which suggests that some cells

continue to spiral to the right, while others either cease to spiral, or reverse their direction of spiral growth.

The cell whose rotational growth was followed photographically, continued to spiral to the right until just before it ceased to elongate at a length of 27 mm. The rotation of a number of other internodal cells was followed during elongation and it was found that they generally exhibited a reversal of growth at a somewhat shorter length. Of the eleven cells whose growth history was followed, there was a reversal of direction at the following lengths; 27 mm; 14 mm; 13 mm; 10 mm; 10 mm; 8.5 mm; 8 mm; 8 mm; 7 mm; 7 mm.

In general, therefore, it appears that Nitella opaca Ag. exhibits much the same sort of spiral growth behaviour as Nitella axillaris Braun.

A further point which arises out of these measurements of spiral growth is that the empirical relationship;

$1/d = a + b \cot \theta$, which was derived earlier (Part II "Polarising Microscope"), is only an approximation, since b is now shown to be a function of length.

TORSION OF CYLINDRICAL CELL CAUSED BY PRESSURE CHANGES

It has been suggested that spiral growth in Nitella might be due to mechanical stresses which arise when the elastically anisotropic wall is stressed by turgor pressure. This suggestion would be very greatly strengthened if it could be shown that the end of the cylindrical cell describes a torsional movement when the turgor pressure is changed. The demonstration of a measurable twist would not only show that anisotropy of the wall and the inclination of the elastic axes was of sufficient magnitude to cause an effect worthy of serious consideration, but it would also provide a means of comparing the sign of the static twist against the sign of the growth spiral.

A suitable experiment to test the theory was therefore devised. The cell to be tested was suspended vertically from a fixed support and allowed to hang free in a beaker of distilled water. A light pointer was attached to the lower end of the cell to indicate rotation about the cell axis. The test cell was intact and undisturbed, the mounting to the support and to the pointer being made via the empty cells above and below it (Fig.77).

The cell with attached apparatus was then transferred to a Mannitol (or, Sucrose) solution which was just sufficient to plasmolyse the cell. As the turgor pressure dropped the free end of the cell rotated about the cell axis showing clearly a coupling between shear and extension. The direction of twist was reversed when the cell was again placed in

water and the turgor pressure increased.

The magnitude and direction of rotation, as pressure increases from a state of plasmolysis to full turgor, is shown plotted against the length of the cell in Fig. 78. The points are considerably scattered, but one feature emerges quite strongly; for cell lengths less than about 12 mm. There are 16 points which indicate a right-hand rotation and none which indicate left-hand rotation, while for lengths greater than this arbitrary figure there are 13 indicating left-hand rotation and 2 indicating right-hand rotation. The very great scatter of points is not difficult to understand. The measurements are not easy to make, particularly since one is dealing with fairly delicate cells. There are, therefore, quite large measurement errors. Again, it is to be expected that there will be some variation from cell to cell (particularly when grown under different conditions) of these mechanical features of the wall which control magnitude of strain, viz. elastic modulii (particularly the shear modulii) wall thickness, turgor pressure, angle of m.e.p., length/diameter ratio, etc.,

There is, therefore, no reason to suppose that when plotted against length, the magnitude of the twist should lie on a smooth curve. Length just happens to be a convenient parameter to indicate age, stage of development, etc..

Because the very great scatter of points tends to obscure the main features, the data have been replotted in Fig. 79. In this figure <u>direction</u> only is shown, and it is plotted on a logarithmic length

length scale. This scale has been chosen merely to avoid crowding at the lower end, and not because it has any special significance. This method of plotting the results brings out the very notable behaviour of the cells more clearly. The following observations have been made:-

(a) The cells show an easily discernable torsion when the internal pressure is increased from zero (gauge pressure) to full turgor.

(b) The direction of rotation is consistent with the direction of spiralling observed in spiral growth. All young cells appear to twist to the right while most of the older cells twist to the left. There is no inconsistency in the behaviour of the three cells which show a right-hand twist at lengths of up to 4 cm. One cell of the Nitella opaca internodes spiralled to the right up to a length of 27 mm., although the majority reversed at lengths far short of this. This distribution of directions is not one which would have arisen by pure chance with any reasonable degree of probability.

In Fig. 80 the direction of twist has been plotted against the angle of the m.e.p. (with due regard as to whether the m.e.p. is directed so as to make a left or a right-hand spiral around the wall). Those cells in which the m.e.p. makes a left-hand spiral around the cylindrical wall exhibit a right-hand twist, while the twist is opposite for cells where m.e.p. makes a right-hand helix. There is one exception;

one cell with a right-hand m.e.p. also showed a right-hand rotation. (This result has been rechecked as far as was possible from the figures and there was no inconsistency such as would suggest an error).

The change-over from right to left-hand twist does notseem to occur when the m.e.p. is zero, but at a point where itmakes a small left-hand helix (about 2°). This could either be due to a small error in the setting of the cross-wires of the microscope; or, it might well be that the axis of elastic symmetry does not exactly coincide with the m.e.p.

A very indirect, and certainly not very accurate, check on this "change-over" point has been obtained in the following way. It appears from Green's work, and from the measurements on spiral growth made during this investigation, that reversal of spiralling quite frequently (but by no means always) takes place at a length of <u>about 1 cm.</u> Further, the measurements of direction of twist suggest that at about 12 mm. there is a critical point (perhaps in the form of a lower limit). From the records it has been possible to calculate a mean chloroplast angle for a cell 1 cm. long. The mean angle is about 14° (as a matter of interest, for the cell data given by Green (1954) in his fig. 4, the angle at 1 cm. is 11°). If a straight line of best fit is fitted to the points in Fig. 6 (streaming angle v angle of m.e.p.), the m.e.p. angle corresponding to a chloroplast angle of 14° is 93°. In terms of the scale used in Fig. 80, this corresponds to helix angle of 3° in the left-hand sense. Too much weight cannot be given to this value, but

it is nevertheless in very good agreement with the change-over point as indicated in Fig. 80.

DISCUSSION

It has been demonstrated that Nitella opaca Ag. exhibits much the same sort of spiral growth behaviour as Nitella axillaris Braun. Further, it has been established that when the turgor pressure is changed the cylindrical wall twists about its own longitudinal axis, and this is interpreted as being due to a coupling between shear and extension arising out of the anisotropy of the wall. This is the first time, to the author's knowledge, that this effect has been observed and interpreted in this way.

Consideration has been given to the possibility that this "pressure-change torsion" might be due to some other mechanism unrelated to the anisotropy of the wall. For example, the possibility has been considered that it is due to a protoplasm/wall interaction which occurs as the protoplasm contracts away from the wall during plasmolysis. It is unlikely, however, that the protoplasm has the requisite mechanical properties. It is just possible that its properties might be such as to cause a small torsional effect of short duration, but it is unlikely that a material of the nature of protoplasm could maintain such a twist. Further, even if the possibility of such a mechanism was entertained, there seems to be no obvious explanation of reversal of twist, since the only (known) directional characteristics of protoplasm in Nitella (streaming) is always in the right-hand sense.

Another possibility considered was that "pressure-change

torsion" is due to anisotropic swelling arising from a change in "bound" water content of the cell wall when placed in the sucrose solution. Again, this is not a very likely cause; and it is rendered even less likely, by the fact that the twisting effect is also observed when the cell is kept in distilled water and the turgor pressure is reduced by puncturing the cell wall.

It seems very likely, therefore, that twisting of the wall is due to the elastic anisotropy of the wall itself.

The question now arises as to whether "pressure change torsion" and spiral growth are linked. The direction of twist as a function of cell length agrees very well with the observed direction of spiral growth when it also is considered as a function of cell length. There is, however, a small discrepancy in that, from the twist measurements reported here, one could expect that the cells would tend to show a reversal of sprialling at a slightly longer length than in fact they appear to. This might be due to the fact that the growth observations, of necessity, were made on tank-grown material, while the twist measurements were made on material freshly collected from the pond. That the twist measurements were not also made on tank material is due to the fact that, at the time these measurements were made, the tank-grown material was in rather a poor state. It is possible, therefore, that this discrepancy is due to a different growth condition. If one refers to fig. 76, however, it does appear from these data, which also refer to freshly collected material

that spiralling continues at least up to 10 cm. or beyond it. This is completely consistent with the twist evidence.

In general, therefore, the agreement between direction of twist and direction of spiral growth is extremely good, and is consistent with the idea that they are causally linked.

Independent evidence that this is the case has been obtained by Preston & Frei (private communciation) working with the filamentous alga, Chaetomorpha. This alga also exhibits the phenomena of spiral growth, and it has been shown that the direction of twist (as the pressure rises) is in the same direction as the direction of spiral growth.

The evidence that the phenomena of "pressure-change torsion" is linked with the phenomena of spiral growth is, therefore, very strong indeed.

There is, also, quite good evidence, already presented, that pressure-change torsion is caused by the sort of mechanism proposed in this thesis. To establish this more surely it is necessary to see if it could also be the mechanism of spiral growth in other types of cell.

The wall structure of the growth zone of sporangiophores in Phycomyces seems to be, at least superficially, similar to that of Nitella, and it is not unreasonable to propose a similar mechanism of spiral growth in this case. A difficulty arises, however, due to the fact that the direction of spiral growth in Phycomyces reverses without any (known) change in wall structure (e.g., direction of m.e.p.). The sign of the shear/

extension coupling coefficients can be changed without changing the sign of Θ by choosing suitable values of the elastic coefficients. Whether such suitable values of the elastic coefficients are physically realizable, however, is at present in doubt.

The wall structure of Chaetomorpha is quite different from that of Nitella and from that of the sporangiophore of Phycomyces. It is possible that the cross-fibrillar wall structure (angle between crossed fibrils ~~plants~~ is 80°) could be regarded as having the elastic symmetry of a monoclinic crystal. This type of crystal requires 13 independent elastic coefficients to completely specify its elastic behaviour. These 13 coefficients include as independent coefficients the coupling coefficients which appeared in the orthorhombic case only when there was a rotation from the principal axes. It is to be expected, therefore, on the basis of the ideas expressed earlier in this thesis, that Chaetomorpha will exhibit a "pressure-change torsion" effect and the phenomena of spiral growth. It is known to exhibit both.

CONCLUSION

The aim of this investigation was to examine the molecular structure and mechanical properties of the plant cell wall in relation to growth. The internodal cell of Nitella opaca was selected for this study because, being a single cell, it was free from tissue interactions and being large, it was possible to determine the mechanical properties of the wall directly.

Experimental work was divided into three main parts and this subdivision has been preserved in the presentation of this thesis. In the first phase of the experimental work (Part II of thesis) the structure of the wall was examined in order to provide a basis for the interpretation of later work, and to provide information on the nature of the material to be submitted to mechanical test. Apart from supplying this basic information, these structural investigations extended existing knowledge of the wall structure of Nitella (see summary at end of Part II).

In Part III, mechanical properties of the wall (tensile moduli and creep behaviour in direct tension) were determined, and examined in relation to the structure revealed in Part II, and in relation to the measured growth rate of the cells tested.

In Part IV evidence was presented which suggested that, at least in the case of the phenomena of spiral growth, mechanical forces play a part in determining or modifying the course of growth. A theory

of spiral growth was proposed in which the process was interpreted in terms of the structure determined in Part II and the mechanical properties determined in Part III.

The results of the experimental work on the mechanical properties of the wall, might be summarized as follows:

(1) The wall was found to be elastically anisotropic, the tensile modulus being higher in the transverse direction than in the longitudinal direction. It is known from the work on wall structure that there is a preferred transverse orientation of the crystalline component. The high transverse modulus is, therefore, consistant with this preferred orientation of the cellulose microfibrils, which, by the nature of their crystal structure, can be considered as regions having a high tensile modulus. A high tensile modulus was also observed in the wall of Valonia, in the directions of the main cellulose chains.

(2) Again, by virtue of their crystal structure, the microfibrils will not be readily subject to flow and it has in fact been found that the rate of creep in transverse wall strips is negligible compared with the rate of creep of longitudinal strips. It is interesting to note in this connection that the rate of growth in the transverse direction is small compared with that in the longitudinal direction.

(3) The anisotropy of the wall as indicated by the ratio E_T/E_L was positively correlated with the growth rate and the correlation was highly significant. Reasons were given for thinking that this might, in some degree, be due to increasing disorientation of the microfibrils

in cells nearing the end of their elongation. The proportion of water - soluble materials present in the wall was also positively correlated with growth rate (significant at the 0.02 level), and this might also have a bearing on the observed fall in the ratio E_T/E_L. As the growth rate diminished, the ratio E_T/E_L appeared to approach the value 2. This is the ratio of the principal stresses in a thin-walled pressurised cylinder. This may be a coincidence but is noted in passing.

The correlation between E_T/E_L and cell length was negative but not significant.

(4) The stress at which permanent set was first observed was considerably higher for transverse strips than for longitudinal strips.

(5) For uniaxial stress in the longitudinal direction, the creep rate increased rapidly beyond a certain limiting stress. This observation is limited to the effect of uniaxial stress and cannot necessarily be accepted as a literal statement of the behaviour of material when subjected to a multiaxial stress in the wall. It does, however, suggest a possible danger if mis-interpretation of data obtained from experiments on the elasticity and plasticity of whole tissue (e.g., coleoptile tests in which the wall stress is indeterminate).

(6) Evidence was presented which suggested that the rate of creep was higher for longitudinal strips cut from 'high growth rate' cells, than it was for similar strips cut from 'low growth rate' cells. Agreement between creep rate and growth rate was improved if both the

physical properties of the material and the dimensions of the cell were taken into account (the dimensions of the cell, determine the wall stress for a given turgor pressure). This is further evidence that the physical properties of a growing wall of Nitella are rather different from those of a non-growing wall.

(7) Experimental evidence was presented which indicated that the wall contains cationic binding groups. The creep behaviour of some cell walls can be influenced by the nature of the cations to which they have been subjected. Longitudinal strips cut from one cell wall did not show a significant difference in creep behaviour when treated with $CaCl_2$ and KCl. It may or may not be significant that this cell had the highest growth rate of those tested. It was suggested that the magnitude of the difference in creep behaviour between strips treated with $CaCl_2$ and KCl might depend on the degree of esterification of the carboxyl groups. No supporting evidence was presented, but a possible experimental check was suggested.

(8) Removal of water soluble substance from the wall did not significantly alter either the transverse, or the longitudinal, tensile moduli, but the load at which permanent set of longitudinal strips occurred was lowered substantially. Insufficient measurements were made to establish clearly the effect of removal of water soluble substance on the creep rate, but, in one case, for a cell having an intermediate creep rate and growth rate, there was a substantial increase in rate of creep after this treatment.

(9) The elastic properties of the wall were generally consistant with what one might have expected on the basis of their wall structure, viz. a high tensile modulus and low creep rate in the direction of the preferred orientation of crystallites. Cox (1952) has published an analysis of the effect of orientation of the fibres, on the stiffness and strength of materials which derive their strength wholely or mainly from thin fibres which are capable of transmitting high loads along their lengths but which offer no great resistance to loading transverse to their lengths. The results of the analysis were applied to samples of resin bonded fibrous filled materials and moderately good agreement with experimental results was found. The plant cell wall is a material of this general type and it might be profitable to apply Cox's analysis in this case. By measuring those elastic modulii which can be more easily measured and by measuring the distribution function of fibril direction, it might be possible to make reasonable estimates of those elastic constants which are not amenable to measurement. Such an approach might be very useful in a theoretical study of stresses in the cell wall which are thought to lead to spiral growth.

(10) The data summarised above suggests that the mechanical properties of a growing wall are different from those of a non-growing wall. In mechanical theories of growth, extension is held to be due to a disturbance of molecular forces within the wall, causing the physical properties to be changed in such a way that it is passively stretched by turgor pressure. While it has not been proved that the mechanism of

extension growth is of this sort, nothing has been revealed in the measurements of mechanical properties which is inconsistant with such a view. Indeed the evidence is wholely consistant with what one might expect if extension growth was brought about by such a mechanism.

In this connection the observations on spiral growth and 'pressure-change' torsion are relevant. It has been demonstrated that when the turgor pressure rises, the Nitella cell exhibits a torsional twist about its own axis and that the sign of the rotation is consistant with it being connected with spiral growth. This observation has been given added weight by the fact that a similar observation has since been made for Chaetomorpha (Preston and Frei, private communication). A theory has been proposed to account for this 'pressure-change' torsion in terms of the known wall structure.

If one accepts the idea that spiral growth is due to a mechanical force (arising within the wall) causing a lateral displacement of the wall elements - and such a view is widely held - it is difficult to deny that in extension growth mechanical forces play some part.

It might be said, therefore, that the evidence presented so far is generally in favour of mechanical theories of extension growth. As was pointed out in the introduction, however, there are some serious difficulties in accepting such a mechanism of growth - at least in its simpler forms.

Some of these are outlined below:-

(a) It has been pointed out by Preston (1952, 1958) that if growth of a cell involves mechanical stretching of the wall and if no

other overriding factors intervene, then cell form might go hand in hand with wall structure. Although in a number of cases (and Nitella is one) cell form and wall structure are mutually consistant, there are a number of instances which can be cited as evidence for complete independence of cell shape from wall architecture. Preston points out that in Valonia with its very special arrangement of microfibrils, the shape of the vesicle is globular; but Cladophora and Rhizoclonium, which have a very similar, wall structure, have an entirely different growth form. Chadophora and Spongomorpha have an almost identical growth form but entirely different wall structure.

(b) If plastic stretching under turgor is the primary mechanism of extension growth it is difficult to conceive, in the case of the highly crystalline wall of Valonia, how such a mechanism could operate (provided the assumption that the growth process can be interpreted in terms of structure determined on dry material, is correct).

(c) The wall stress of a thin-walled pressurised cylinder is proportional to the radius of the cylinder. If, therefore, in one section of a very long cell (e.g. Nitella) the radius is momentarily greater than that of the rest of the cell, the resulting increase in wall stress in this region will tend to preserve, or increase, the radius difference. The measurements of diameter listed in table 7 are remarkably uniform (except where measurements are made near a node where there is a slight swelling), and it is difficult to account for this uniformity on the basis of growth by simple mechanical stretching.

(d) It has already been pointed out that it is difficult to reconcile the constancy of the direction of the m.e.p. and of the magnitude of the birefringence, with the idea of passive reorientation of the wall elements as the cell elongates.

There are, therefore, serious difficulties in the way of accepting mechanical theories of growth in their simplest form. Preston (1958) has suggested that it seems likely that an understanding of growth processes is to be sought by combining the concept of the wall as a living system with a purely mechanical hyphothesis of the type proposed by Roelofson & Houwink. He goes on to say, "If for instance, loosening (and even partial dissolution) of microfibrils were caused by some active metabolic factor, with subsequent repair, and combined with the type of displacement envisaged in the multinet growth hypothesis, then the disorientation of microfibrils from the transverse might be expected not to keep place with elongation. The rate of disorientation would depend upon the relation between the rate of synthesis and the rate of elongation. This would further allow for the known intervention of metabolic factors in growth processes." The present series of measurements offer no evidence as to whether a more complex process, of the type outlined by Preston, is involved in extension growth. Such evidence as has been presented on the growth of Nitella internodes, however, seems to indicate that mechanical considerations do play some part in the growth process.

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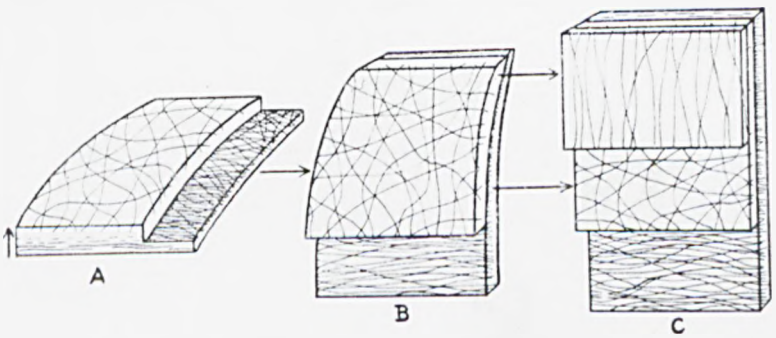


Fig. 2. The fibrillar structure in the growing cotton hair. A: near the top, B: where the tip merges into the tubular part, C: in the tubular part. Layer boundaries are drawn for illustrative purposes only; the transition is gradual.

B will gradually shift to the periphery and will acquire a random, or perhaps even a more nearly axial, orientation.

FIG. 1.

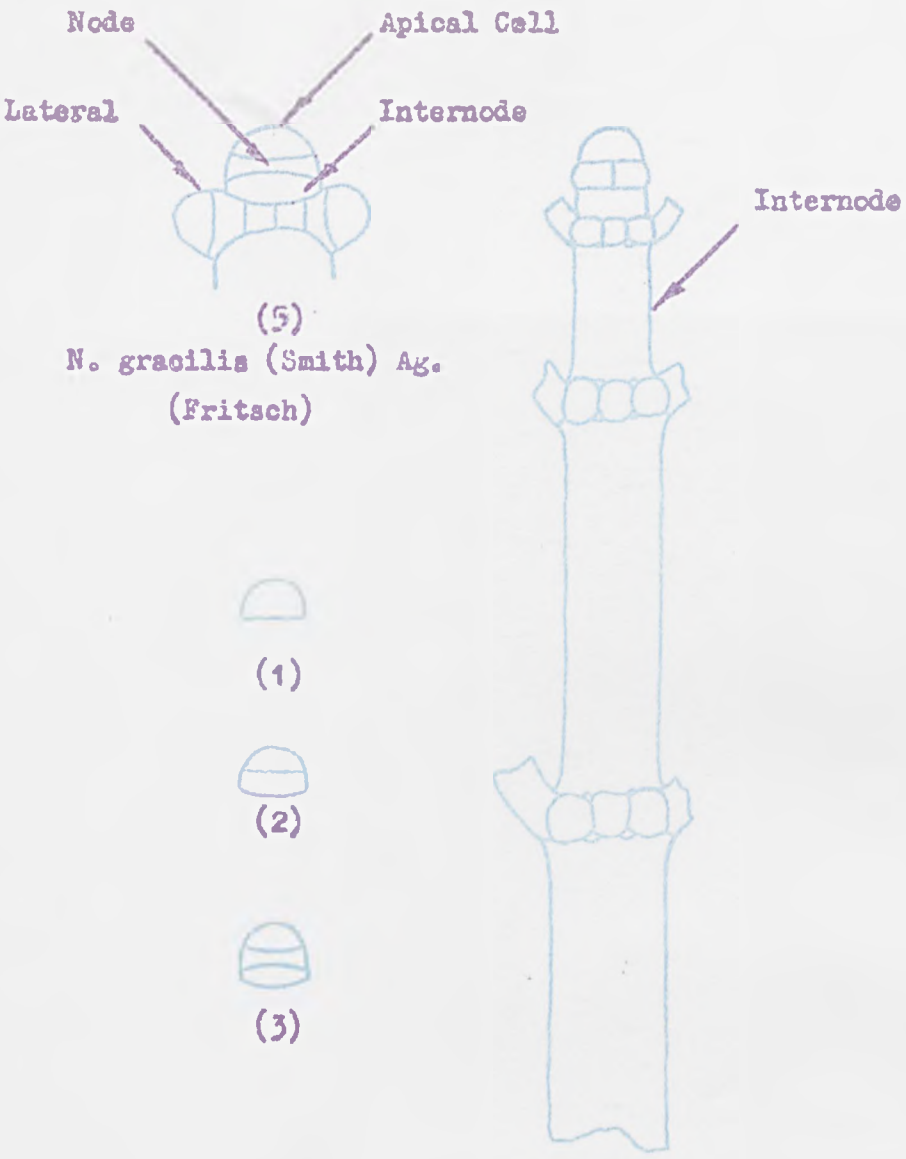


Diagram of a Nitella Shoot (After Green)

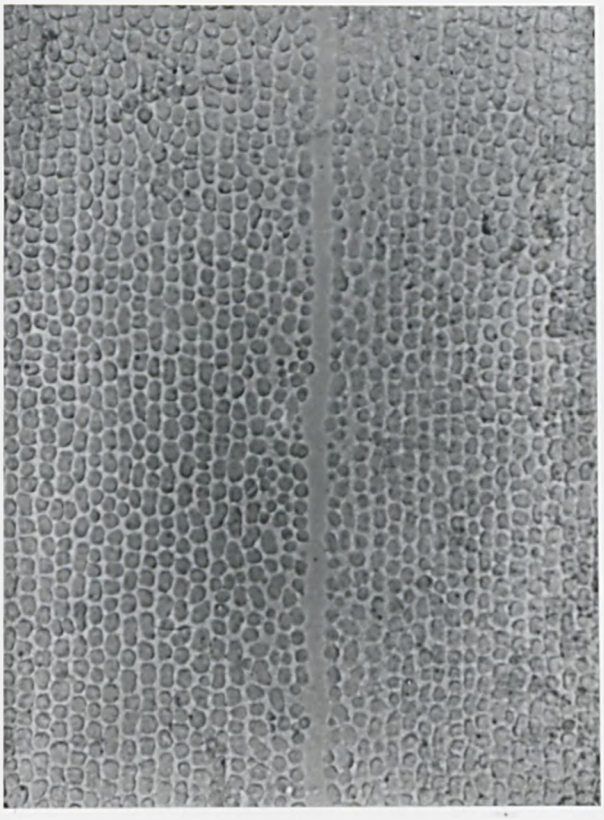
(6 - 9)

Transverse Sections of Young Stem-Node of

Chara Delicatula

(After Groves and Bullock-Webster)

FIG. 2.



CLEAR STRIATION BETWEEN FILES OF CHLOROPLASTS

FIG. 3.

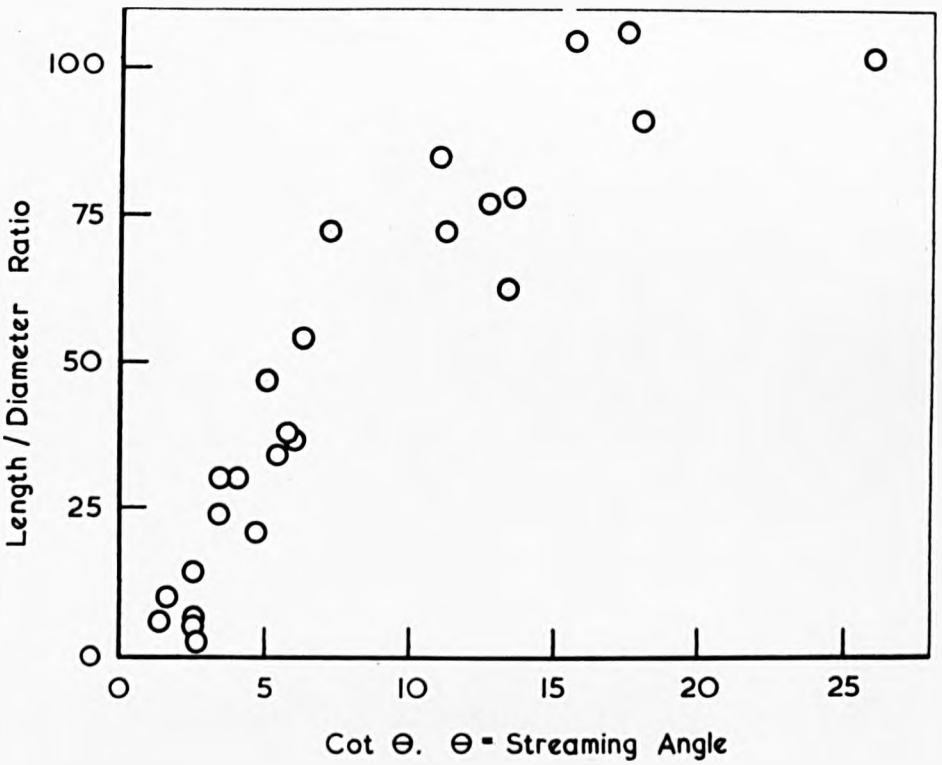
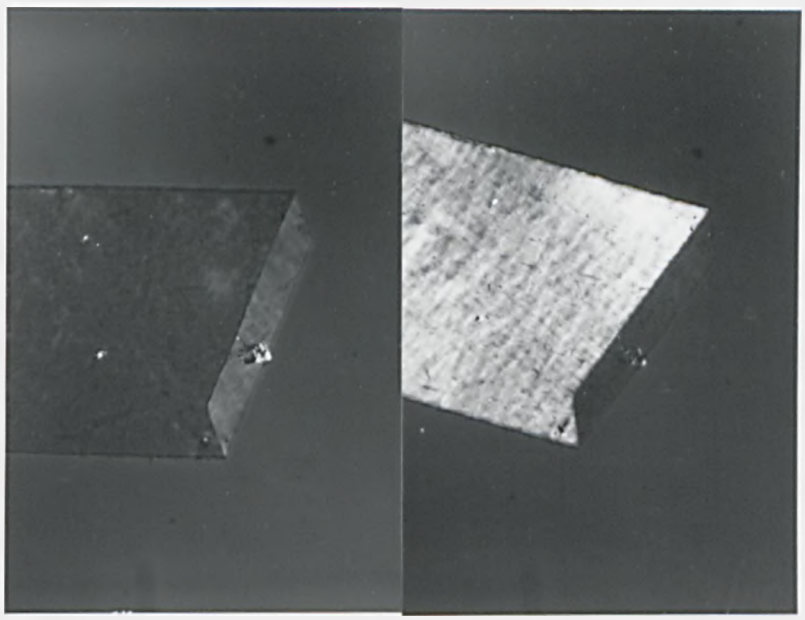

100
75
50
25
0
Length / Diameter Ratio
0
5
10
15
20
25
Cot Θ. Θ = Streaming Angle

FIG. 4.



(a) (b)

IN (a), DOUBLE WALL IS EXTINGUISHED

IN (b), SINGLE WALL IS EXTINGUISHED

AFTER ROTATION OF 14°. (X 95)

FIG. 5.

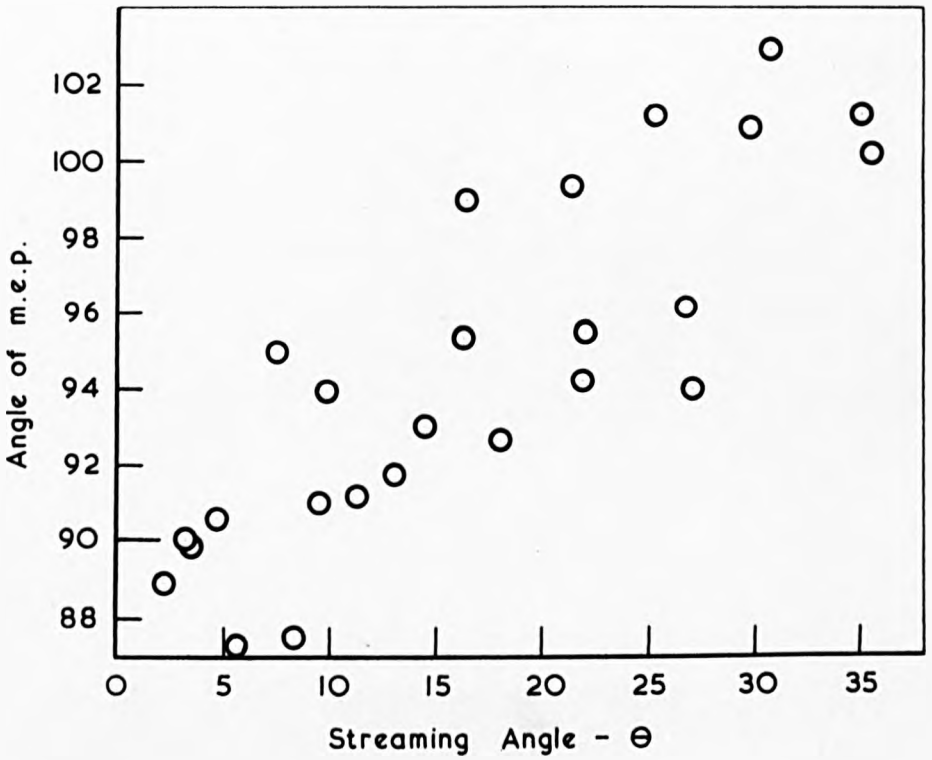
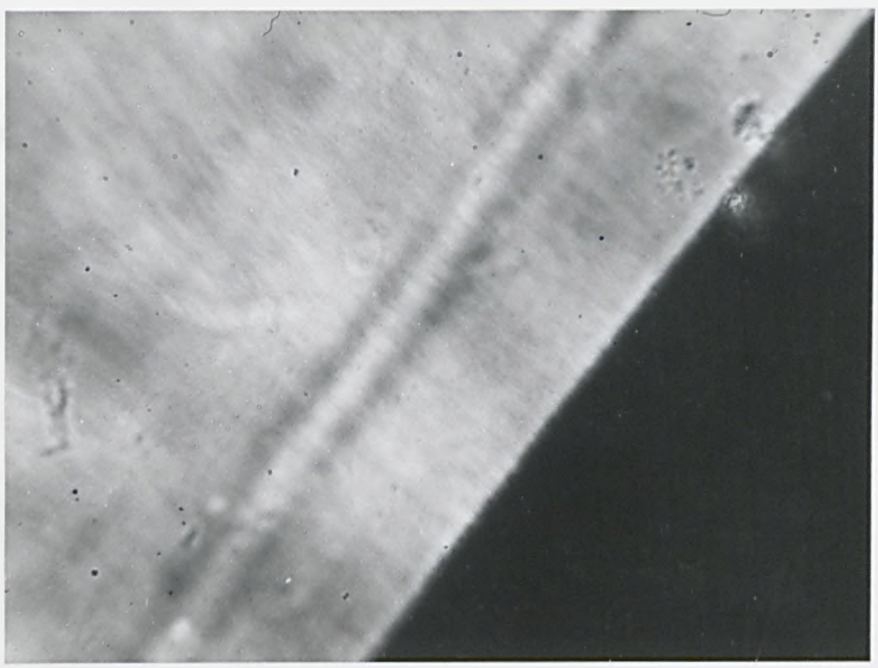

Angle of m.e.p.
102
100
98
96
94
92
90
88
0
5
10
15
20
25
30
35
Streaming Angle - Θ

FIG. 6.



STRIATION BETWEEN CROSSED NICOLS; COMPENSATOR DISPLACED FROM 'WALL EXTINCTION' (X 300)

FIG. 7.

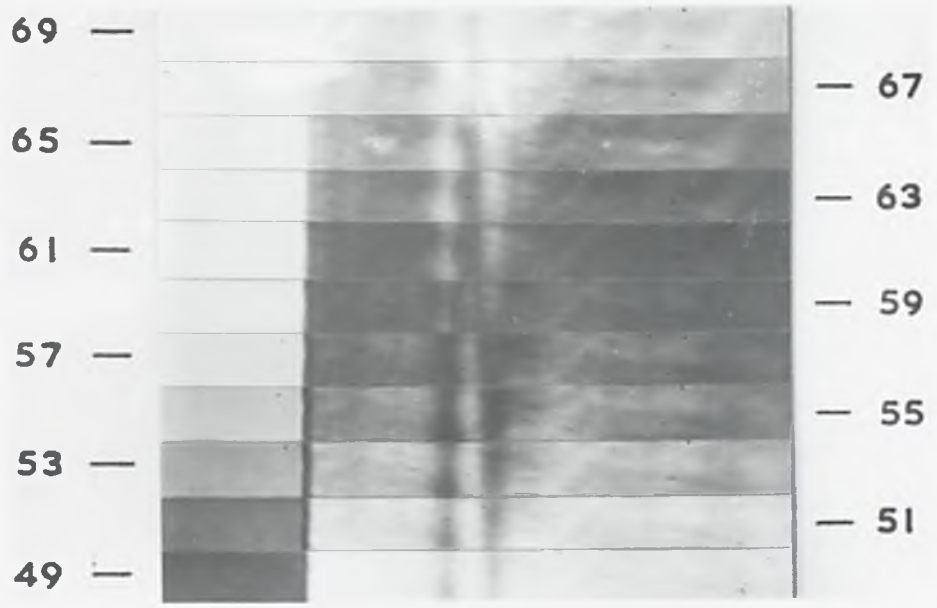
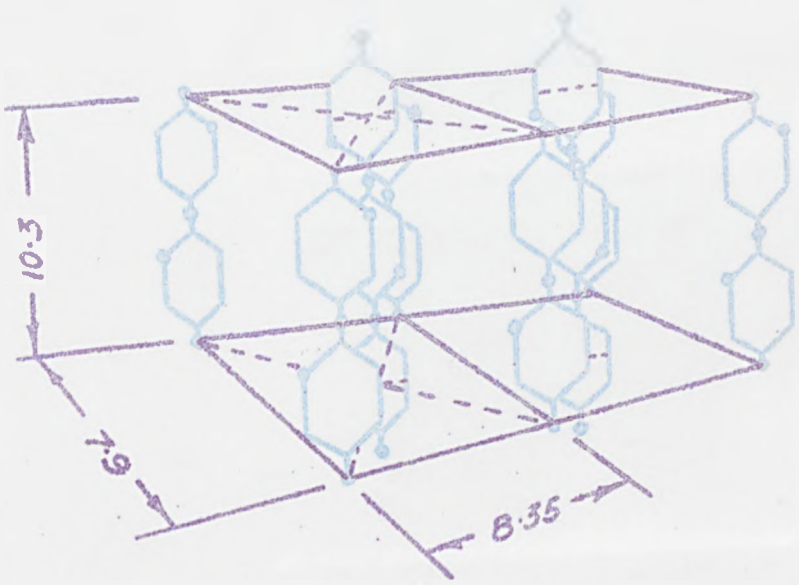


FIG. 8.

FOR EXPLANATION SEE TEXT

RIDGES (FOLDS?) IN REGION OF STRIATION

FIG. 9.



TWO UNIT CELLS OF CELLULOSE

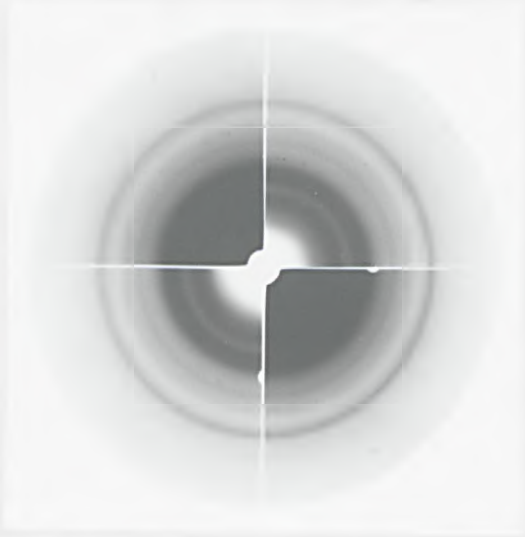
FIG. 10.

AFTER CHLORITE TREATMENT

FIG. 11.

AFTER ALCOHOL EXTRACTION

FIG. 12.

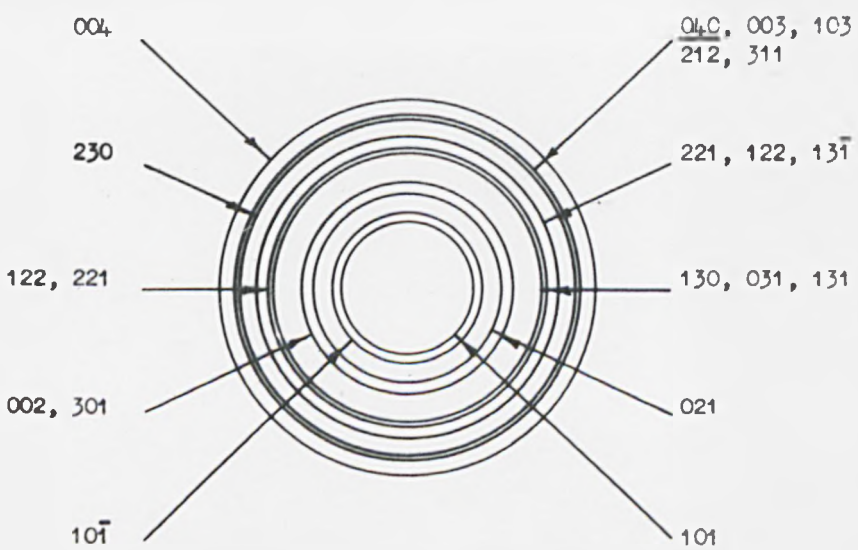


COMPARISON; WITH AND WITHOUT BEAM STOP

FIG. 13.

(a)

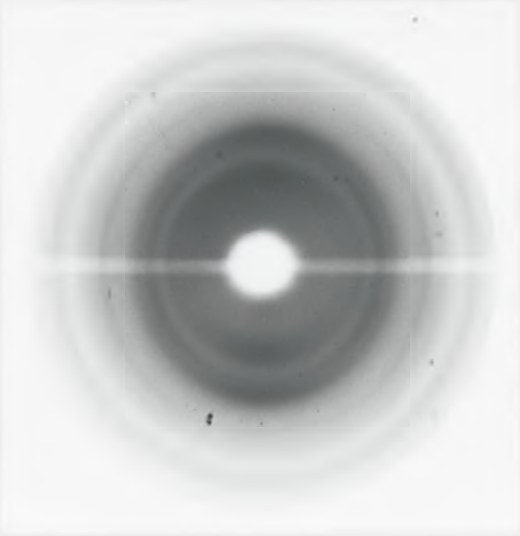
CLADOPHORA / NITELLA COMPARISON



(b)

ALLOCATION OF MILLER INDICES

FIG. 14.



X-RAY BEAM NORMAL TO WALL

FIG. 15.

X-RAY INTENSITY (ARBITRARY UNITS)
0
40
80
120
160
200
240
280
320
360
ANGLE
002 ARC

FIG. 16.

X-RAY BEAM PARALLEL TO PLANE

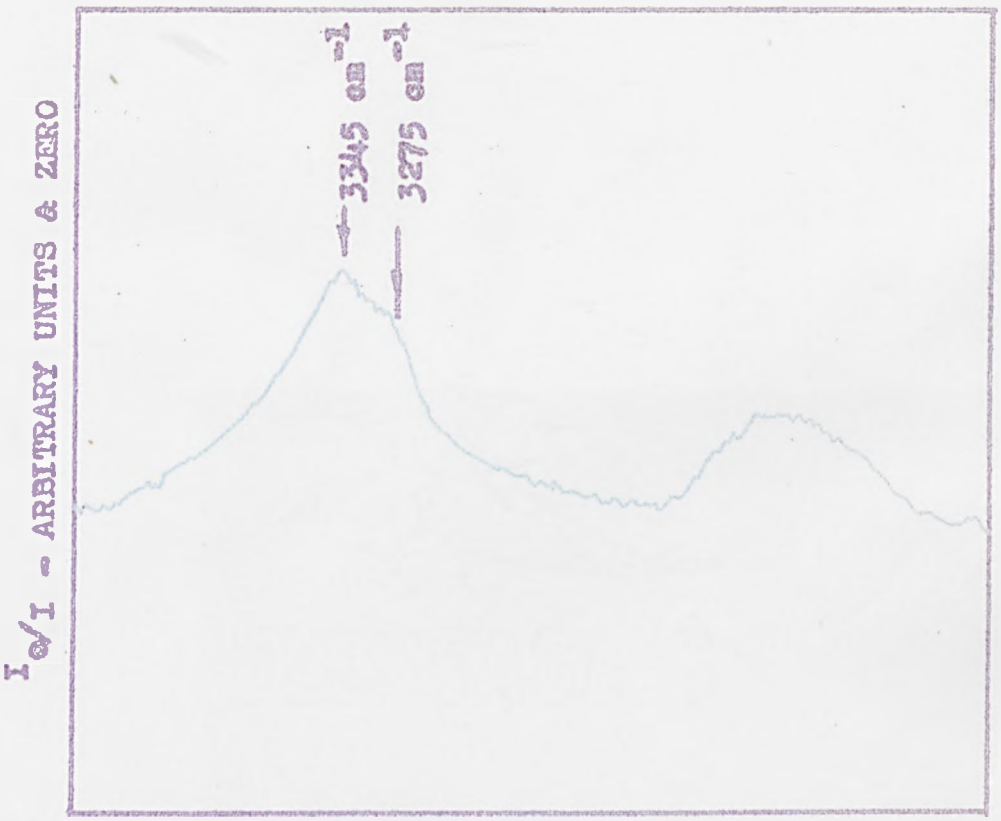
OF WALL AND TO TRANSVERSE DIRECTION

FIG. 17.

X-RAY BEAM PARALLEL TO PLANE

OF WALL AND TO LONGITUDINAL DIRECTION

FIG. 18.

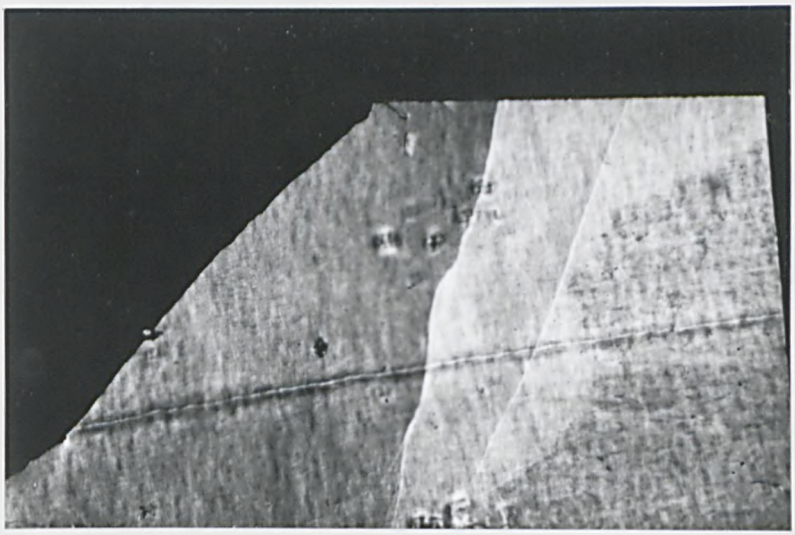


UNTREATED NITELLA AFTER VAPOUR DEUTERATION

NOTE:-

THIS CURVE IS TAKEN DIRECT FROM THE RECORDER CHART. IT IS A CELLULOSE I, TYPE B SPECTRUM (THE BAND AT 3242 cm^{-1} TYPICAL OF TYPE A IS ABSENT). THERE IS NO EVIDENCE OF PRESENCE OF CELLULOSE II.

FIG. 19.



TORN PIECE OF WALL BETWEEN CROSSED NICOLS

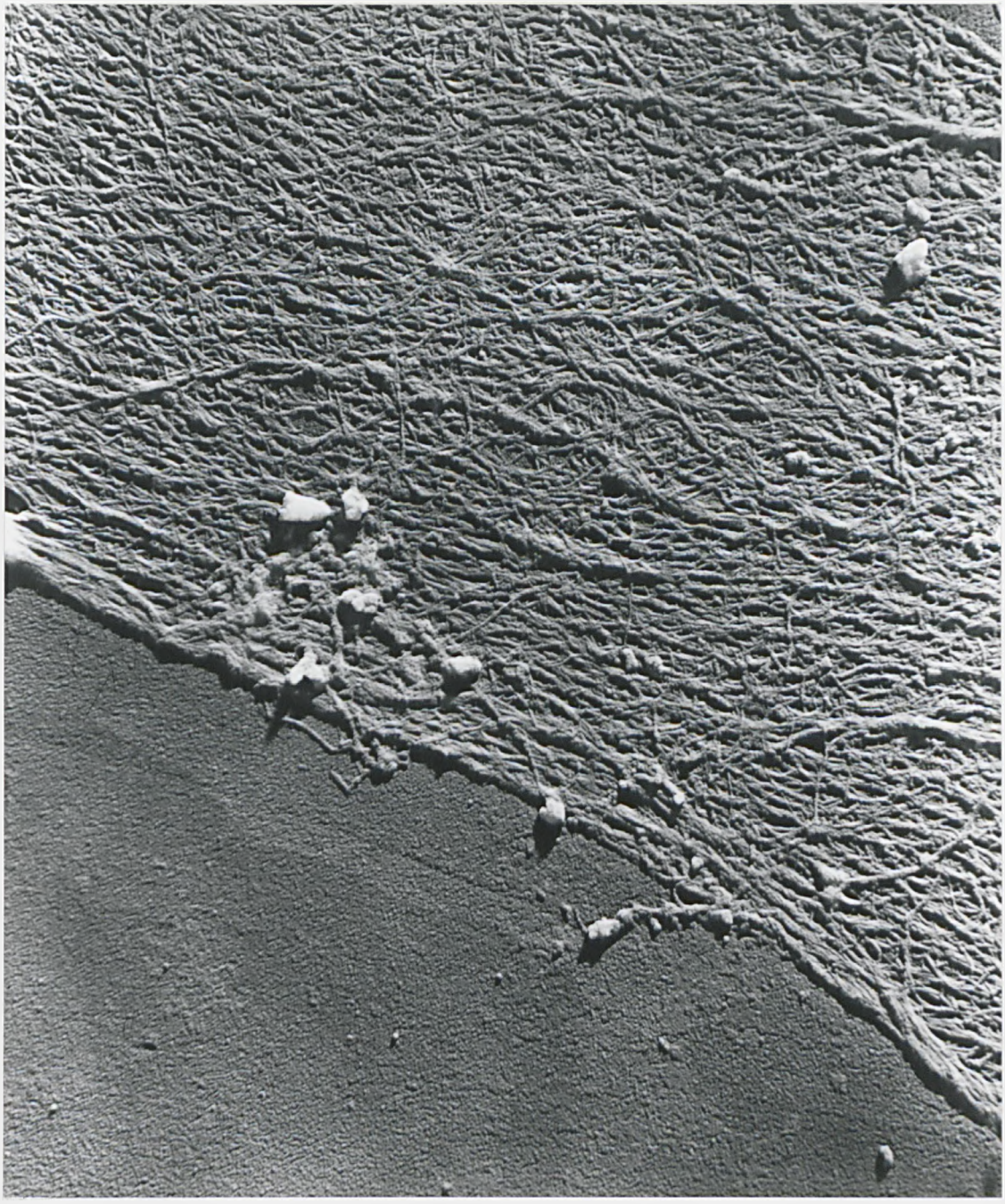
SHOWING STRIATION AND LAMINATION

FIG. 20.

AFTER ALCOHOL EXTRACTION

(Pd-Au SHADOWED)

FIG. 21.



AFTER ALKALI EXTRACTION

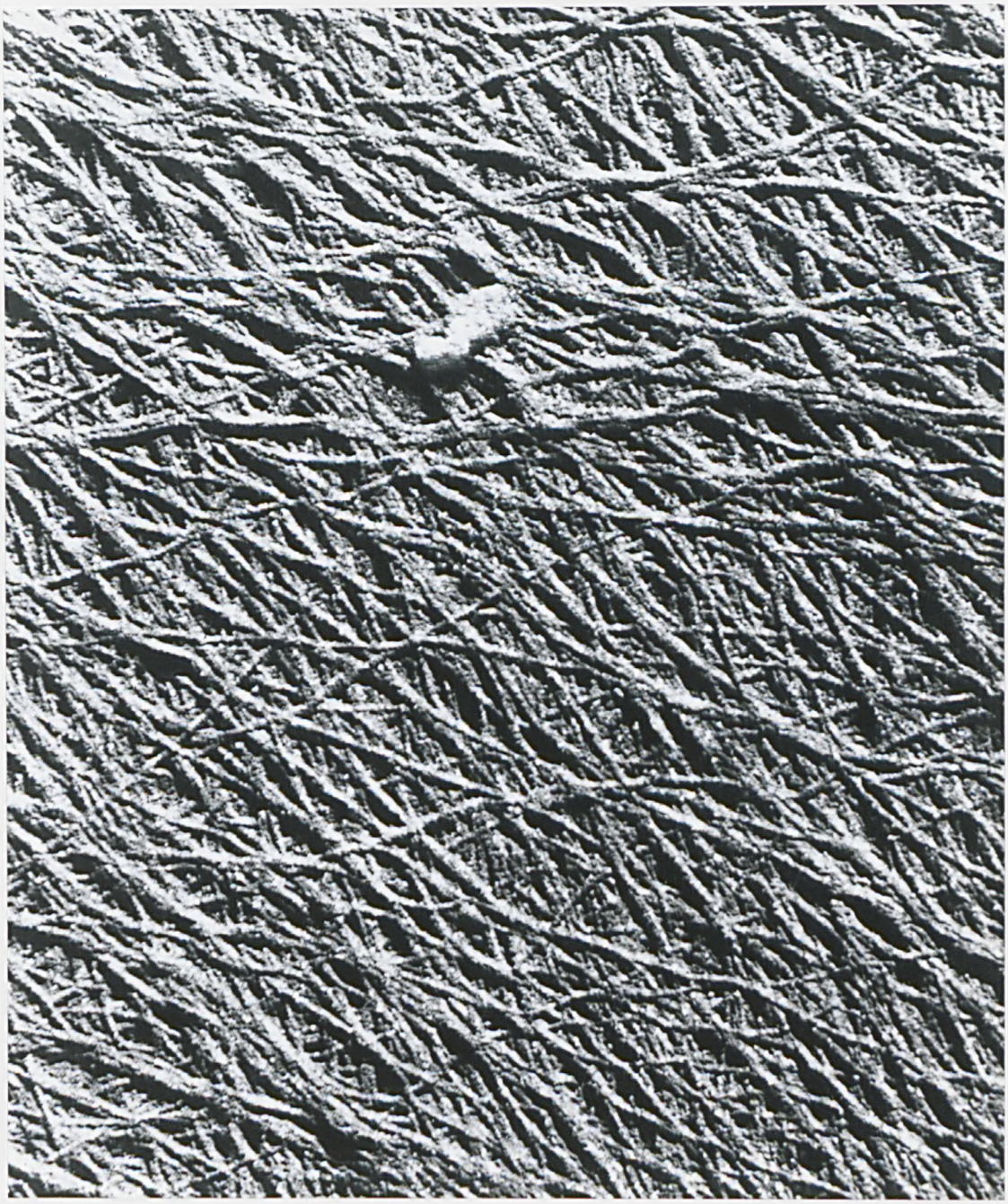
(Pd-Au SHADOWED)

FIG. 22.

'STRIPPED' WALL AFTER AMMONIUM OXALATE TREATMENT

GOLD SHADOWED (X 43,500)

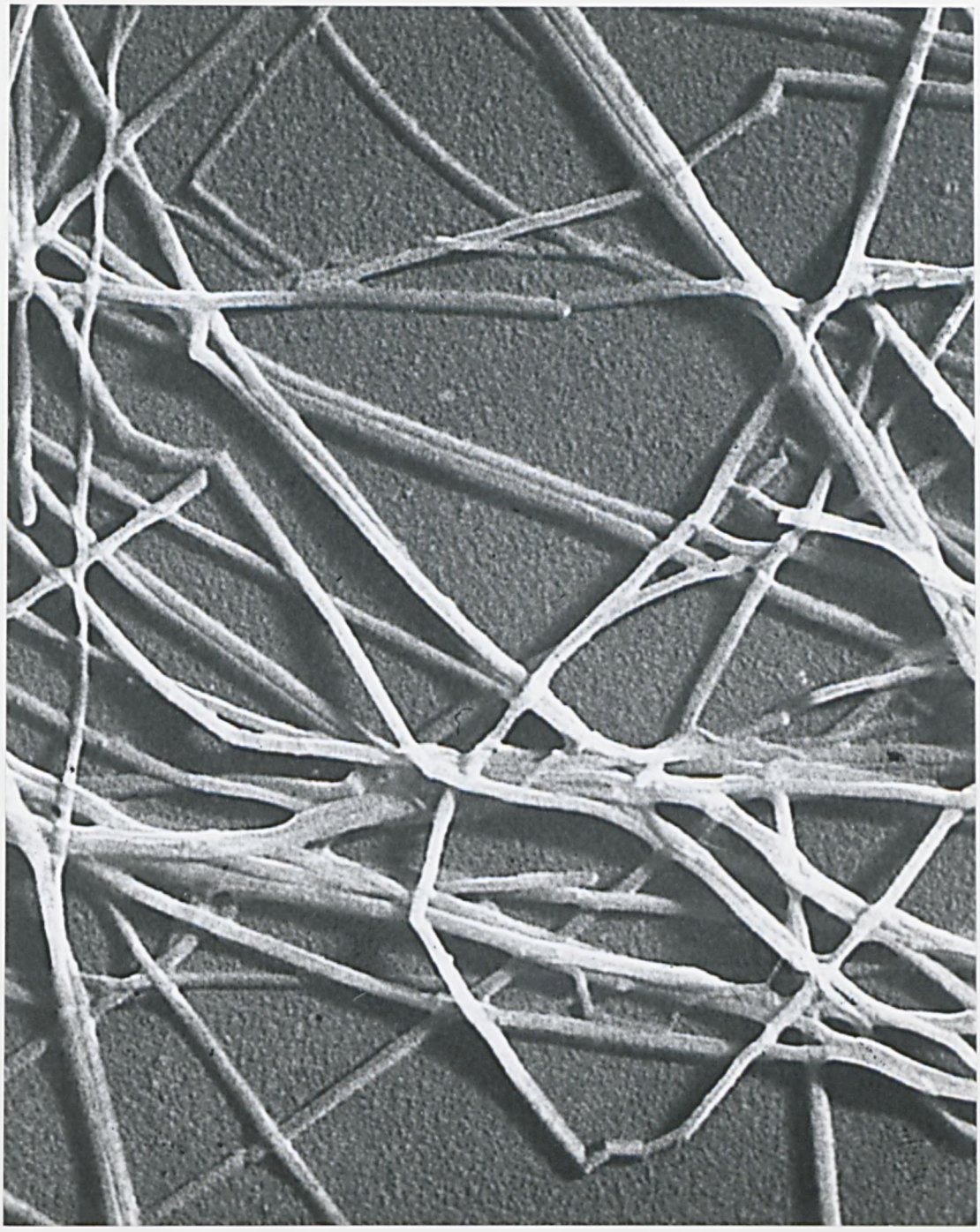
FIG. 23.



NITELLA MICROFIBRILS (X 120,000)

(PLATINUM SHADOWED)

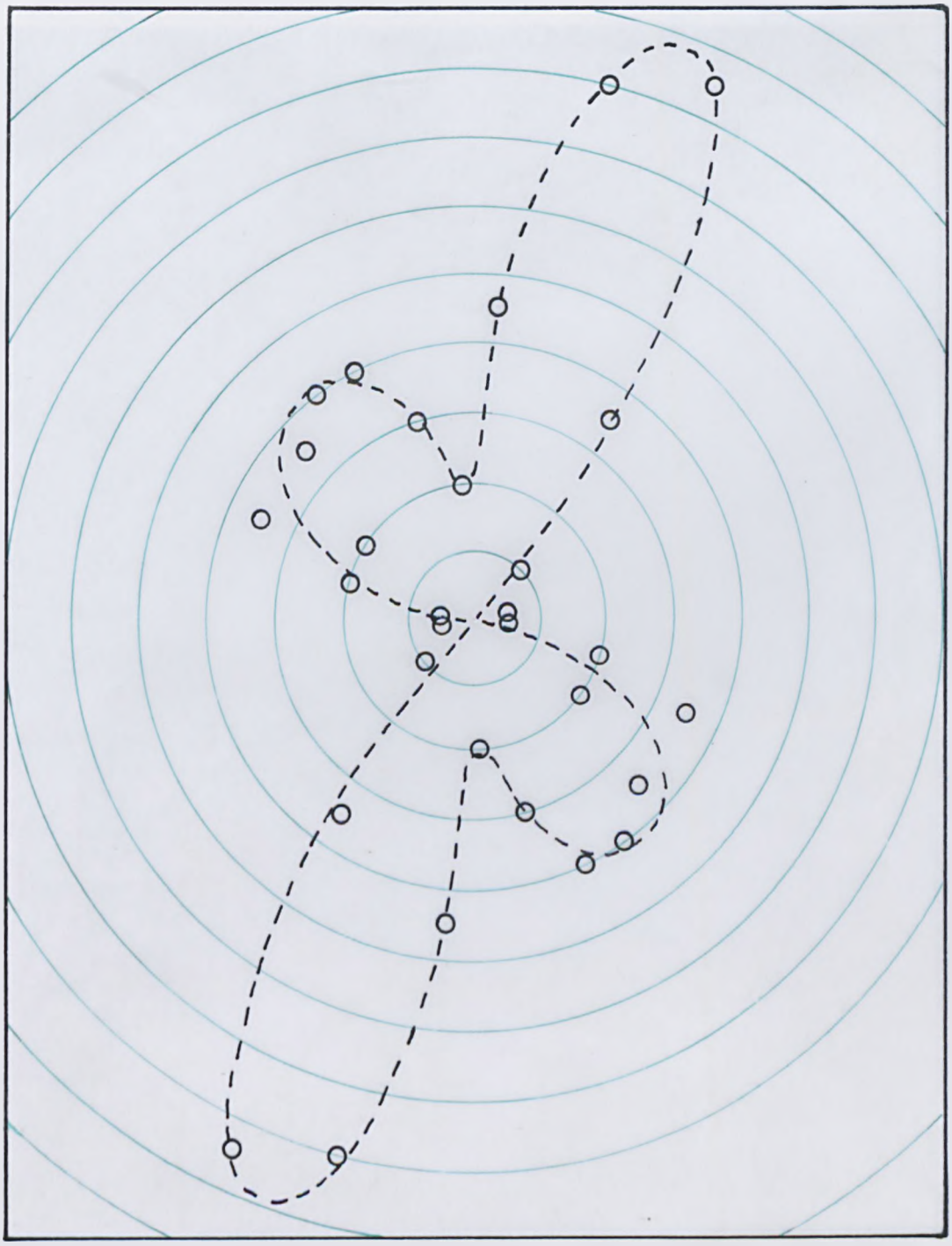
FIG. 24.



HIGH POWER OF VALONIA MICROFIBRILS (X 120,000)

FIG. 25.

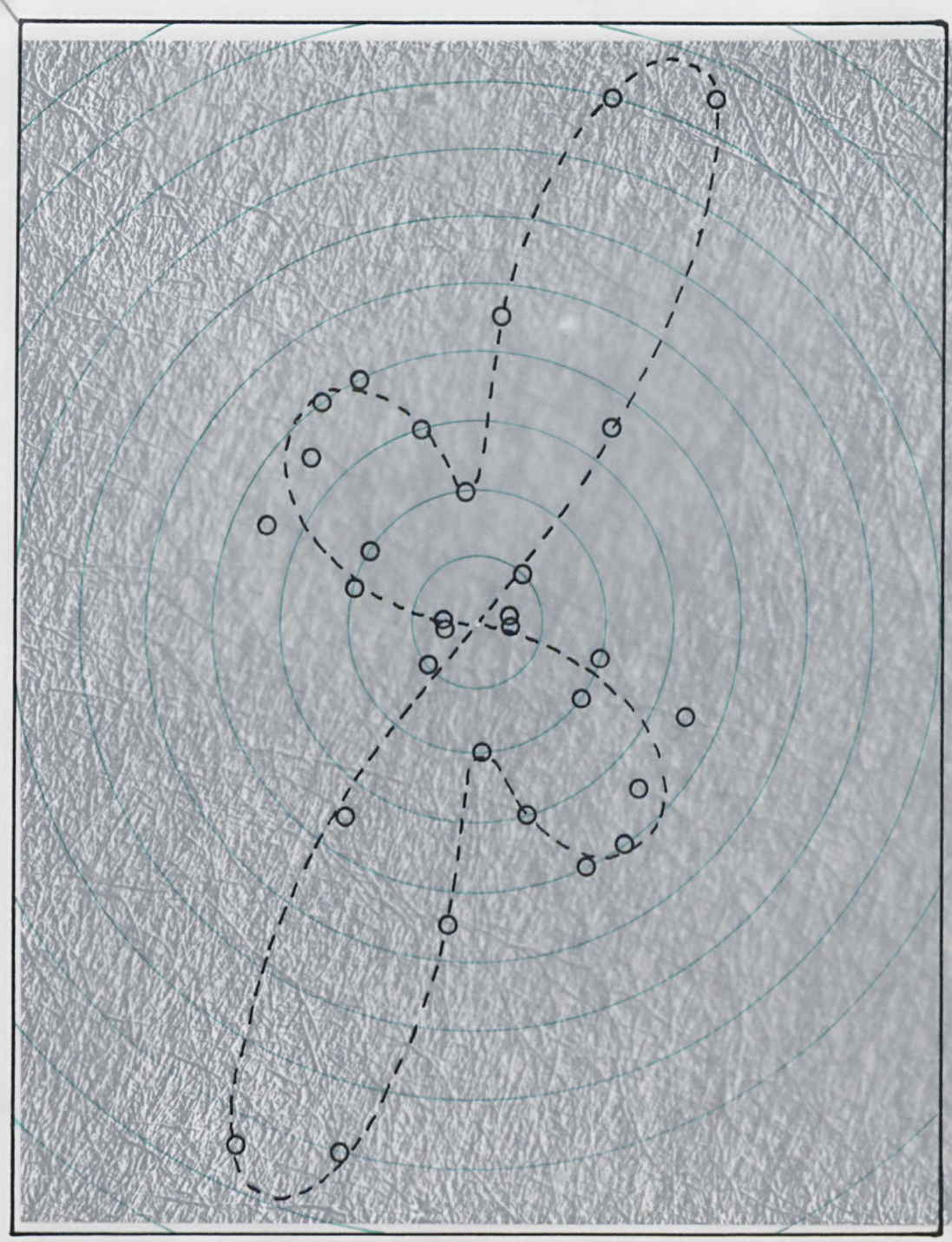
THIS THESIS CONTAINS OVERLAYS AND UNDERLAYS.



INNER LAYER; YOUNG CELL (X 27,900)

(PLATINUM SHADOWED)

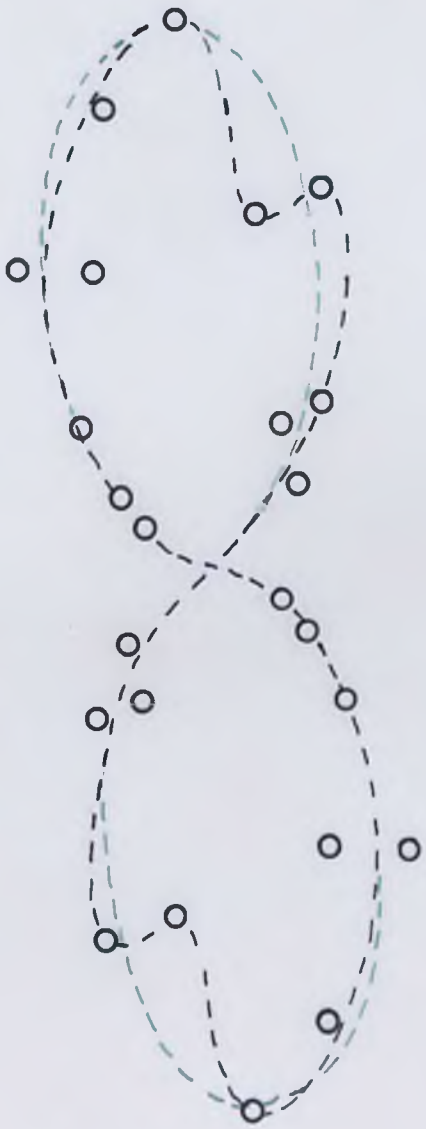
FIG. 26.



INNER LAYER; YOUNG CELL (X 27,900)

(PLATINUM SHADOWED)

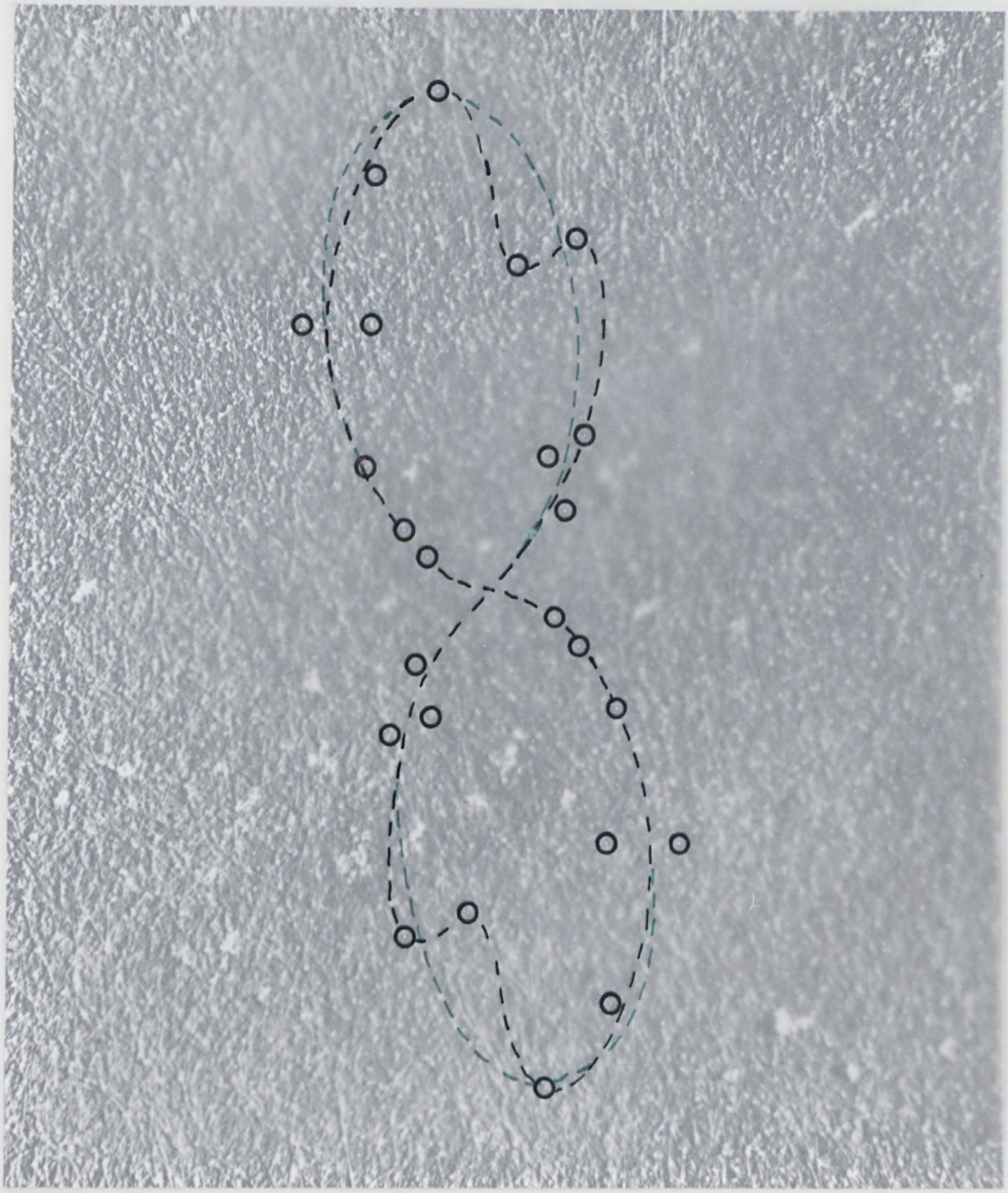
FIG. 26.



LOCATION IN WALL UNKNOWN (X 18,000)

(GOLD SHADOWED)

FIG. 27.



LOCATION IN WALL UNKNOWN (X 18,000)

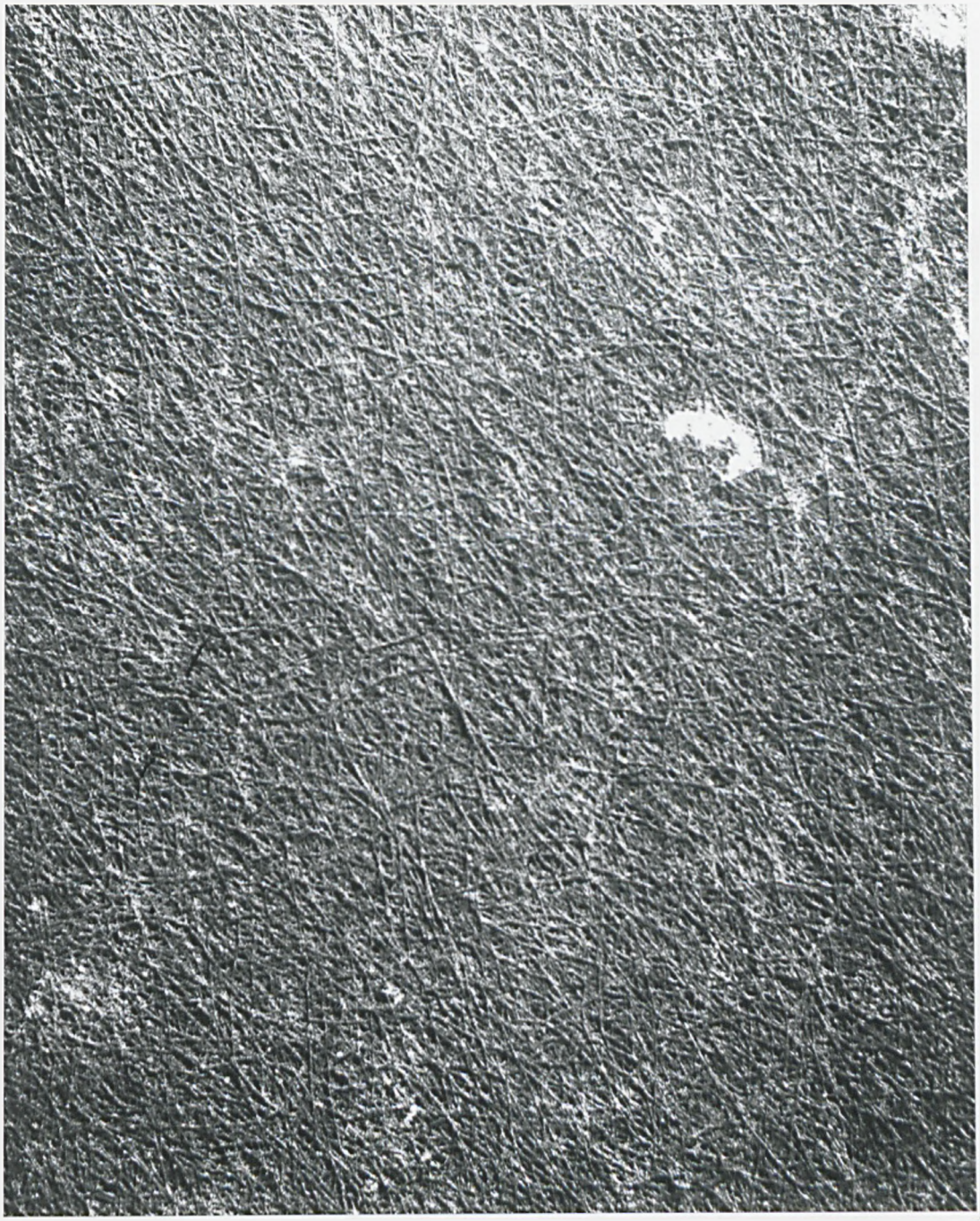
(GOLD SHADOWED)

FIG. 27.

LOCATION IN WALL UNKNOWN (X 27,900)

(PLATINUM SHADOWED)

FIG. 28.



EXTREME OUTSIDE LAYER (X 27,900)

(PLATINUM SHADOWED)

FIG. 29.

OUTSIDE LAYER (X 27,900)

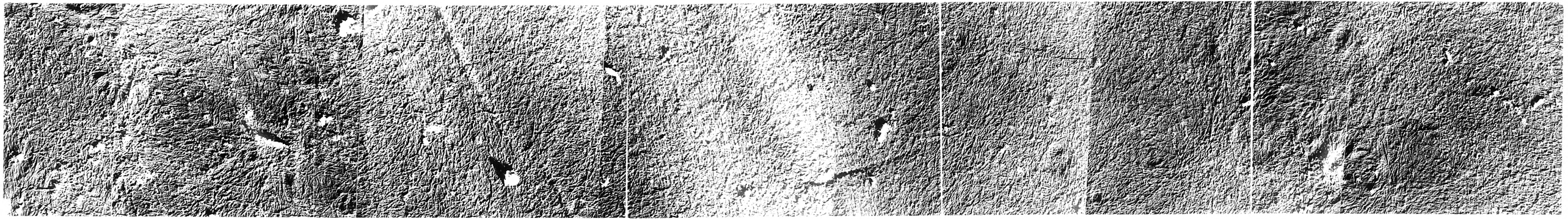
(GOLD SHADOWED)

FIG. 30.

'STRIATION' REGION OF WALL (X 7,200)

(PLATINUM SHADOWED)

FIG. 31(a).



"STRIATION" REGION - 10 MM. CELL (X 15,300)

(PLATINUM SHADOWED)

FIG. 32.

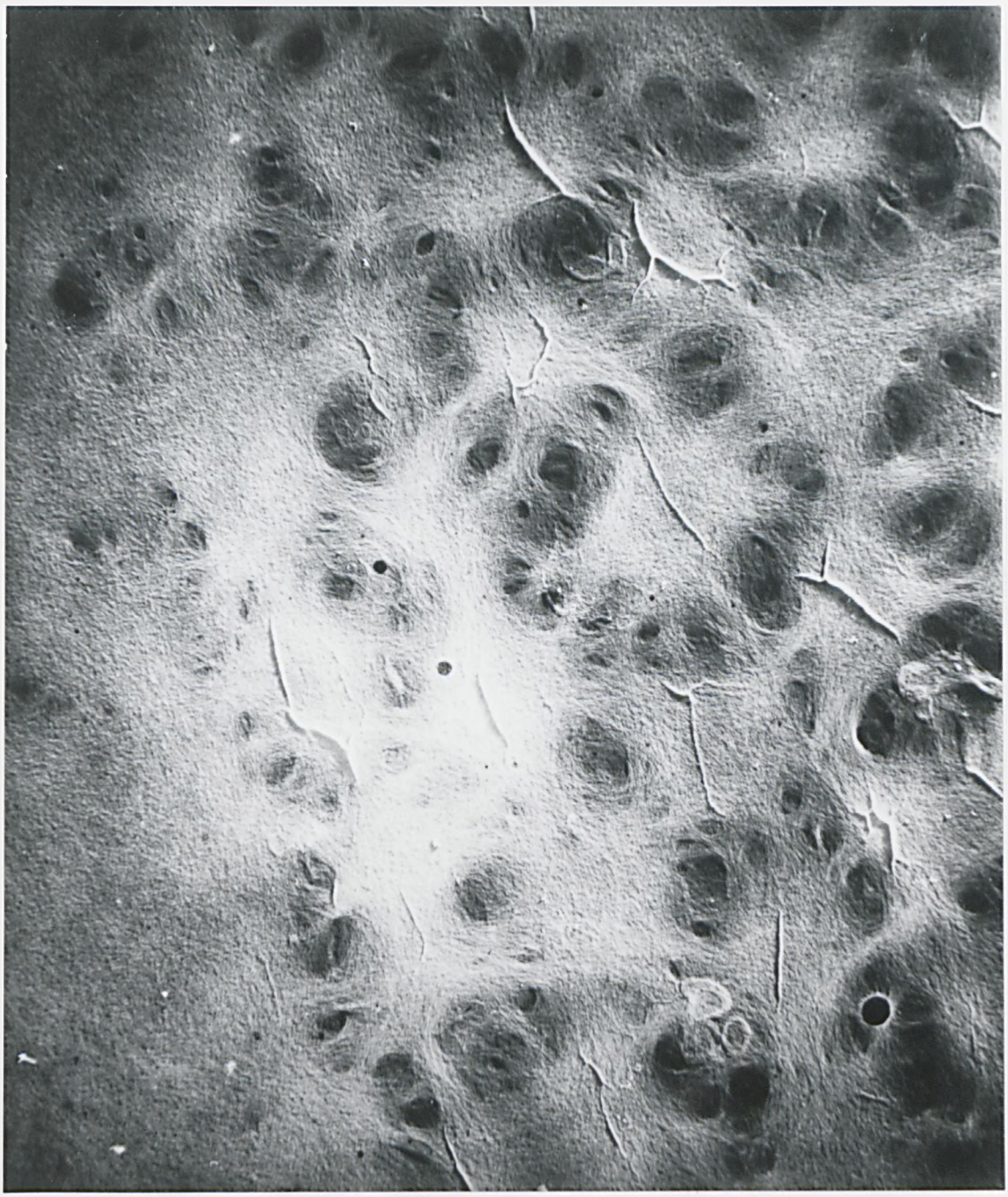
OPPOSITE STRIATION TO THAT REPRODUCED IN FIG. 32.

FIG. 33.

REGION BETWEEN STRIATIONS SHOWN IN FIG. 32, AND 33. (X 15,300)

(PLATINUM SHADOWED)

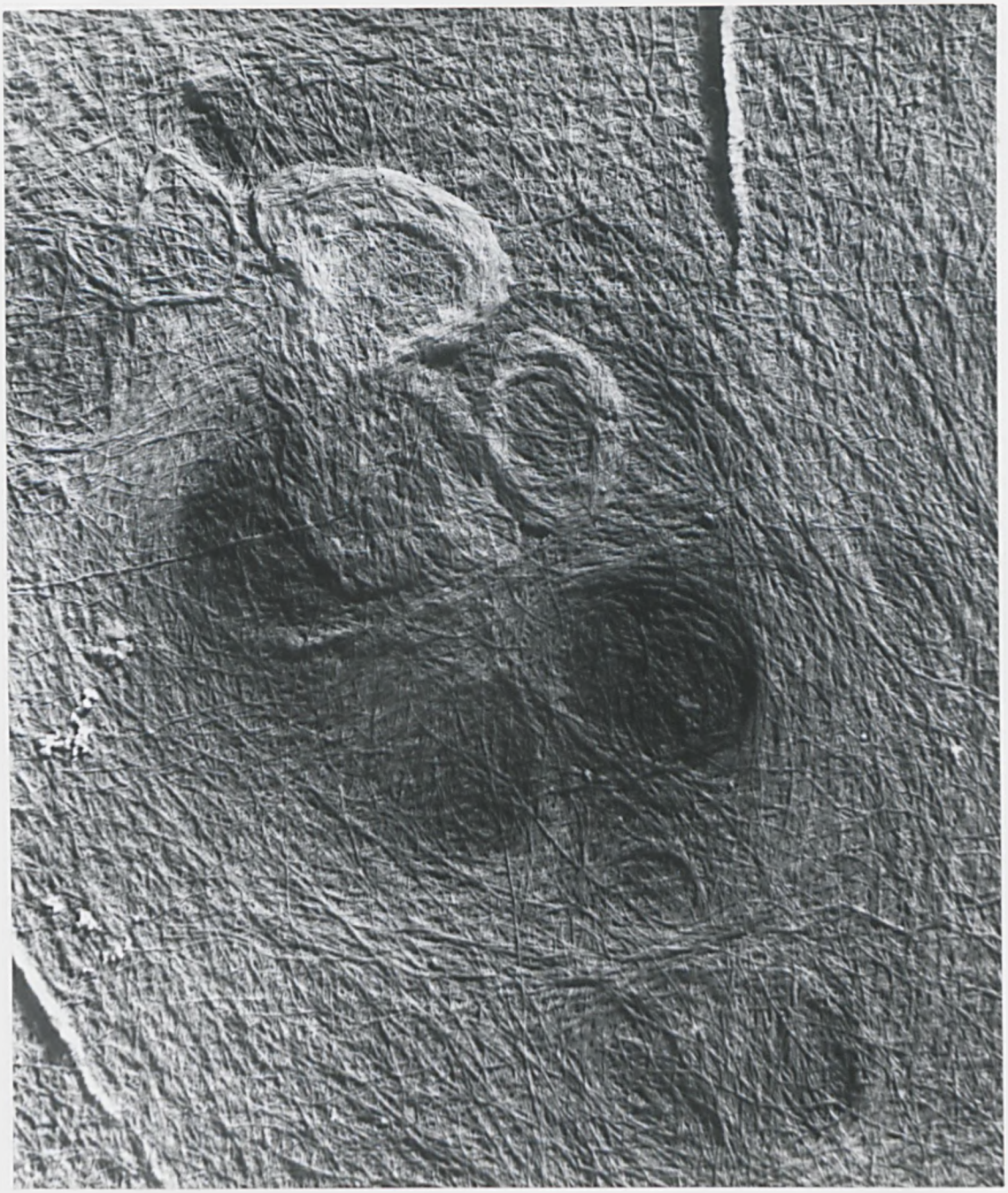
FIG. 34.



OUTER LAYER SHOWING 'DISTURBED' AREAS (X 6,000)

(PLATINUM SHADOWED)

FIG. 35.



HIGH POWER OF 'DISTURBED' AREA SHOWN IN FIG. 35. (X 27,900)

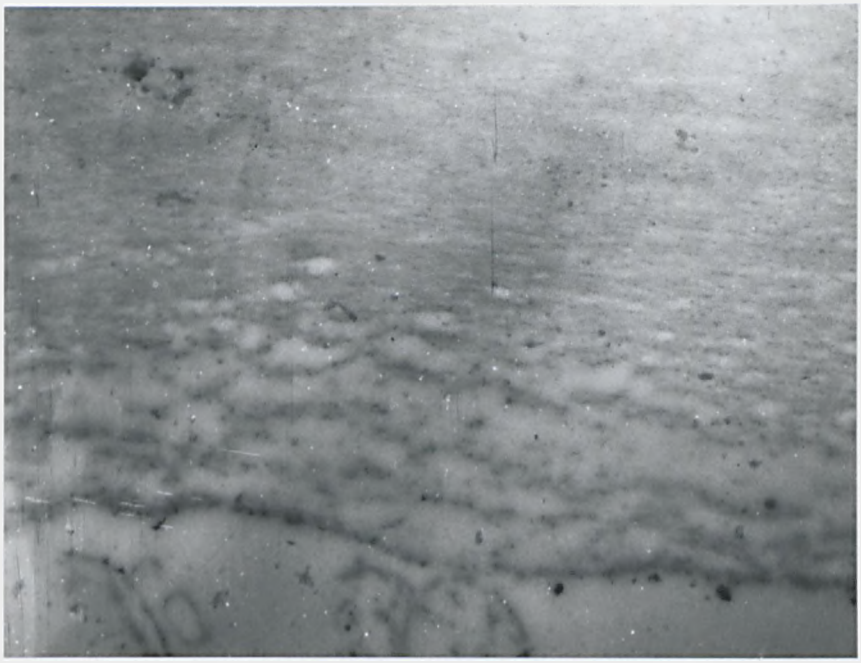
(PLATINUM SHADOWED)

FIG. 36.

SECTION OF NITELLA WALL (X 13,800)

METHACRYLATE NOT REMOVED

FIG. 37.



PART-SECTION OF NITELLA WALL

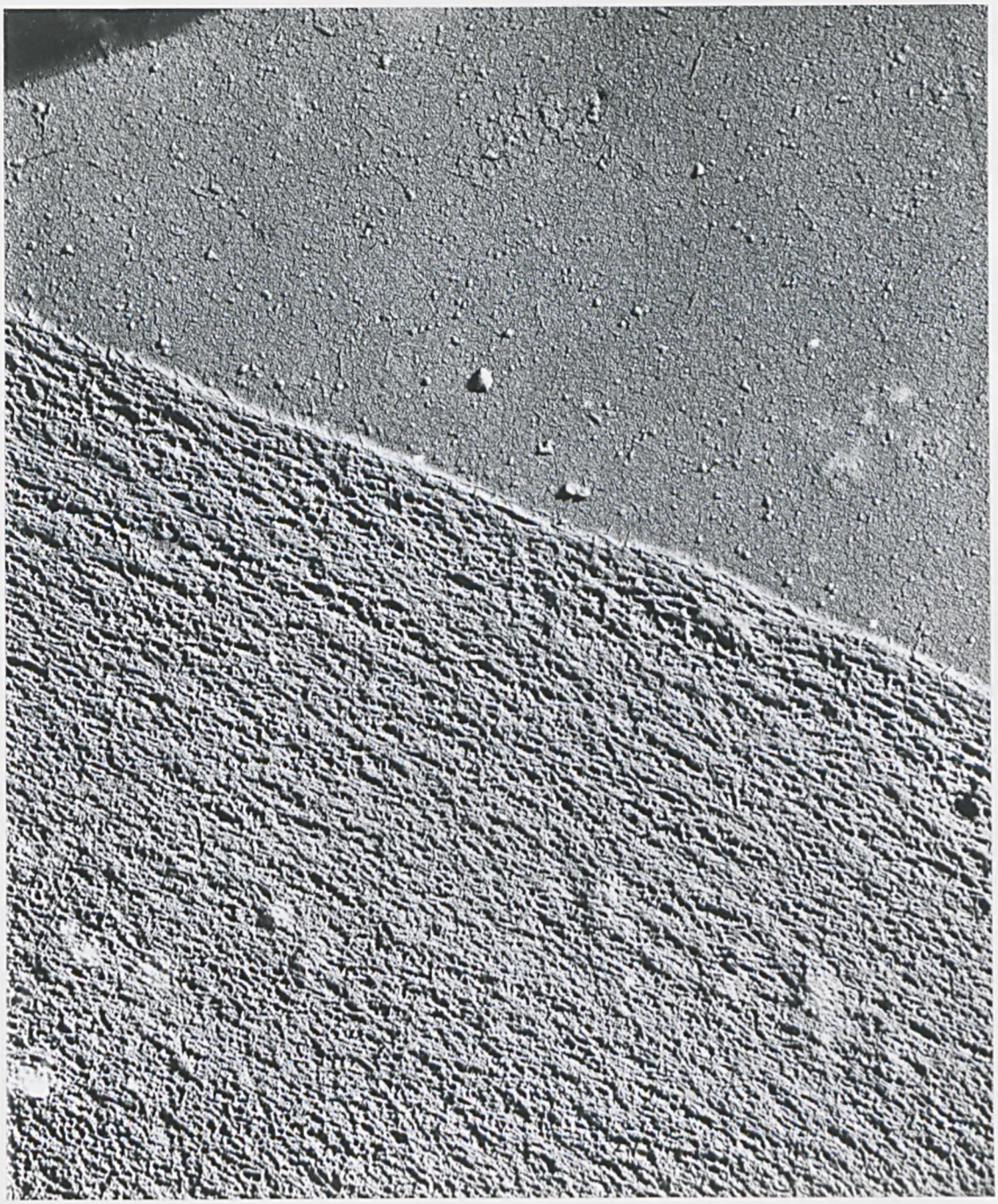
METHACRYLATE NOT REMOVED

FIG. 38.

SECTION OF NITELLA WALL (X 15,300)

METHACRYLATE REMOVED. GOLD SHADOWED

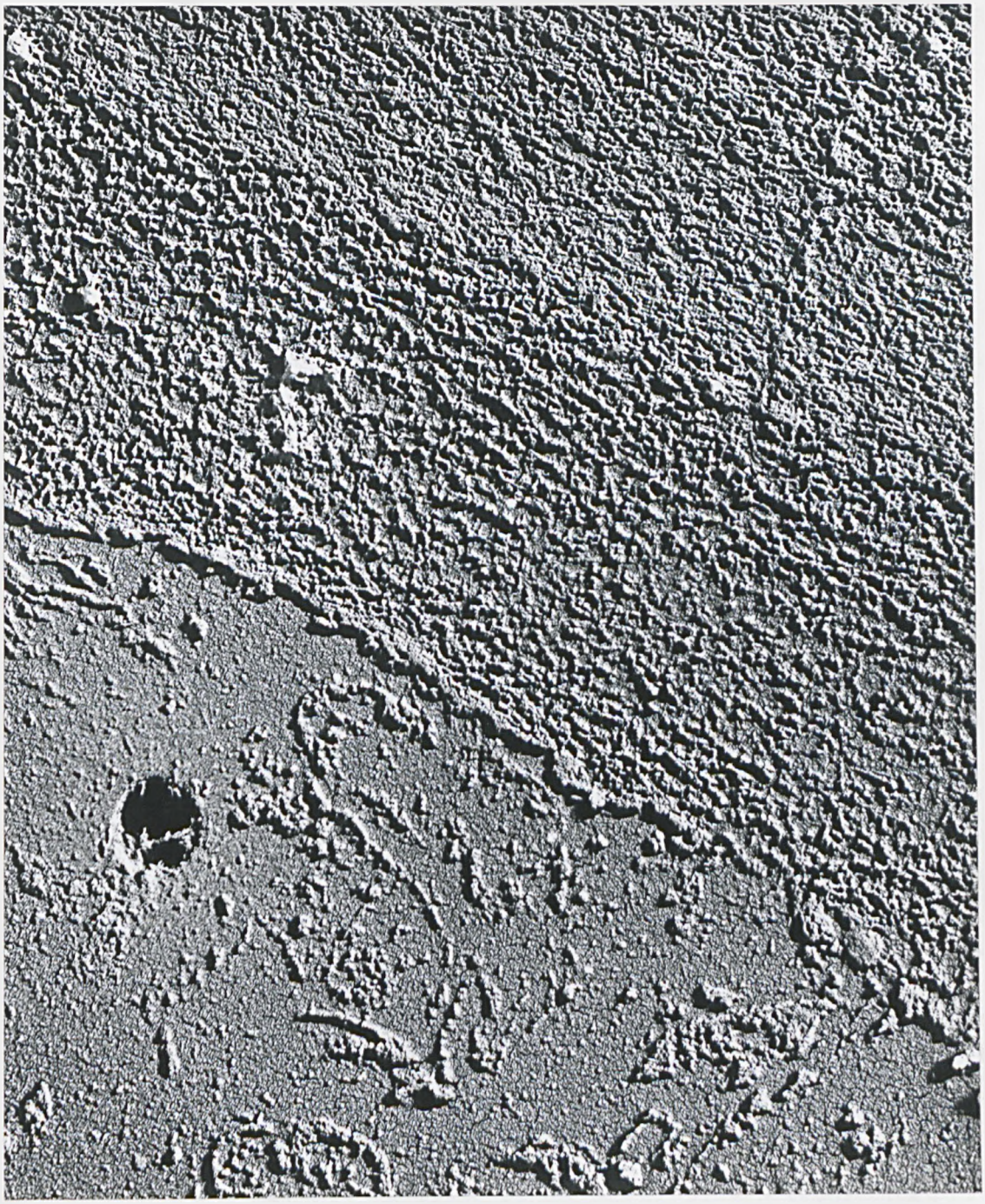
FIG. 39.



SECTION OF NITELLA WALL (X 27,900)

METHACRYLATE REMOVED. GOLD SHADOWED

FIG. 40.



SECTION OF NITELLA WALL (X 27,900)

METHACRYLATE REMOVED. GOLD SHADOWED

FIG. 41.

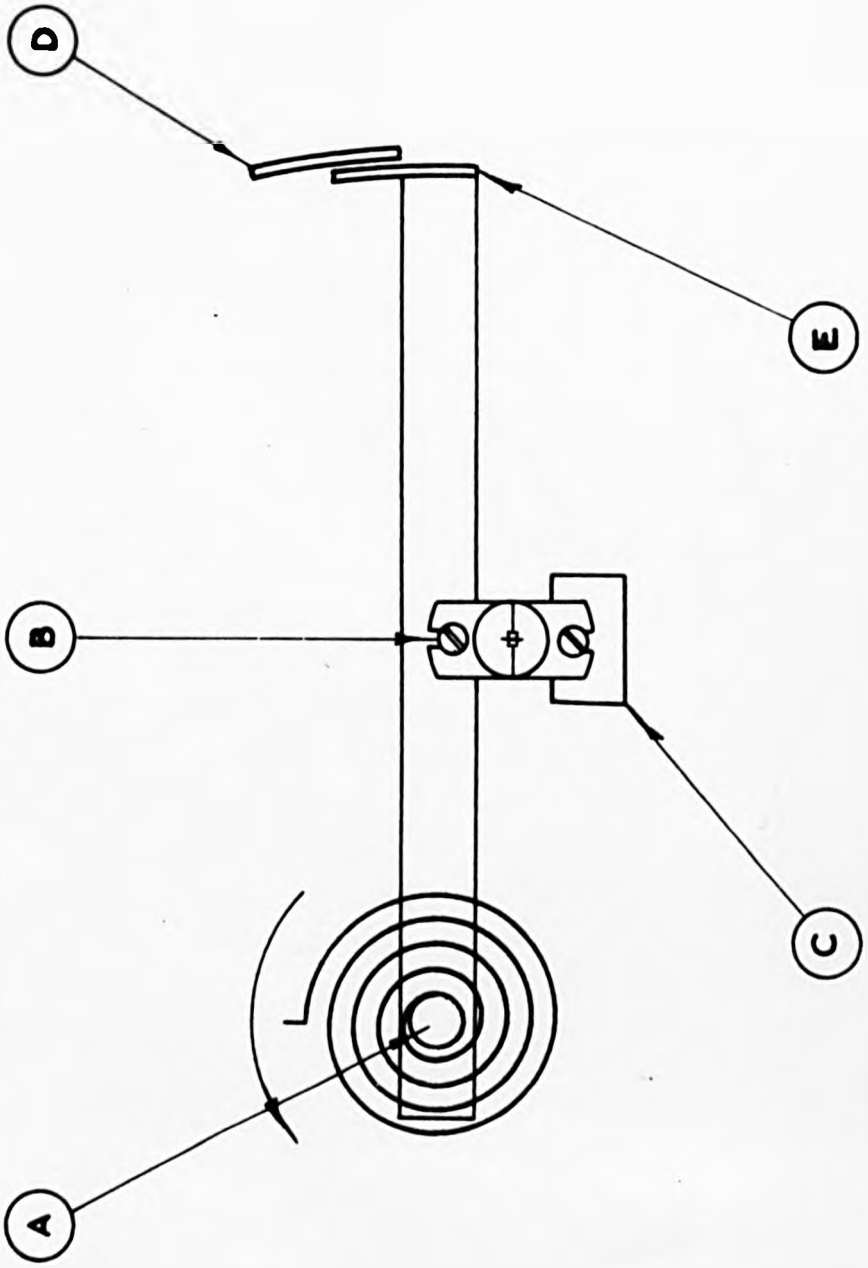
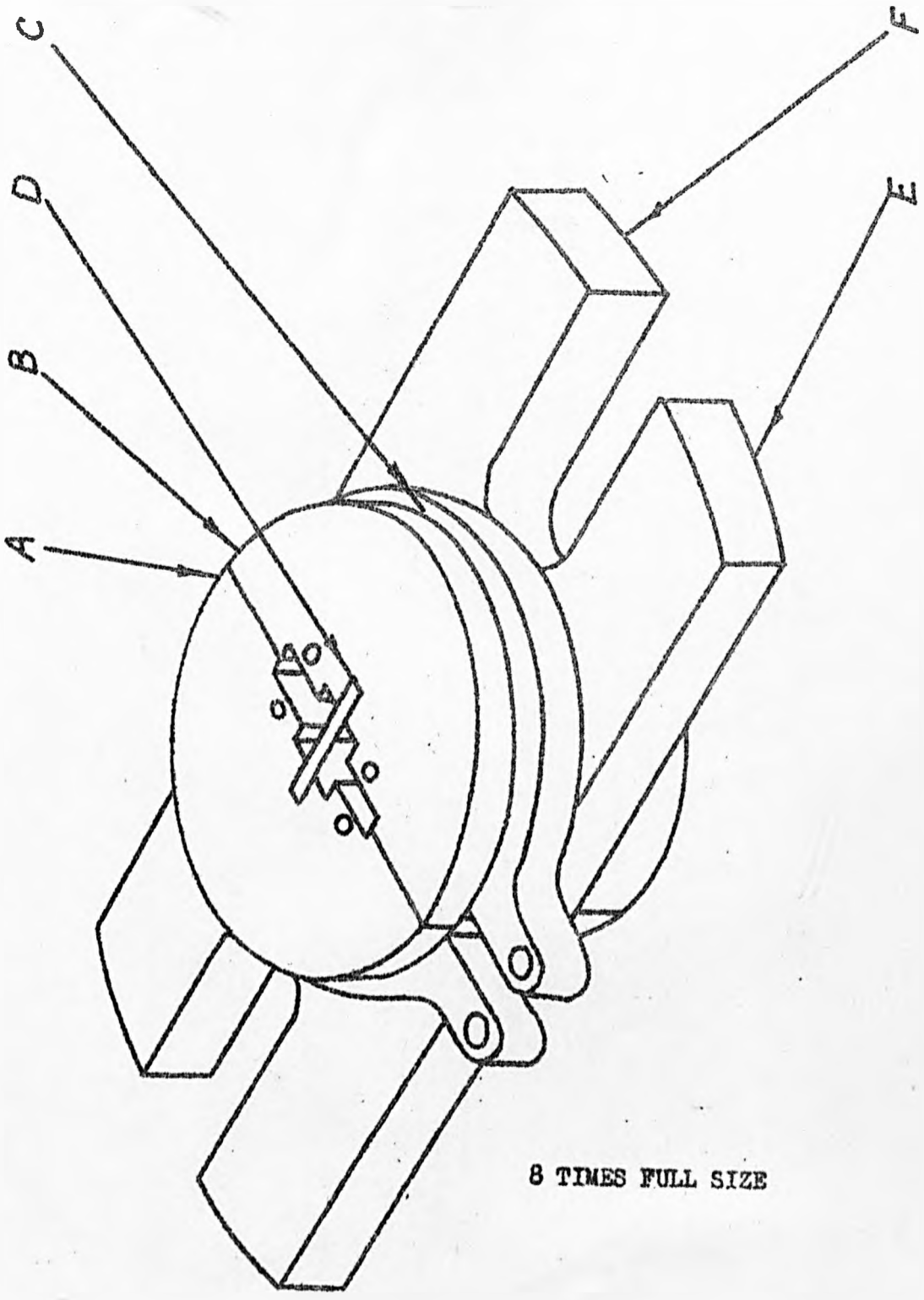


FIG. 42.



8 TIMES FULL SIZE

FIG. 43.

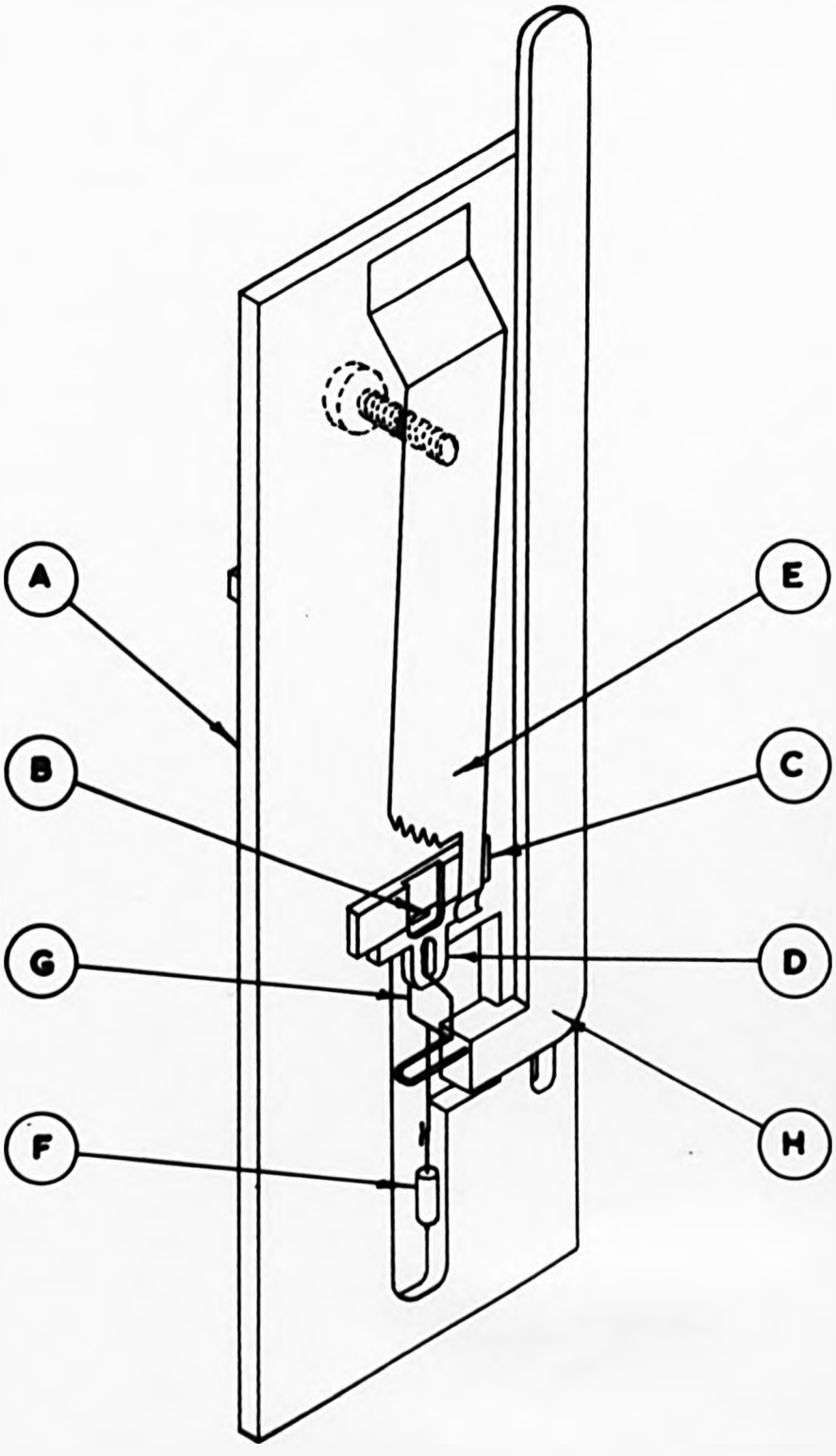


FIG. 44.

TRANSVERSE STRIP; CELL 5.

RATE OF LOADING; 8.75×10^3 DYNES/MIN.

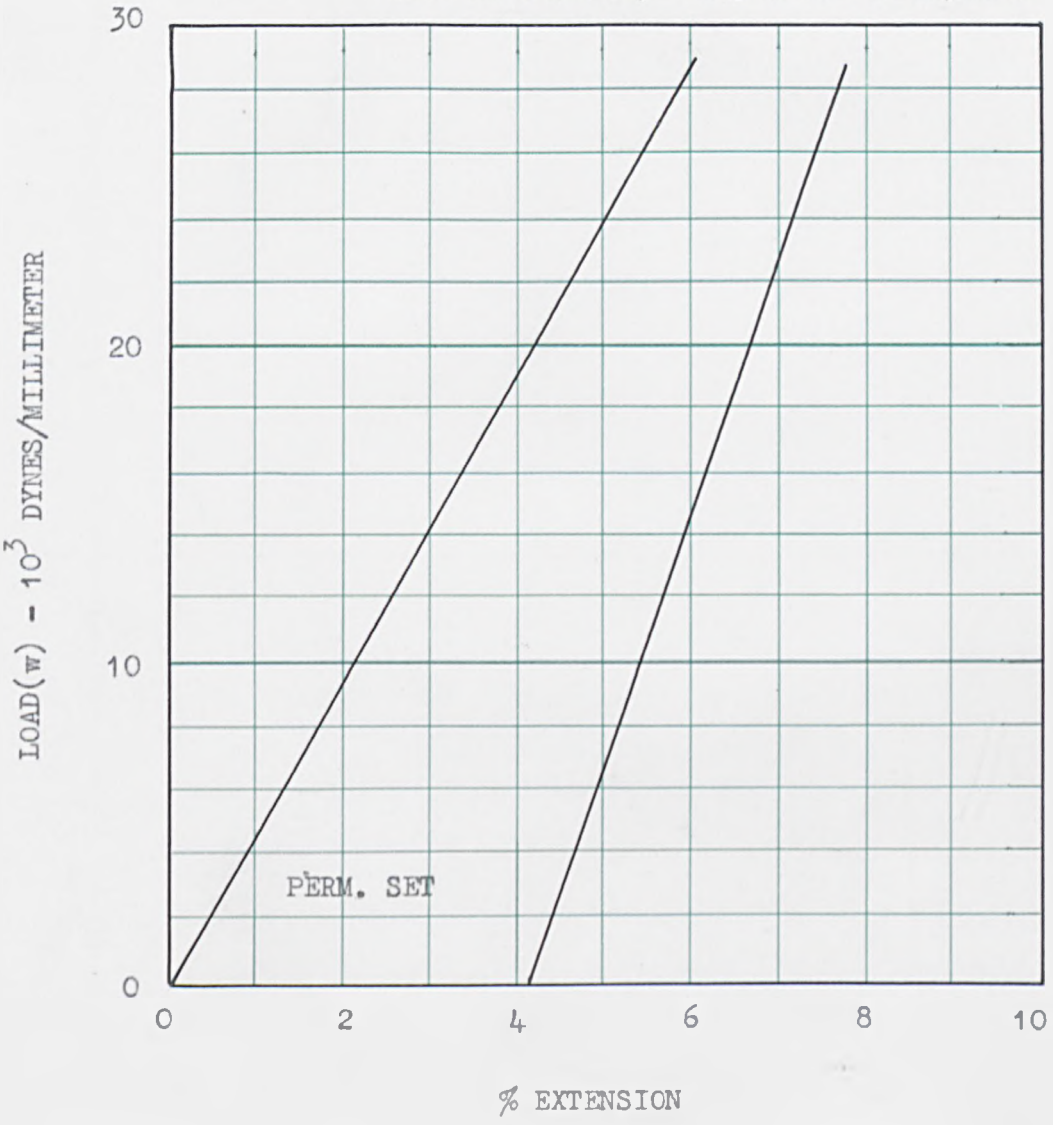


FIG. 45.

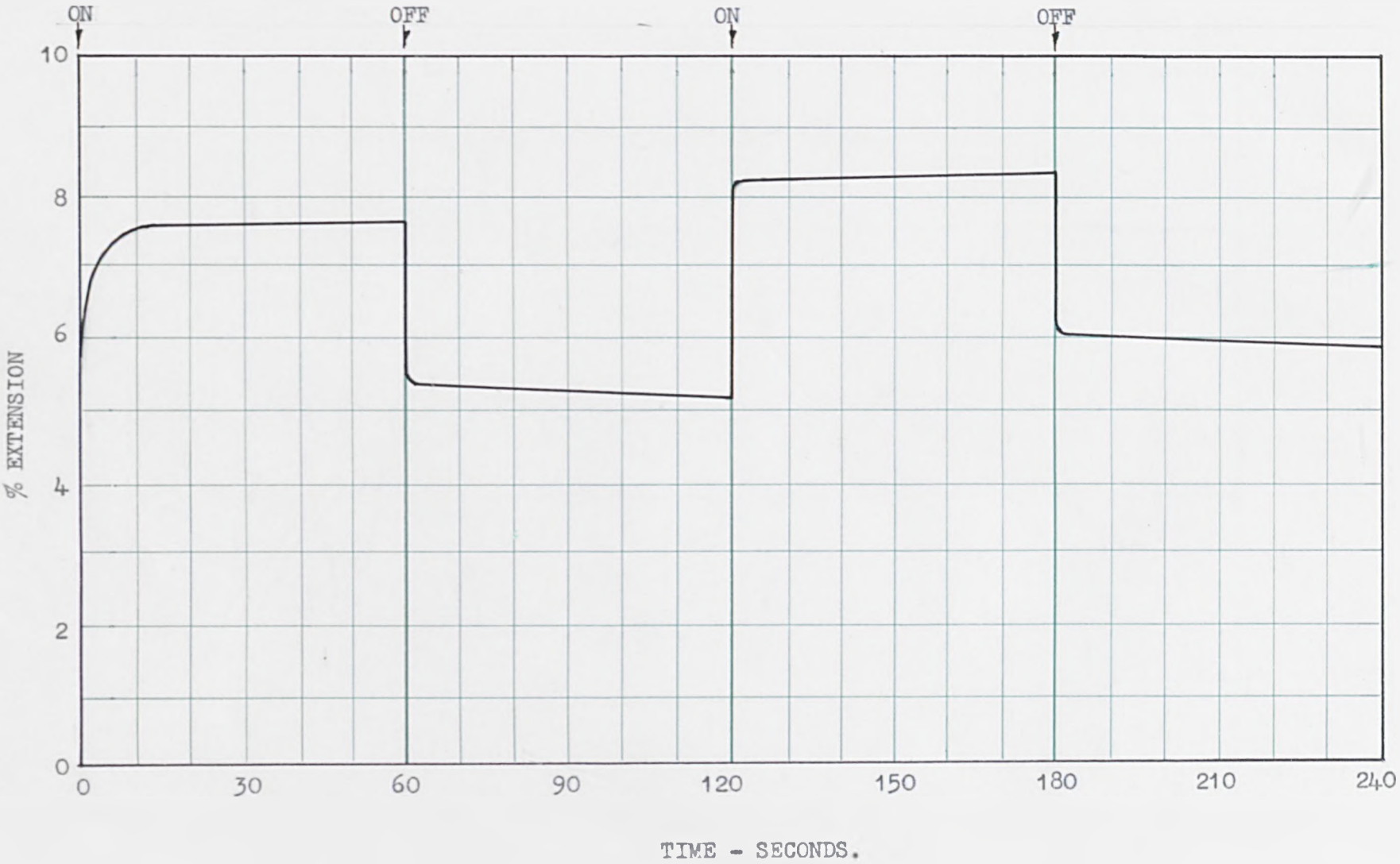

ON
OFF
ON
OFF
10
8
6
4
2
0
% EXTENSION
0
30
60
90
120
150
180
210
240
TIME - SECONDS.

FIG. 46.

STRIPS CUT FROM CELL 14B.

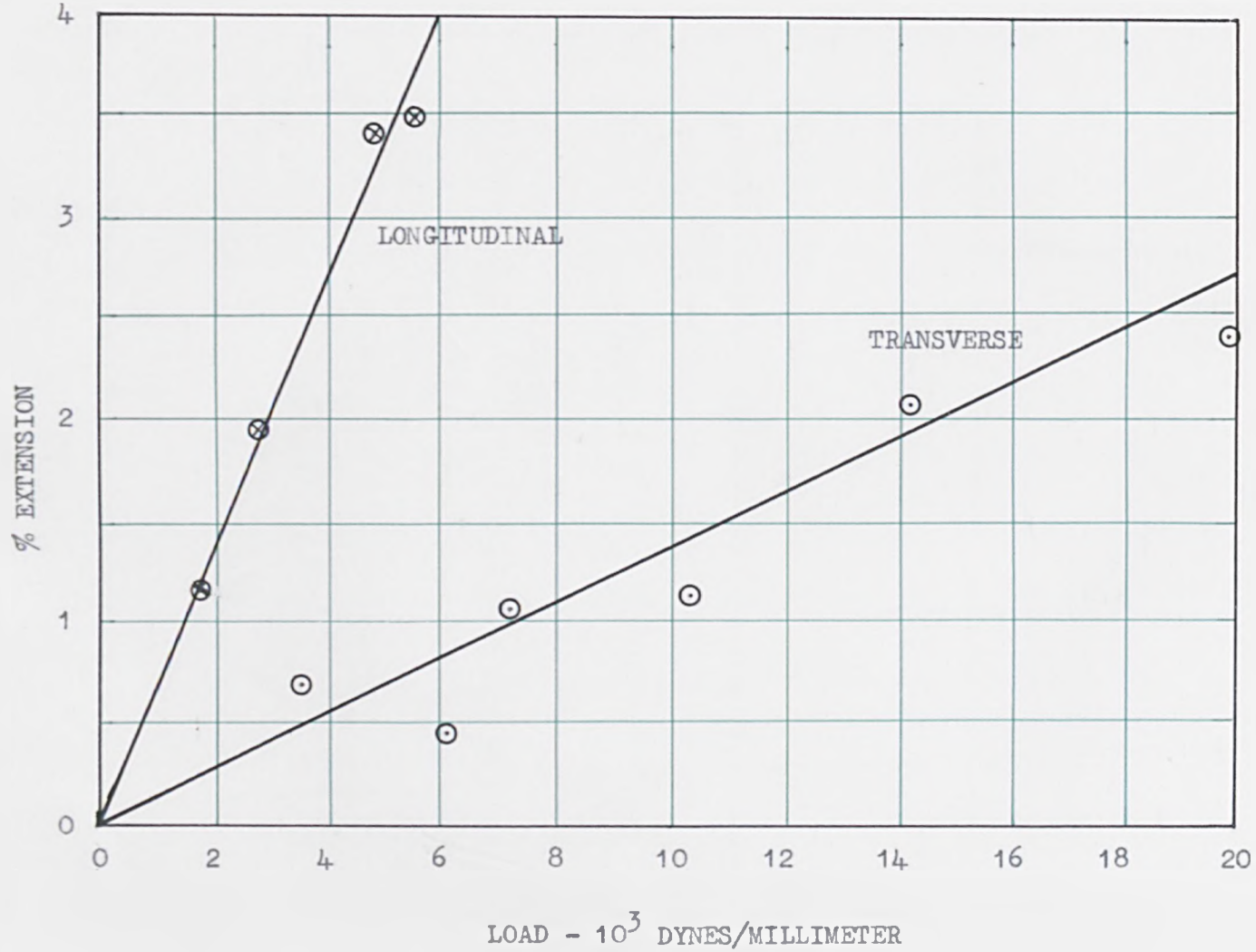


FIG. 47.

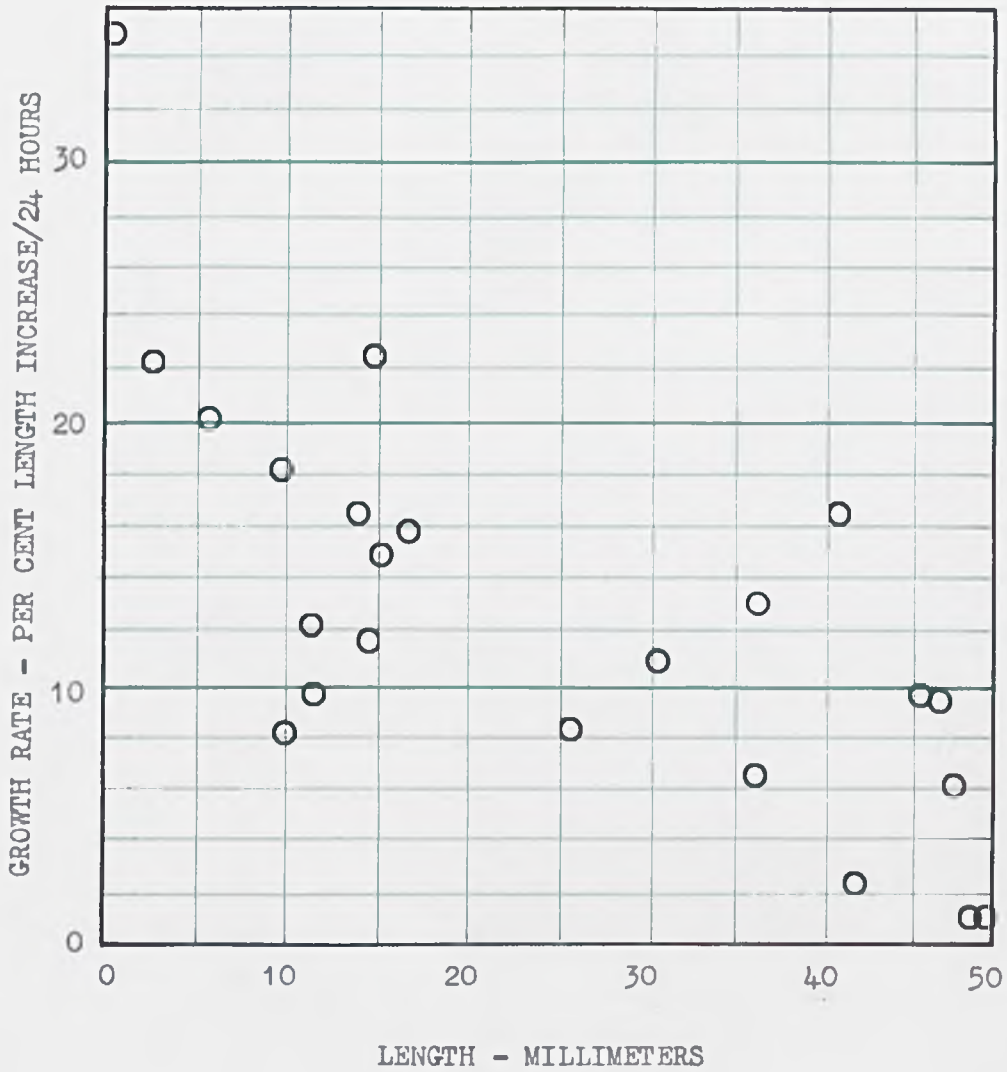

GROWTH RATE - PER CENT LENGTH INCREASE/24 HOURS
0
10
20
30
0
10
20
30
40
50
LENGTH - MILLIMETERS

FIG. 48.

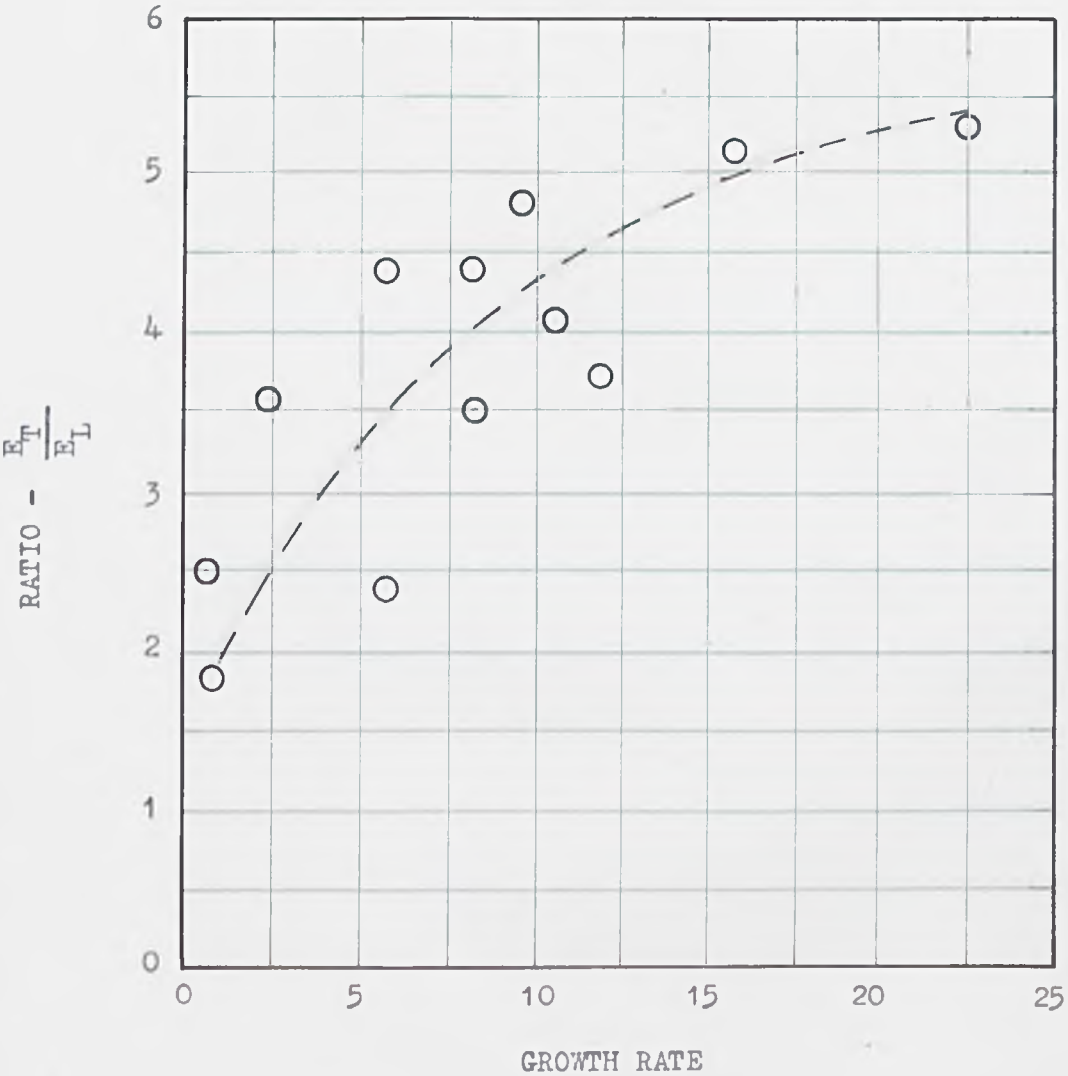

RATIO - $\frac{E_T}{E_L}$
0
1
2
3
4
5
6
0
5
10
15
20
25
GROWTH RATE
PER CENT LENGTH INCREASE/24 HOURS

FIG. 49.

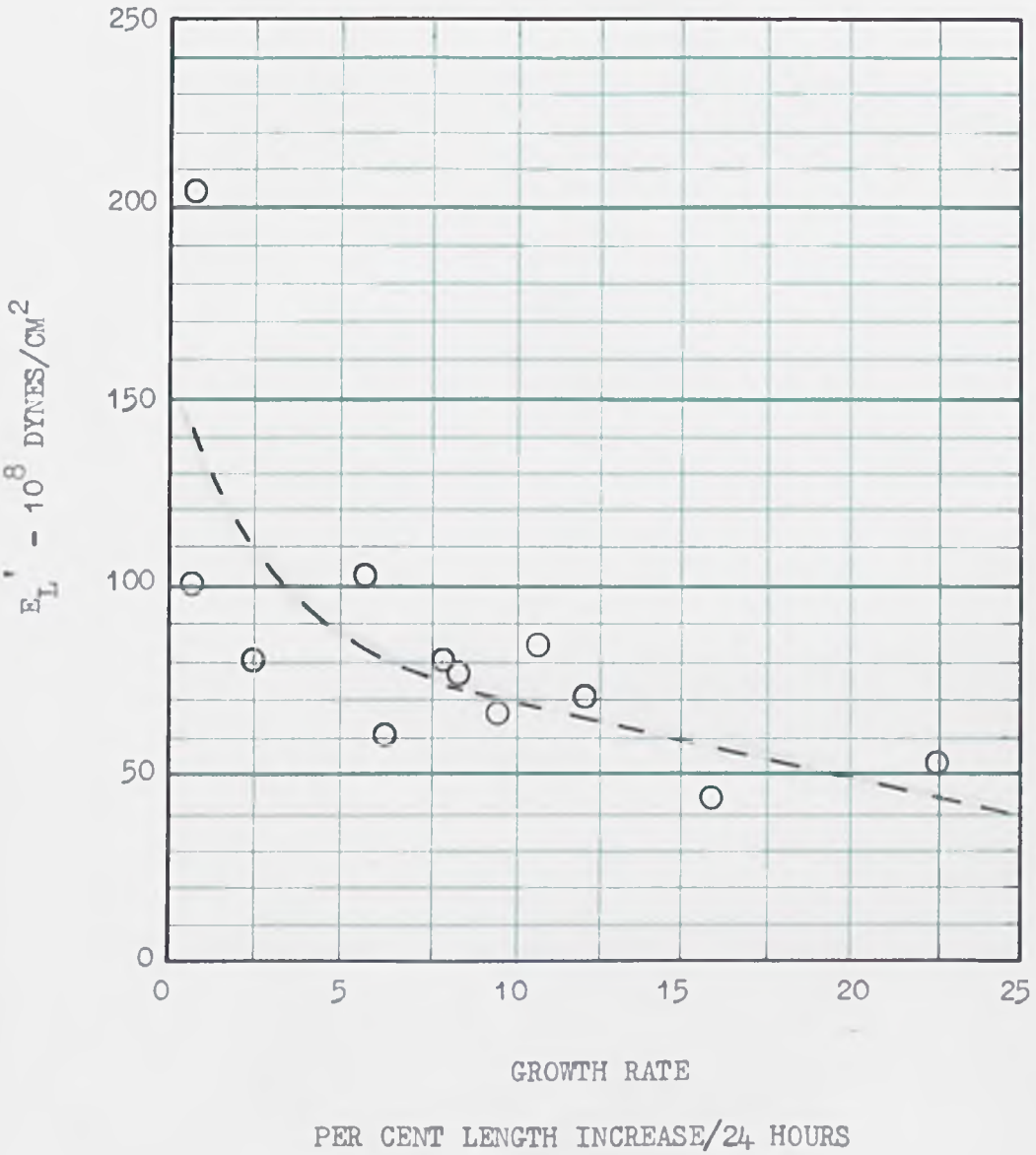

250
200
150
100
50
0
0
5
10
15
20
25
$E_L' - 10^8$ DYNES/CM^2
GROWTH RATE
PER CENT LENGTH INCREASE/24 HOURS

FIG. 50.

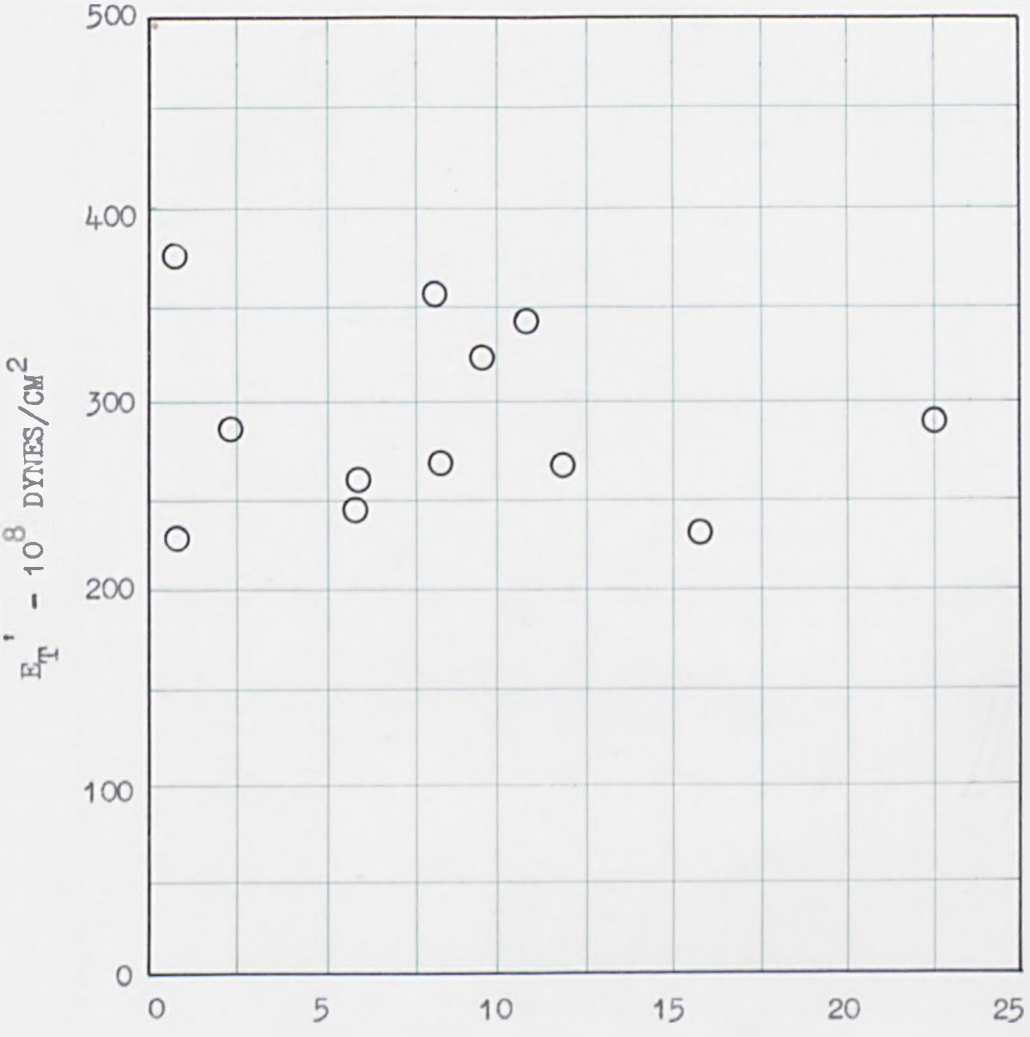

500
400
300
200
100
0
0
5
10
15
20
25
$E_T' - 10^8$ DYNES/CM^2
GROWTH RATE
PER CENT LENGTH INCREASE/24 HOURS

FIG. 51.

CELL 16B.

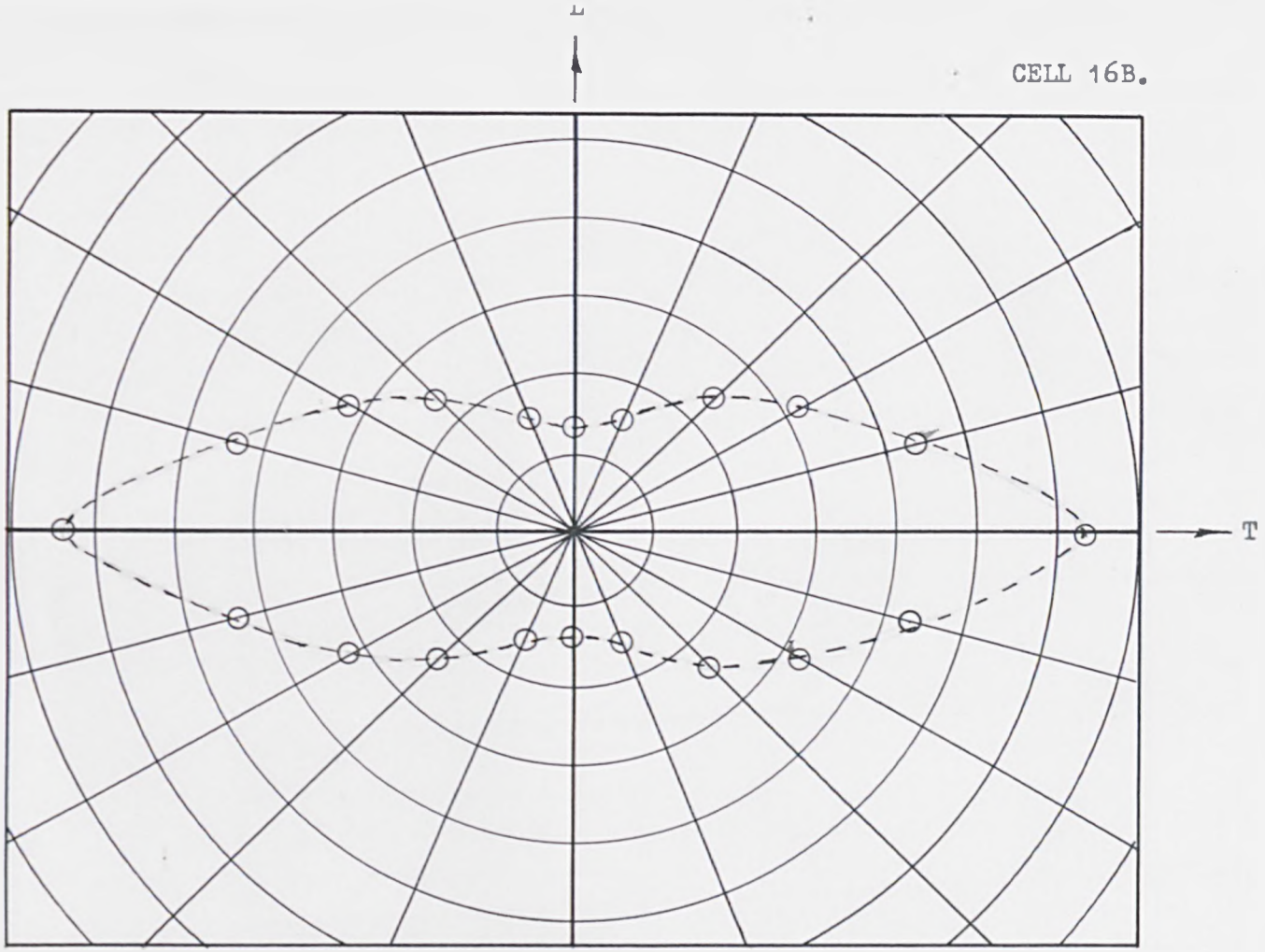


FIG. 52.

VARIATION OF TENSILE MODULUS WITH ANGLE OF TEST STRIP

(UNITS - EACH CIRCLE; 50 X 10^8 DYNES/CM^2)

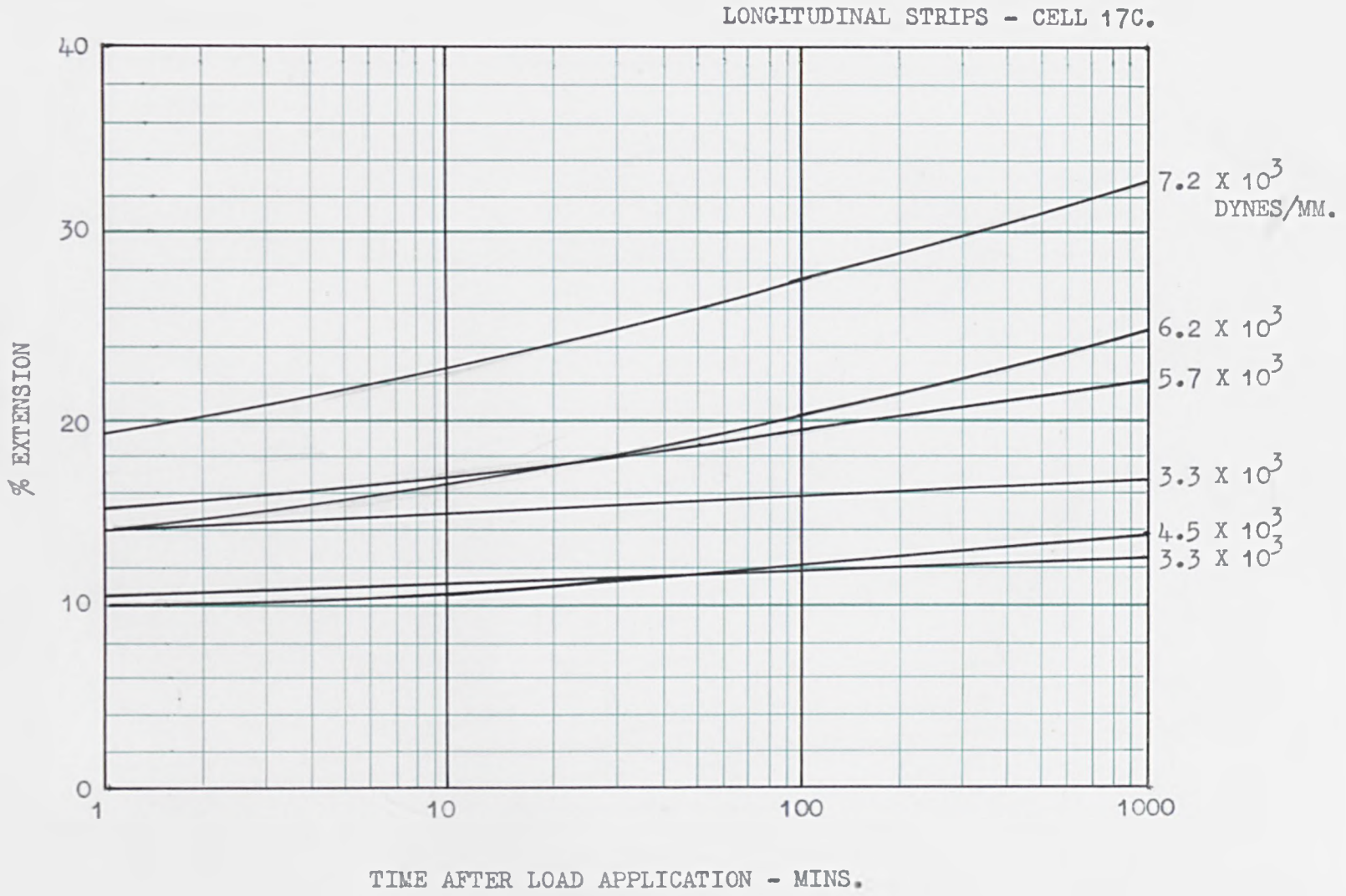
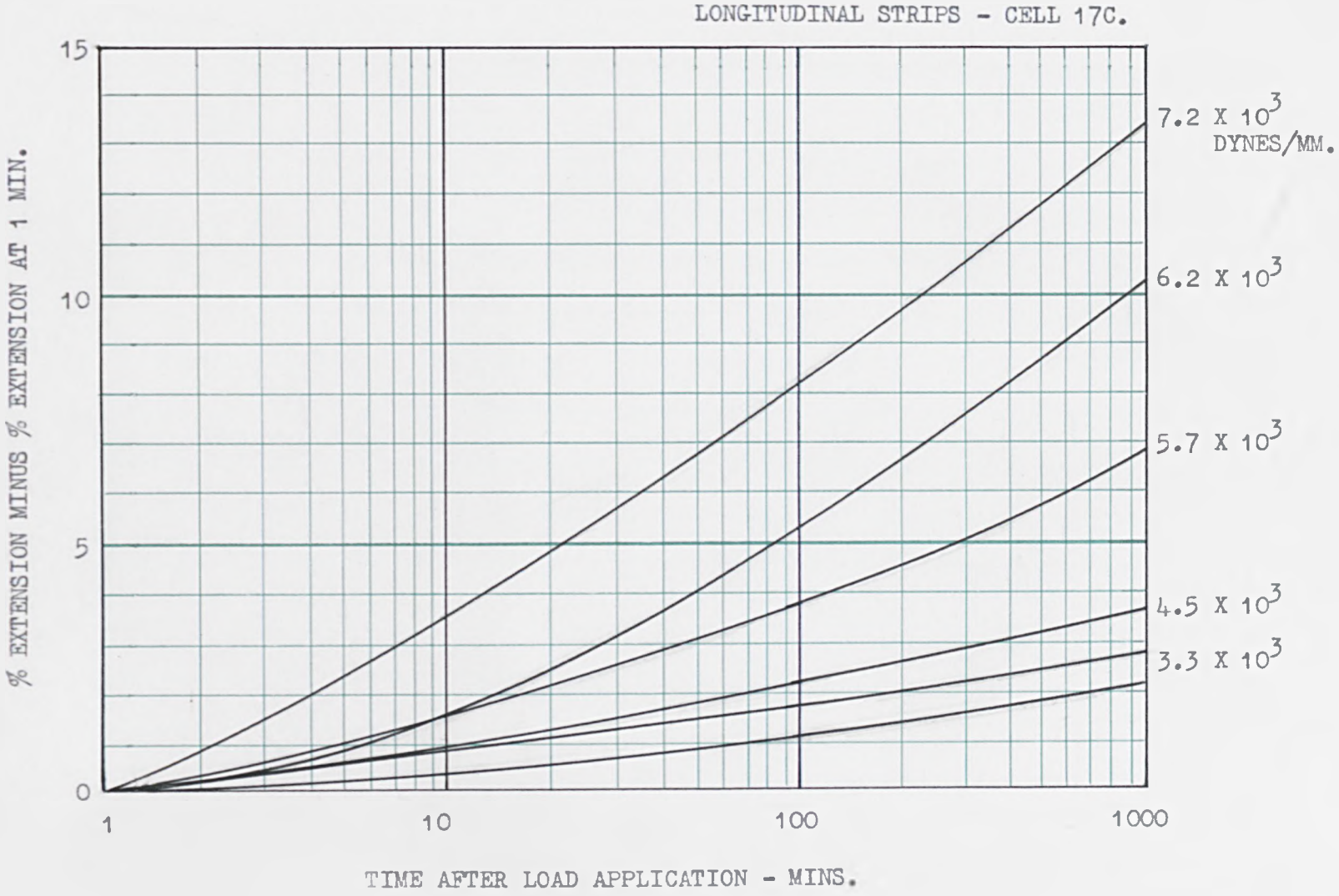

LONGITUDINAL STRIPS - CELL 17C.
40
30
20
10
0
% EXTENSION
1
10
100
1000
TIME AFTER LOAD APPLICATION - MINS.
7.2 X 10³ DYNES/MM.
6.2 X 10³
5.7 X 10³
3.3 X 10³
4.5 X 10³
3.3 X 10³

FIG. 53.

FIG. 54.


LONGITUDINAL STRIPS - CELL 17C.
% EXTENSION MINUS % EXTENSION AT 1 MIN.
15
10
5
0
1
10
100
1000
TIME AFTER LOAD APPLICATION - MINS.
7.2 X 10^3 DYNES/MM.
6.2 X 10^3
5.7 X 10^3
4.5 X 10^3
3.3 X 10^3

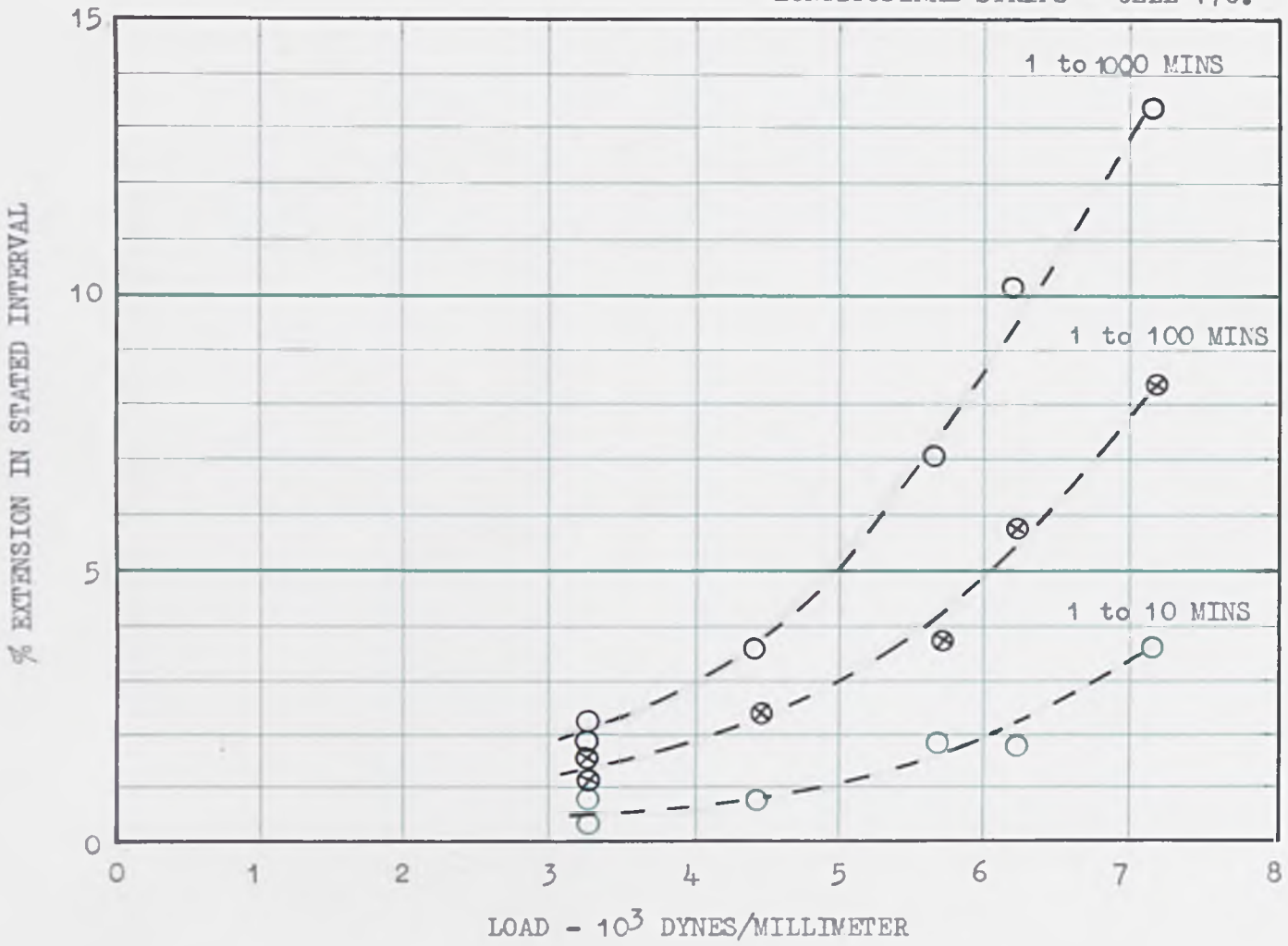

LONGITUDINAL STRIPS - CELL 17C.
1 to 1000 MINS
1 to 100 MINS
1 to 10 MINS
% EXTENSION IN STATED INTERVAL
LOAD - 10^3 DYNES/MILLIMETER
0
5
10
15
0
1
2
3
4
5
6
7
8

FIG. 55.

FIG. 56.

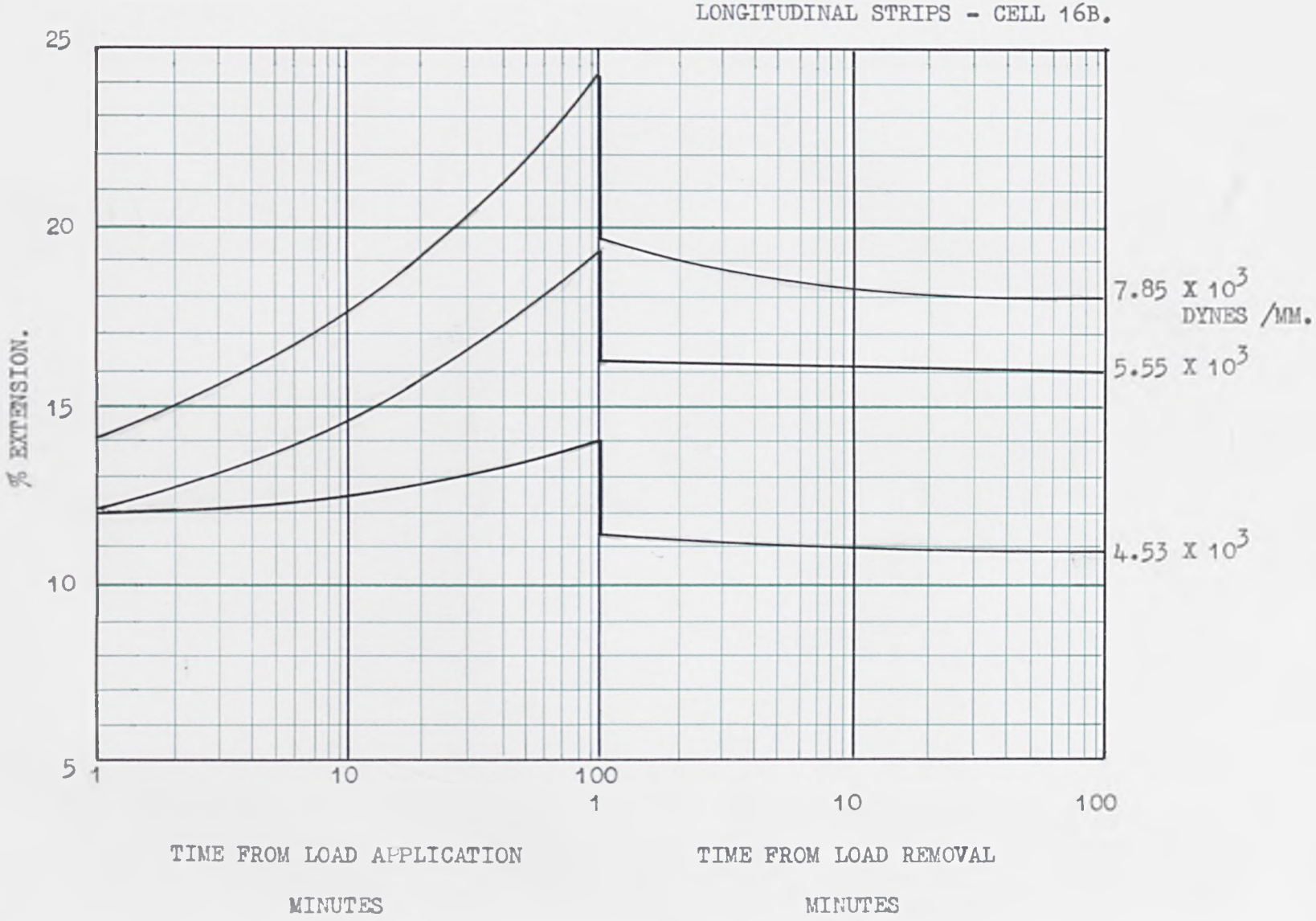

LONGITUDINAL STRIPS - CELL 16B.
% EXTENSION.
25
20
15
10
5
1
10
100
1
10
100
7.85 X 10³ DYNES /MM.
5.55 X 10³
4.53 X 10³
TIME FROM LOAD APPLICATION
MINUTES
TIME FROM LOAD REMOVAL
MINUTES

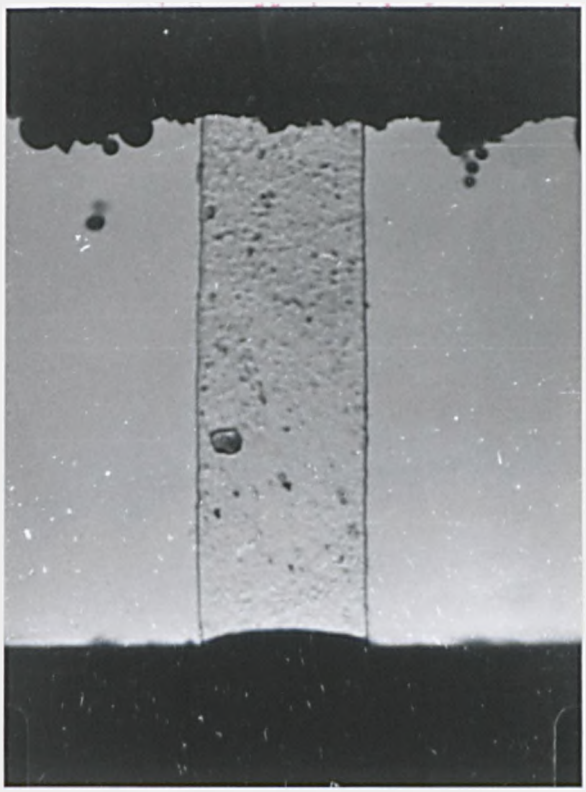
FIG. 57.

% EXTENSION BETWEEN 1 MIN. & 100 MIN.
0
5
10
15
0
1
2
3
4
5
6
7
STRESS - 10^8 DYNES/CM^2
16.3%
15.8 & 9.6%
6.2%
2.4%
1.0%
9.5%
1.0%
▽ 14D 16.3%
□ 17B 15.8%
⊘ 16B 9.6%
○ 17C 9.5%
△ 15C 6.2%
○ 14E 2.4%
⊗ 14C 1.0%
△ 20C 1.0%

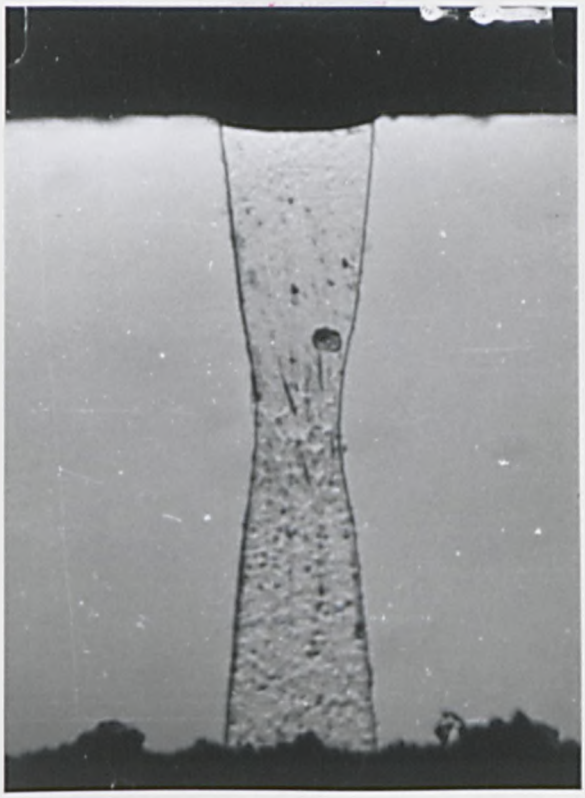
FIG. 58.

% EXTENSION BETWEEN 1 MIN. & 100 MIN.
0
5
10
15
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
TEST STRESS / 'STANDARD' WALL STRESS
16.3 & 15.8 %
9.6%
9.5%
6.2%
2.4%
1.0%
1.0%
▽14D 16.3%
□17B 15.8%
⊘16B 9.6%
○17C 9.5%
△15C 6.2%
○14E 2.4%
⊗14C 1.0%
△20C 1.0%

'LOW CREEP RATE' CELL JUST BEFORE BREAK



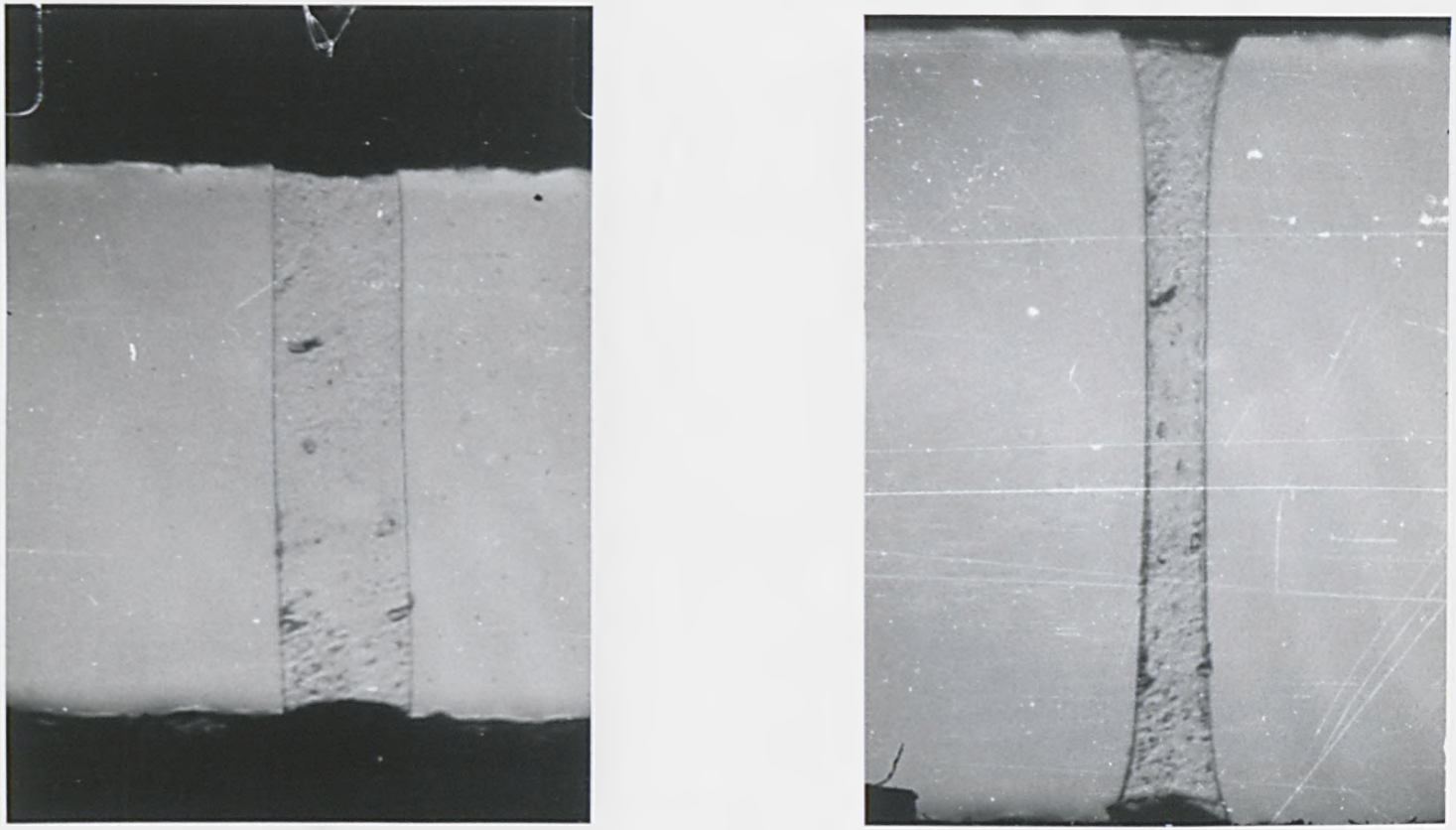
(a)
UNSTRETCHED



(b)
STRETCHED

FIG. 59.

FIG. 60.



(a)

UNSTRETCHED

(b)

STRETCHED

'HIGH CREEP RATE' CELL JUST BEFORE BREAK

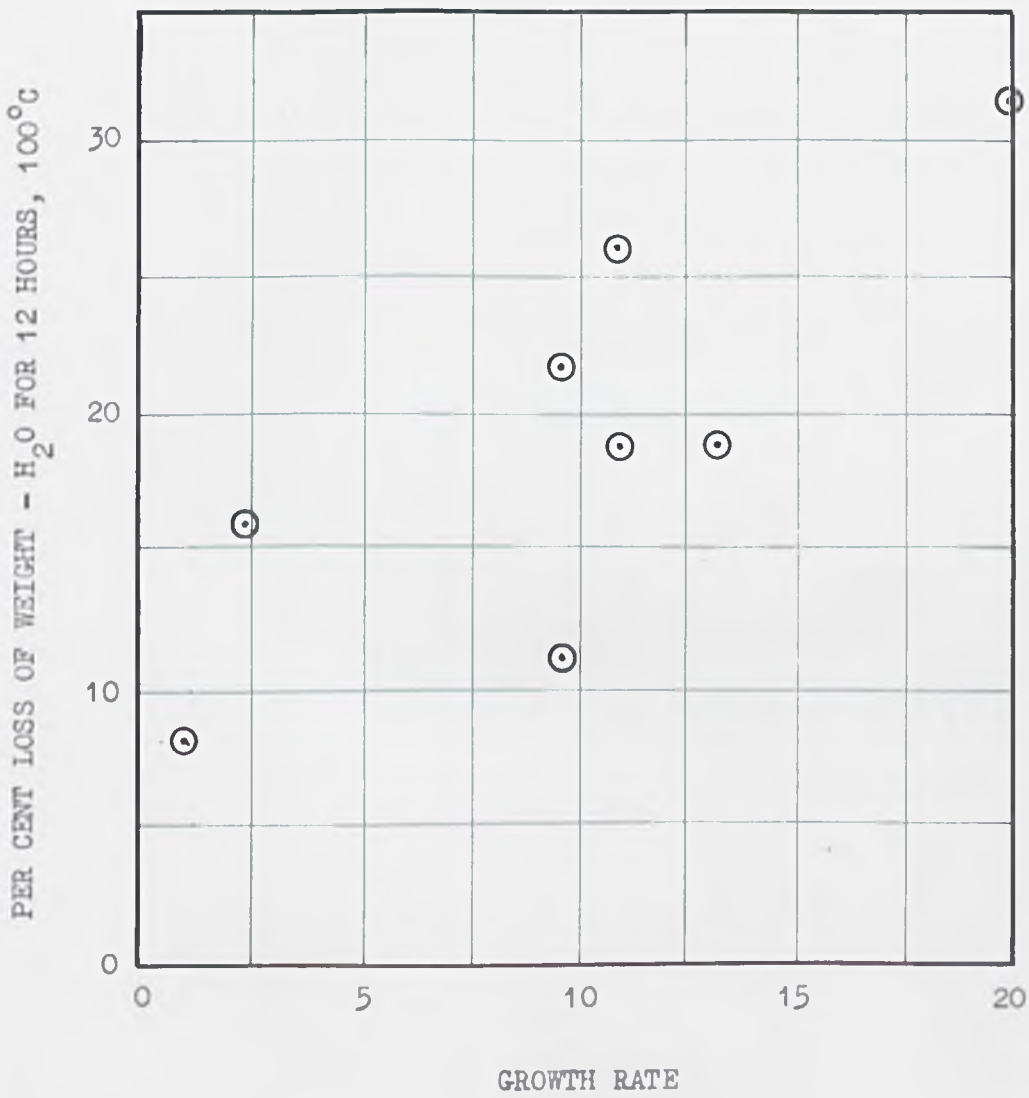

PER CENT LOSS OF WEIGHT - H_2O FOR 12 HOURS, 100°C
30
20
10
0
0
5
10
15
20
GROWTH RATE
PER CENT LENGTH INCREASE/24 HOURS

FIG. 61.

FIG. 62.

INTENSITY - ARBITRARY UNITS

POTASSIUM (K_α)

SAMPLE - KCl TREATED WALL

SAMPLE - $CaCl_2$ TREATED WALL

SAMPLE - UNTREATED WALL

CALCIUM (K_α)

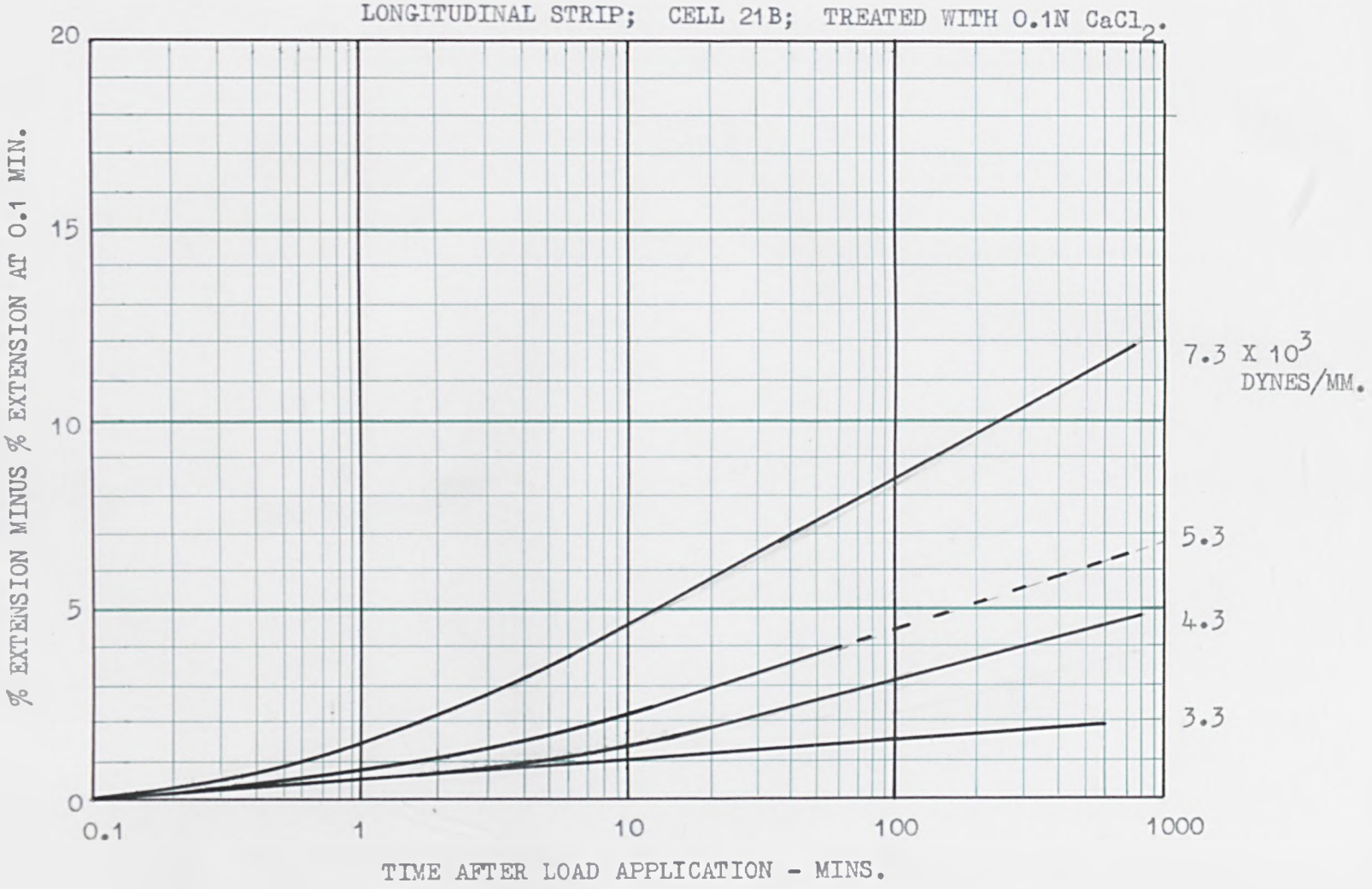
SAMPLE - KCl TREATED WALL

SAMPLE - $CaCl_2$ TREATED WALL

SAMPLE - UNTREATED WALL

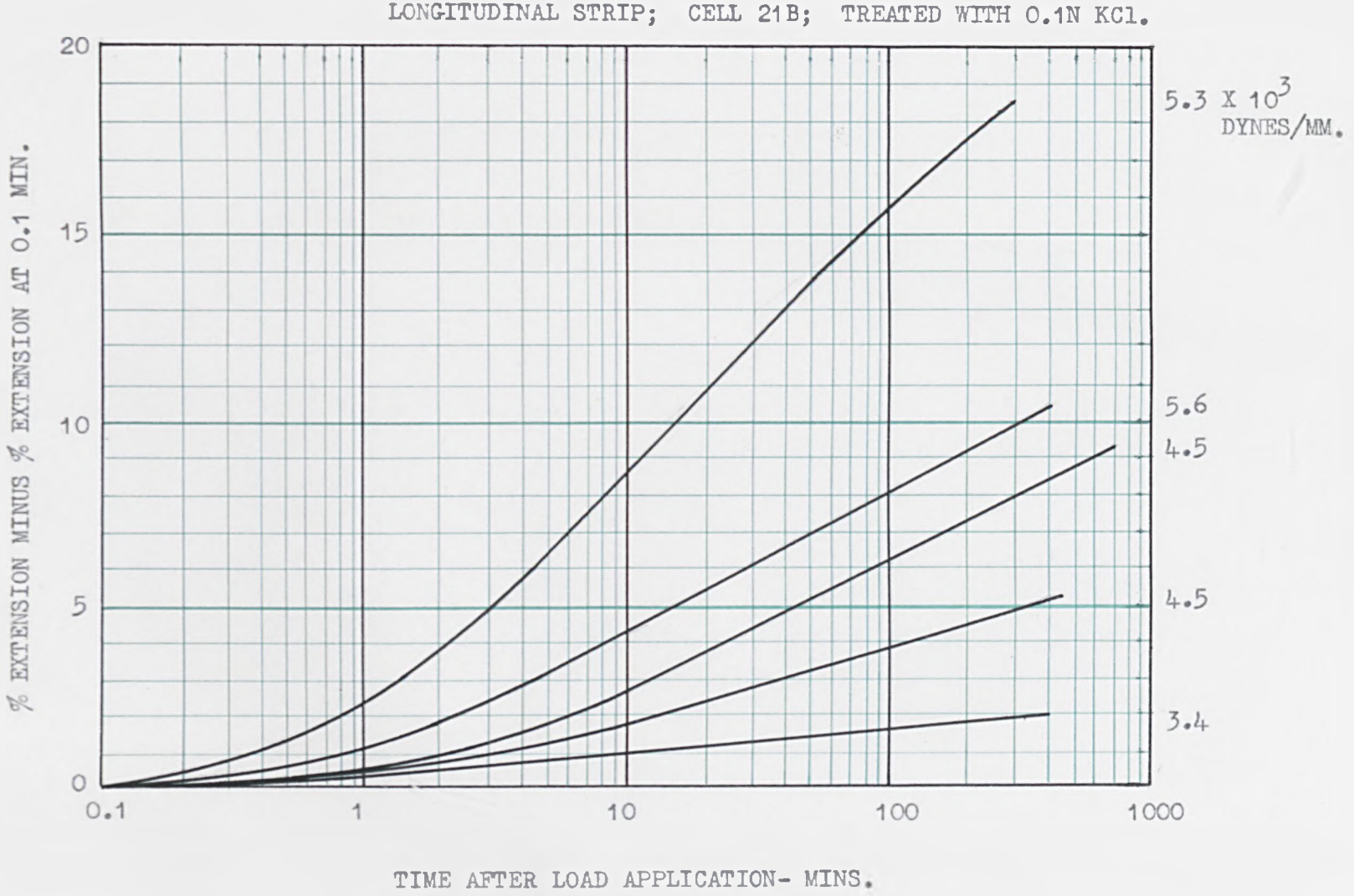
ANGLE - SCALE NOT CONTINUOUS →

FIG. 63.



NOTE:- ONE CURVE (5.3 X 10^3 DYNES/MM.), LOWER THAN THAT SHOWN HAS BEEN OMITTED FOR CLARITY.

FIG. 64.


LONGITUDINAL STRIP; CELL 21B; TREATED WITH 0.1N KCl.
% EXTENSION MINUS % EXTENSION AT 0.1 MIN.
20
15
10
5
0
0.1
1
10
100
1000
TIME AFTER LOAD APPLICATION- MINS.
5.3 X 10^3 DYNES/MM.
5.6
4.5
4.5
3.4

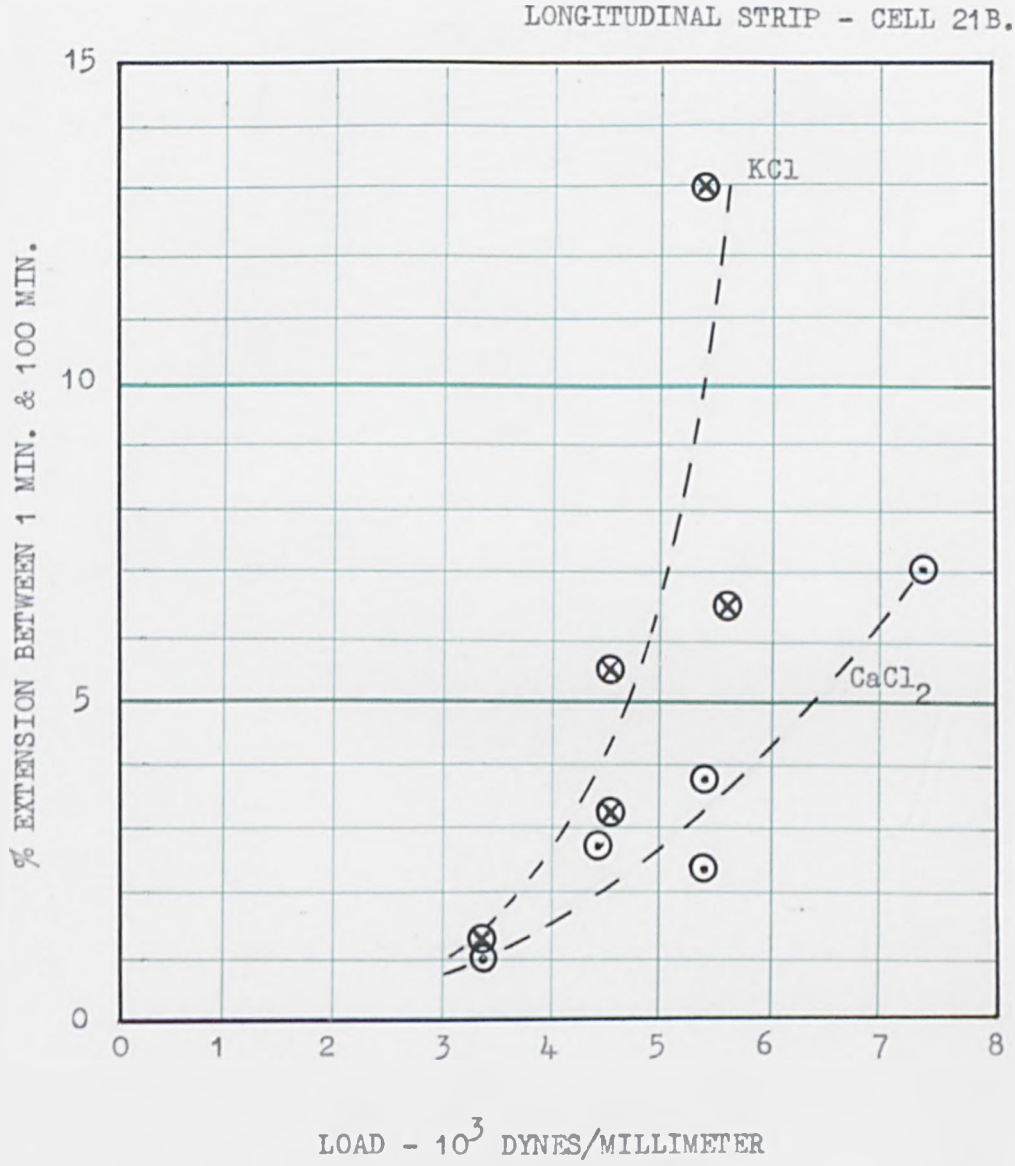

LONGITUDINAL STRIP - CELL 21B.
% EXTENSION BETWEEN 1 MIN. & 100 MIN.
15
10
5
0
KCl
CaCl2
0
1
2
3
4
5
6
7
8
LOAD - 10^3 DYNES/MILLIMETER

FIG. 65.

LONGITUDINAL STRIP - CELL 22B.

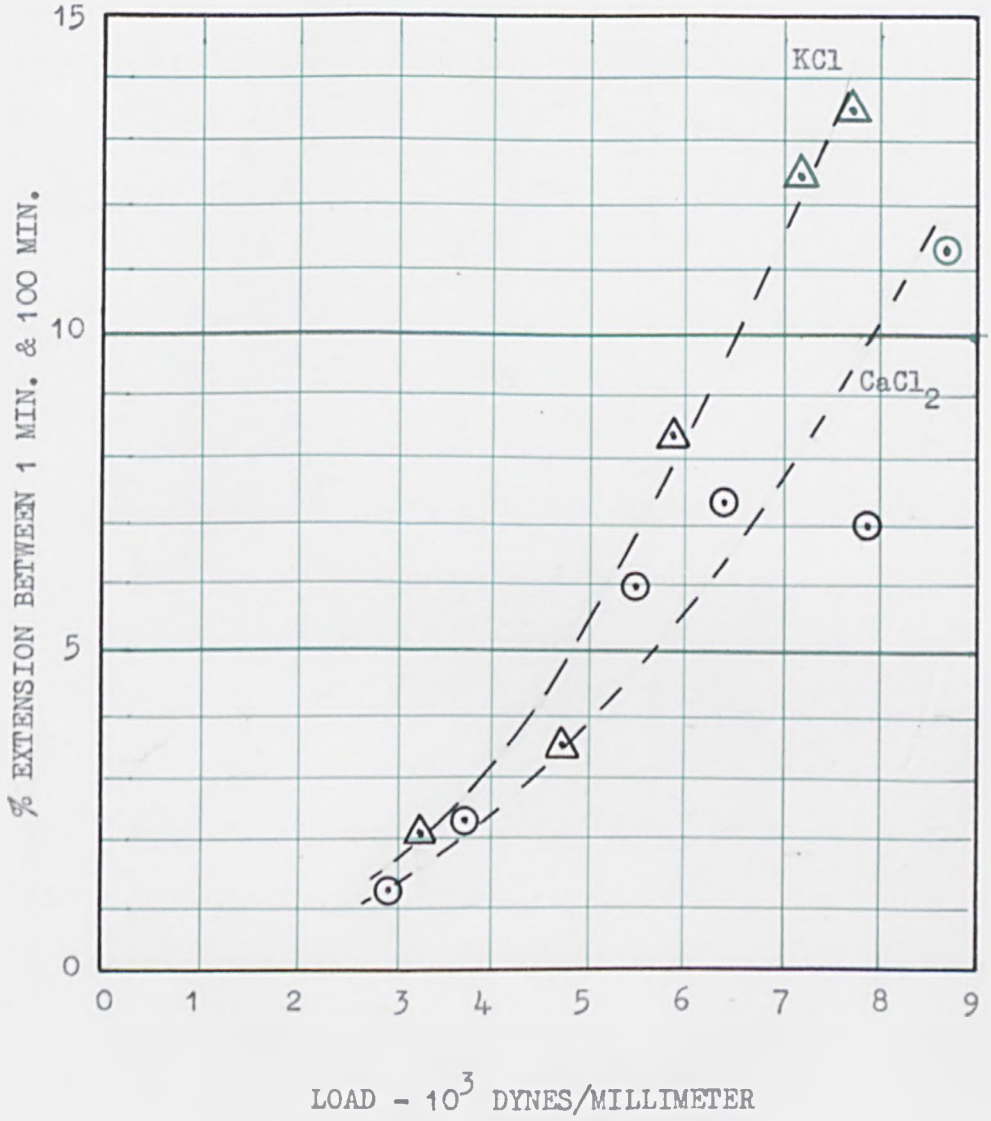


FIG. 66.

N.B. GREEN POINTS OBTAINED BY EXTRAPOLATION FROM BREAK.

FIG. 67.

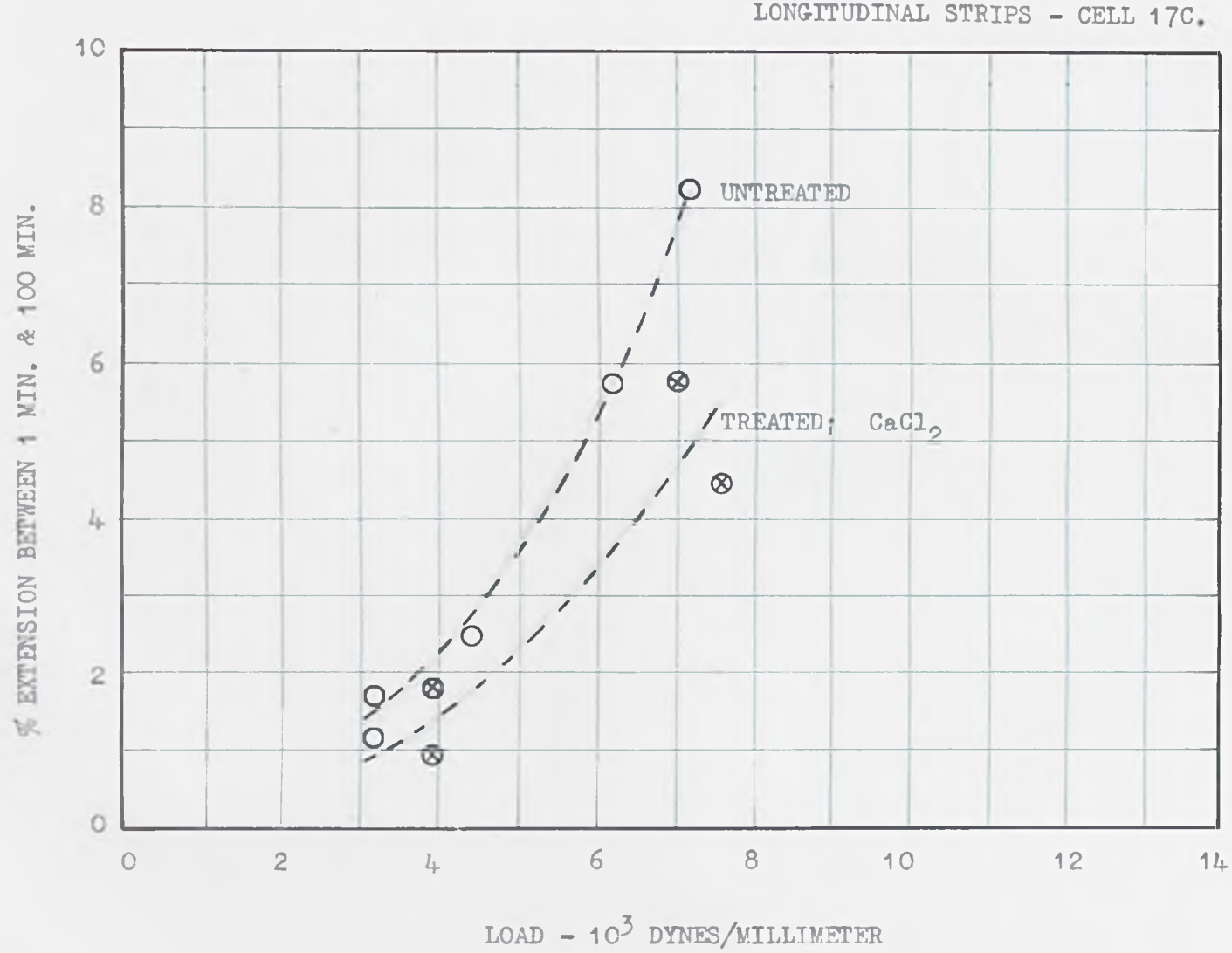

LONGITUDINAL STRIPS - CELL 17C.
% EXTENSION BETWEEN 1 MIN. & 100 MIN.
LOAD - 10^3 DYNES/MILLIMETER
UNTREATED
TREATED; $CaCl_2$
0
2
4
6
8
10
12
14

FIG. 68.

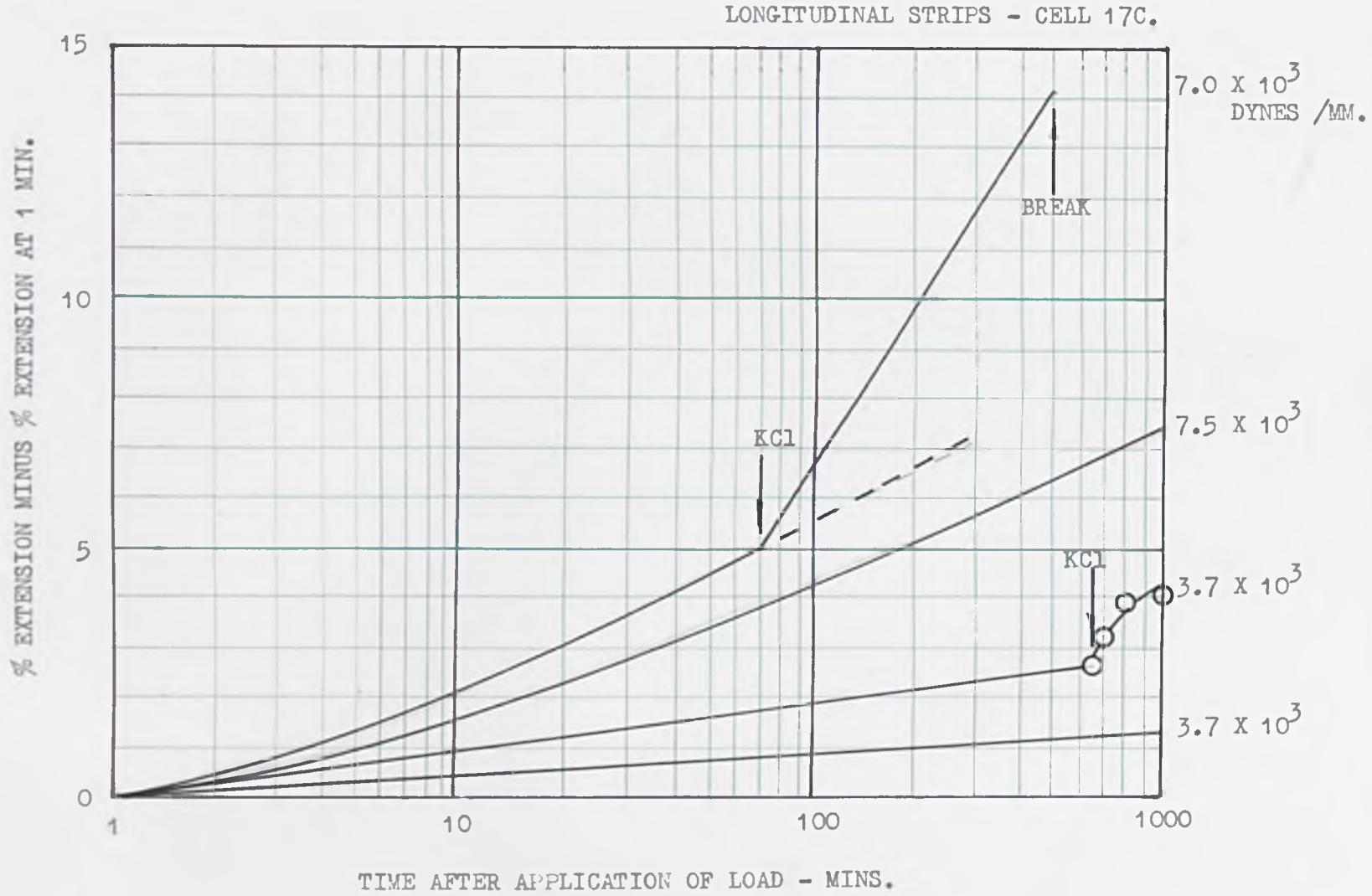
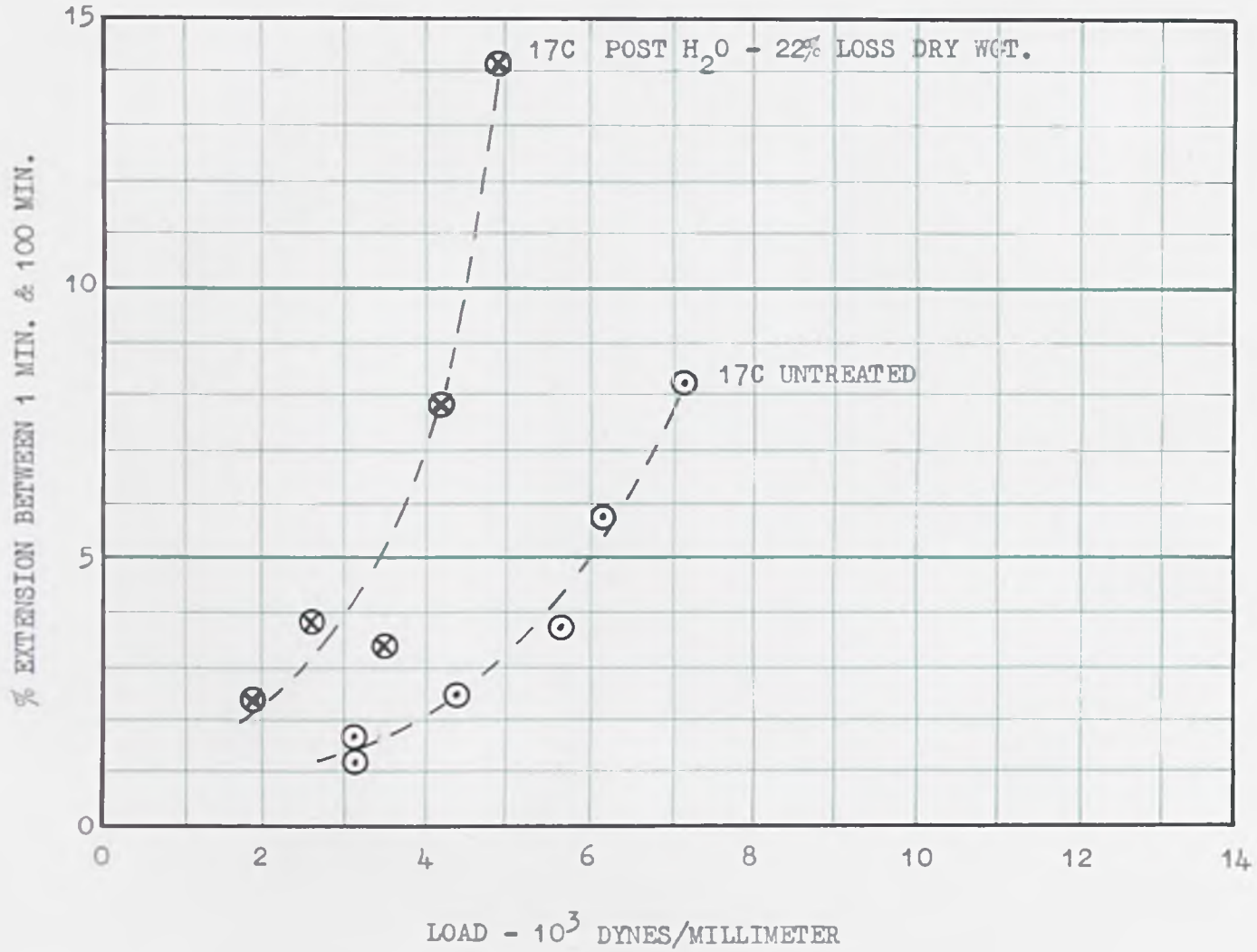

LONGITUDINAL STRIPS - CELL 17C.
% EXTENSION MINUS % EXTENSION AT 1 MIN.
15
10
5
0
1
10
100
1000
TIME AFTER APPLICATION OF LOAD - MINS.
7.0 X 10^3 DYNES /MM.
BREAK
KCl
7.5 X 10^3
KCl
3.7 X 10^3
3.7 X 10^3

FIG. 69.


17C POST H_2O - 22% LOSS DRY WGT.
17C UNTREATED
% EXTENSION BETWEEN 1 MIN. & 100 MIN.
LOAD - 10^3 DYNES/MILLIMETER
0
5
10
15
0
2
4
6
8
10
12
14

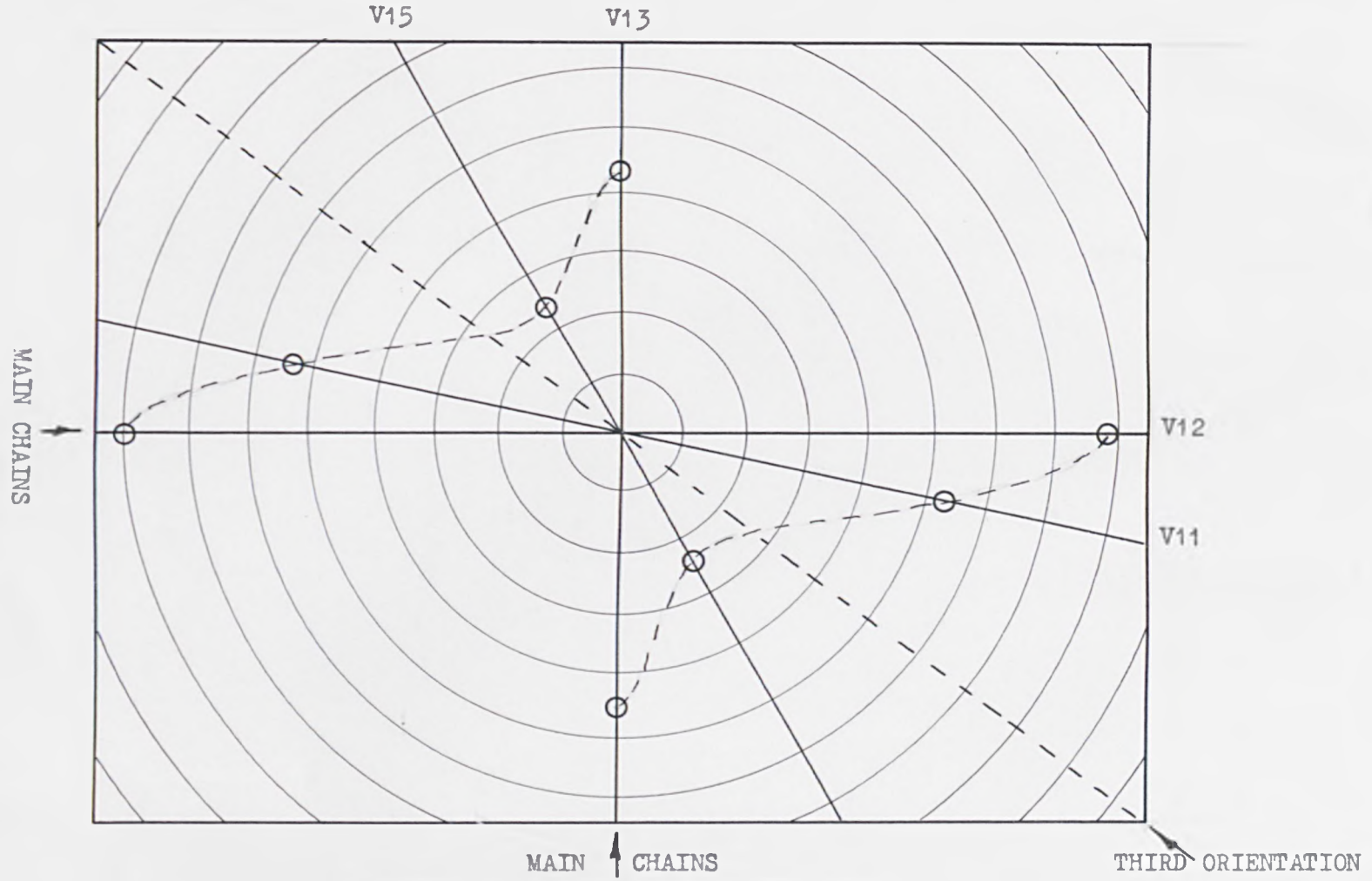


FIG. 70.

VARIATION OF YOUNGS MODULUS WITH ANGLE

ANGLE OF TEST STRIP - REFERRED TO CELLULOSE CHAINS

(ARBITRARY MODULUS UNITS)

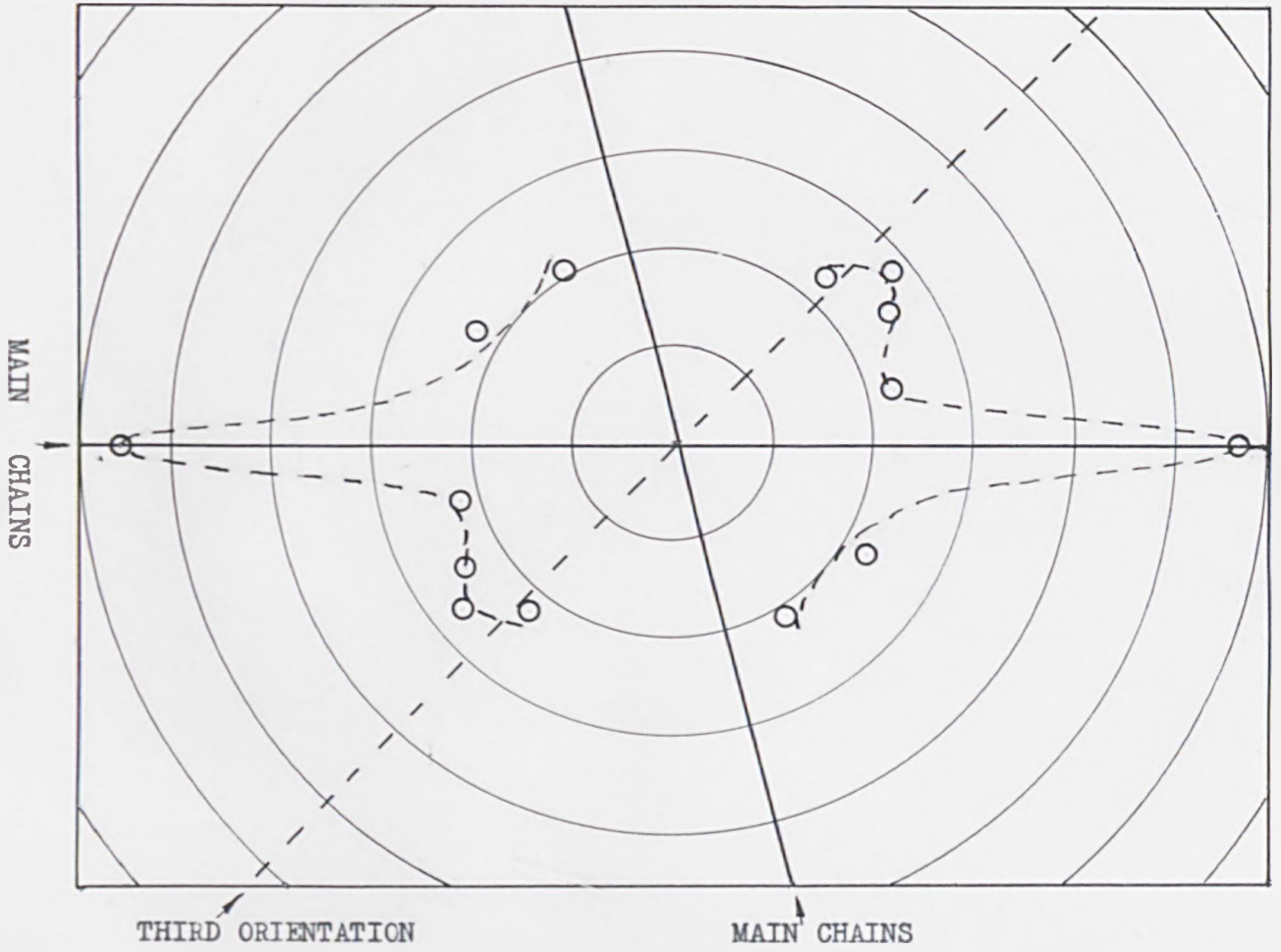
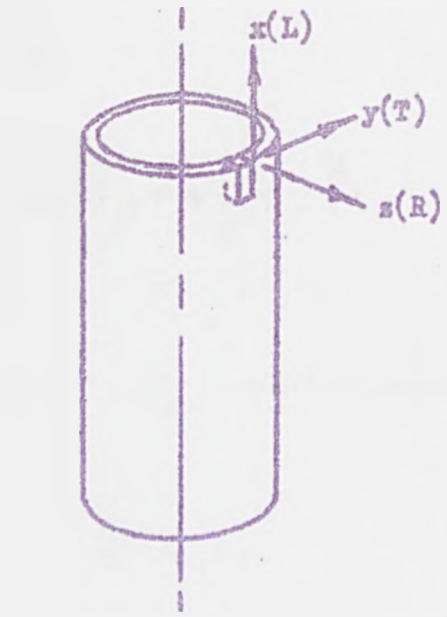


FIG. 71.

VARIATION OF YOUNGS MODULUS WITH ANGLE (VALONIA)

(ARBITRARY MODULUS UNITS)



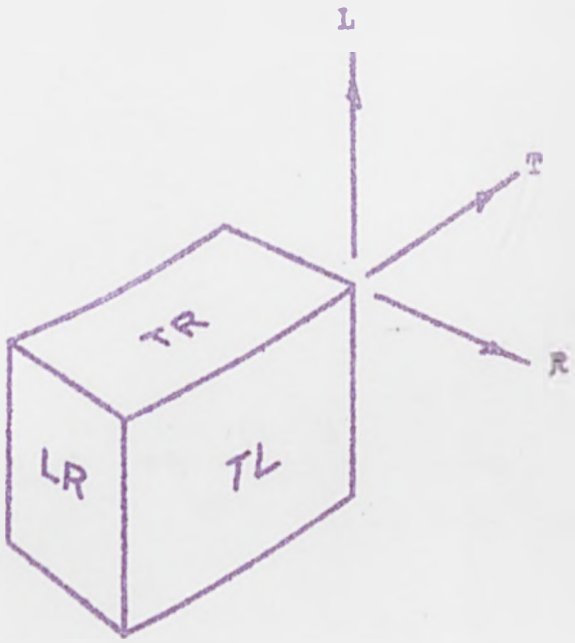


FIG. 72.

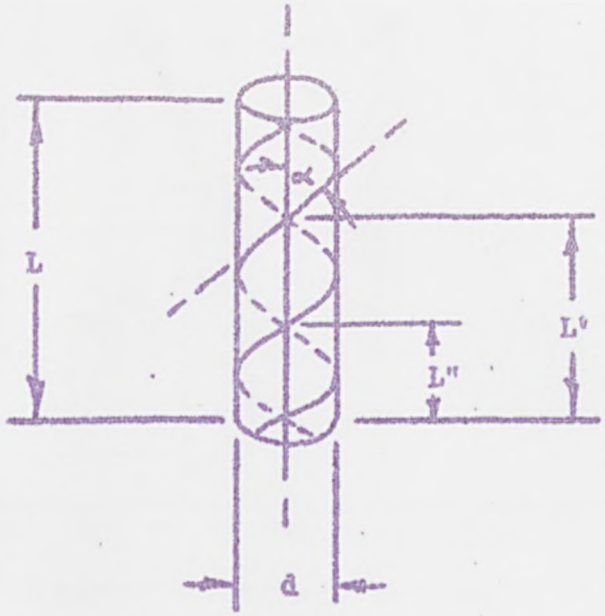
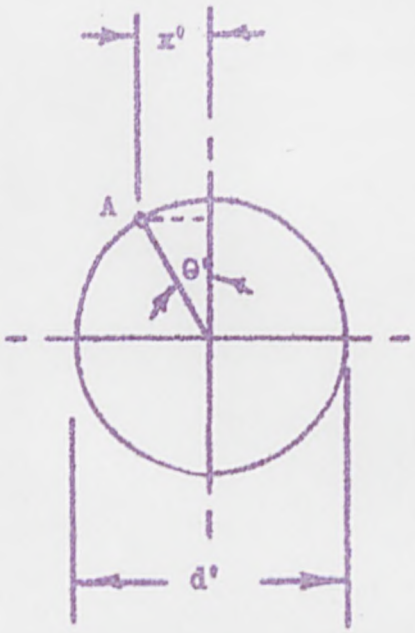


FIG. 73.



POSITION OF A AT TIME t'

POSITION OF A AT TIME t"

FIG. 74.

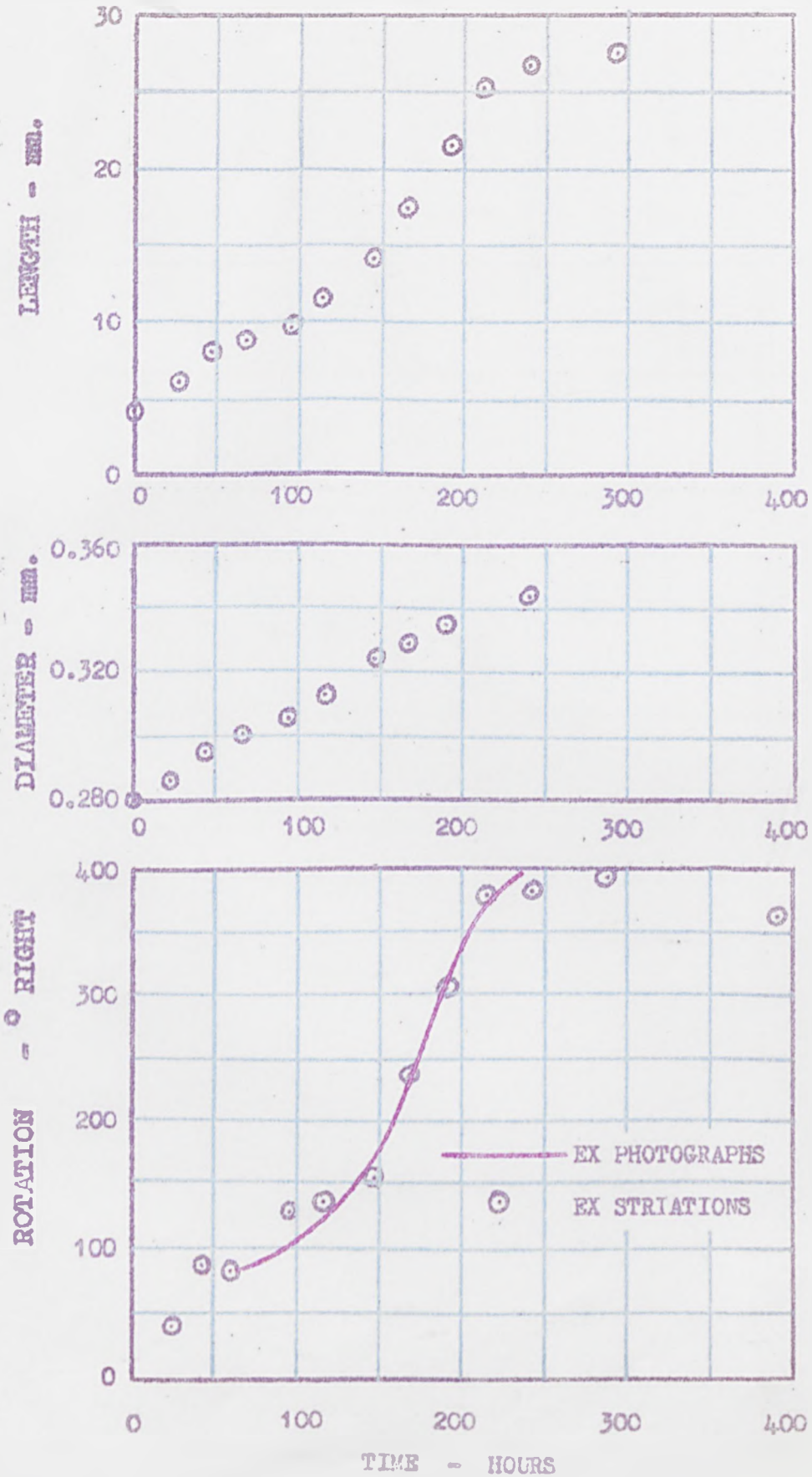

LENGTH - MM.
30
20
10
0
0
100
200
300
400
DIAMETER - MM.
0.360
0.320
0.280
0
100
200
300
400
ROTATION - ° RIGHT
400
300
200
100
0
0
100
200
300
400
EX PHOTOGRAPHS
EX STRIATIONS
TIME - HOURS

FIG. 75.

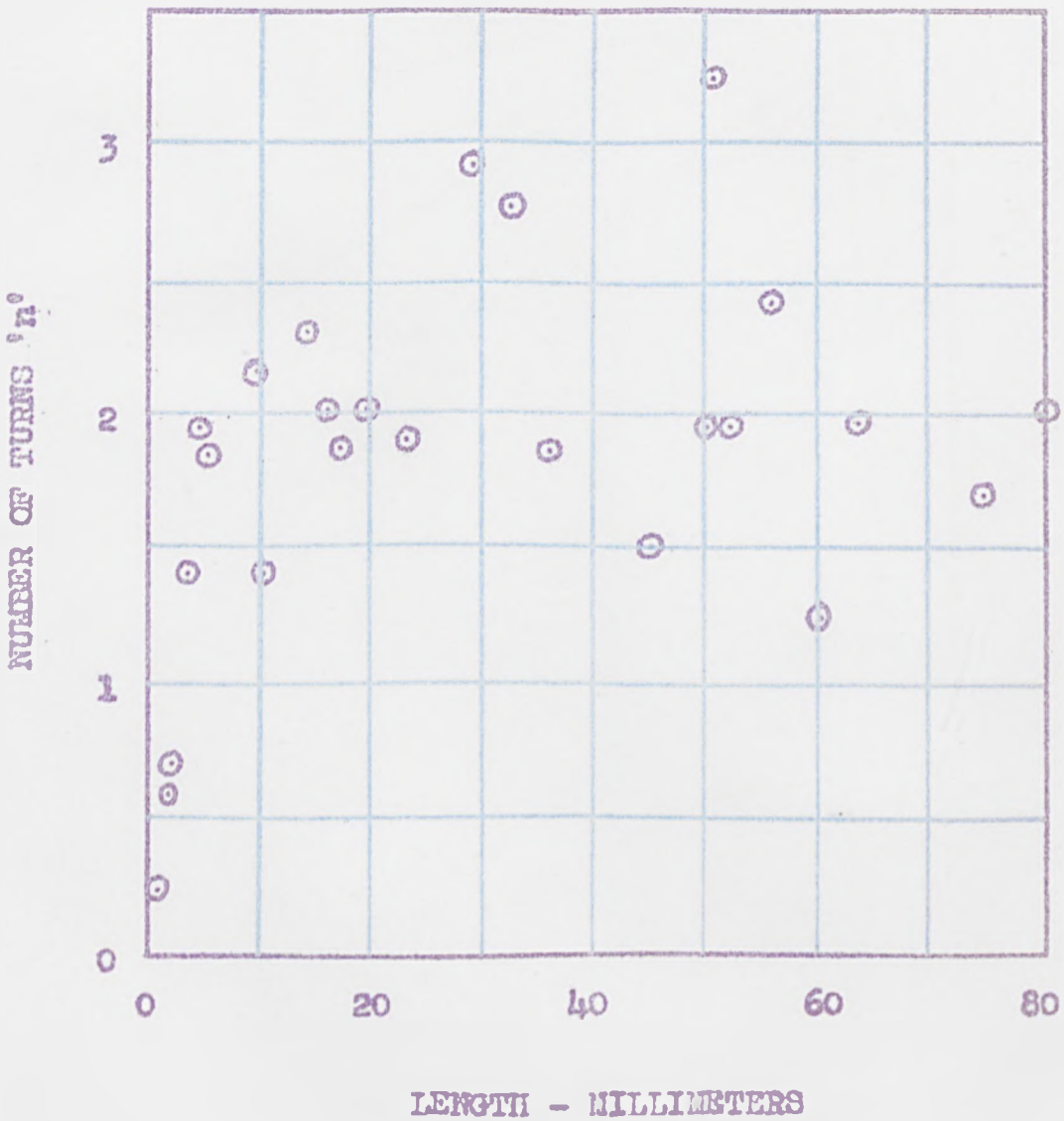


FIG. 76.

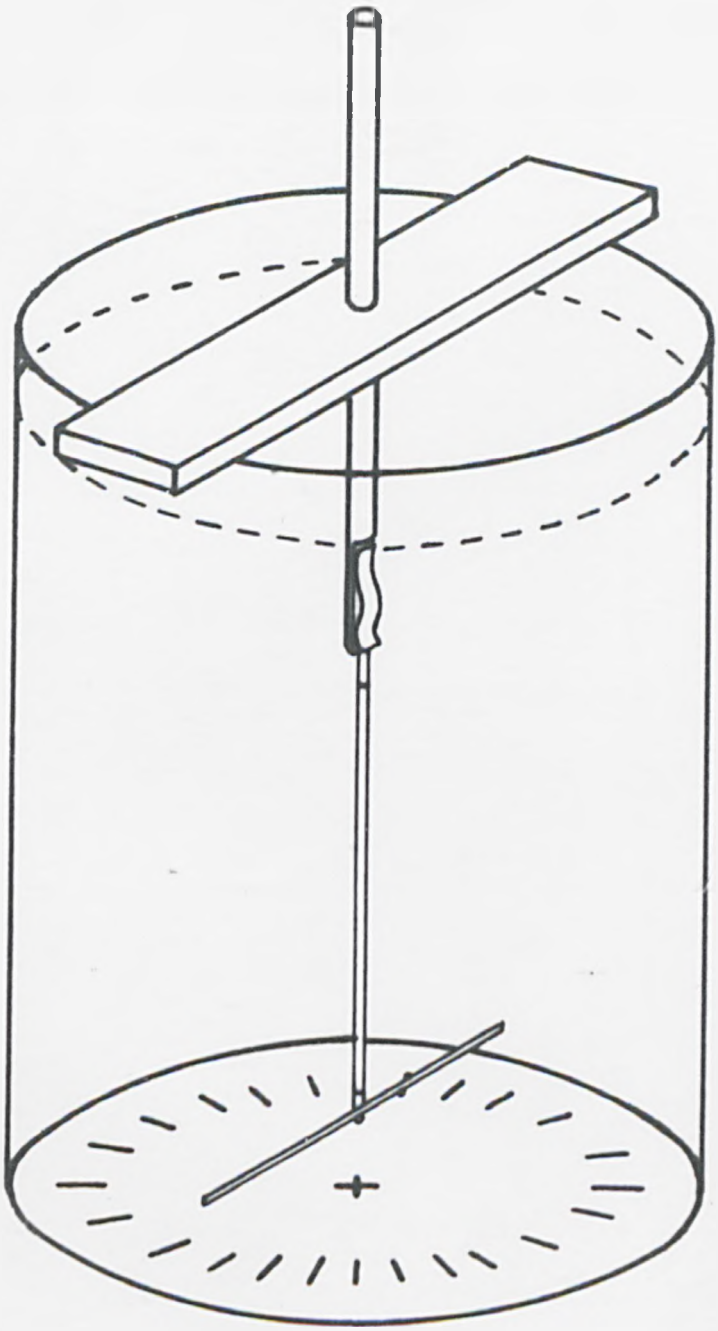


FIG. 77.

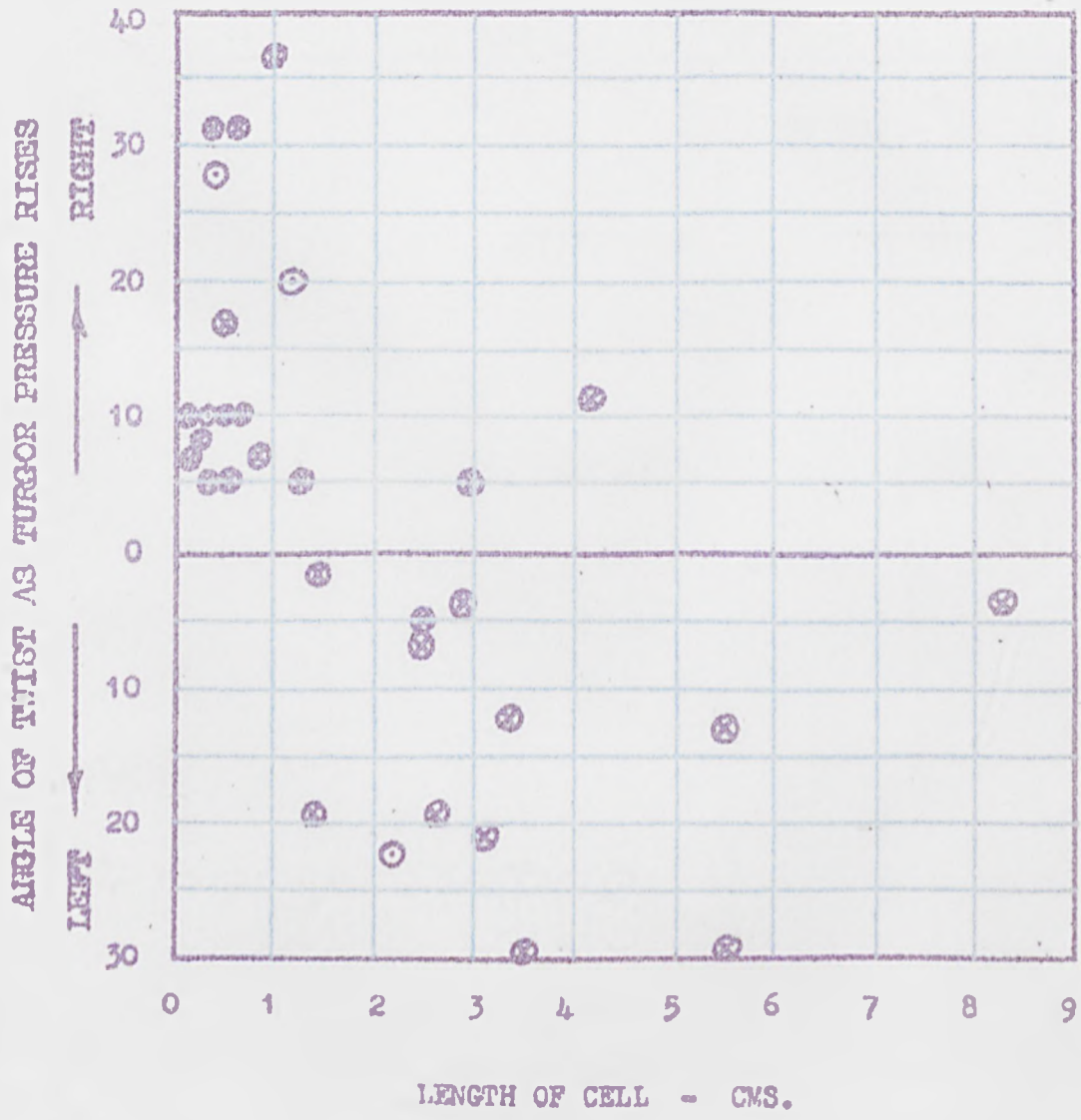


FIG. 78.

⊙ OBSERVATIONS BY MICROSCOPE

⊗ OBSERVATIONS BY INDICATOR

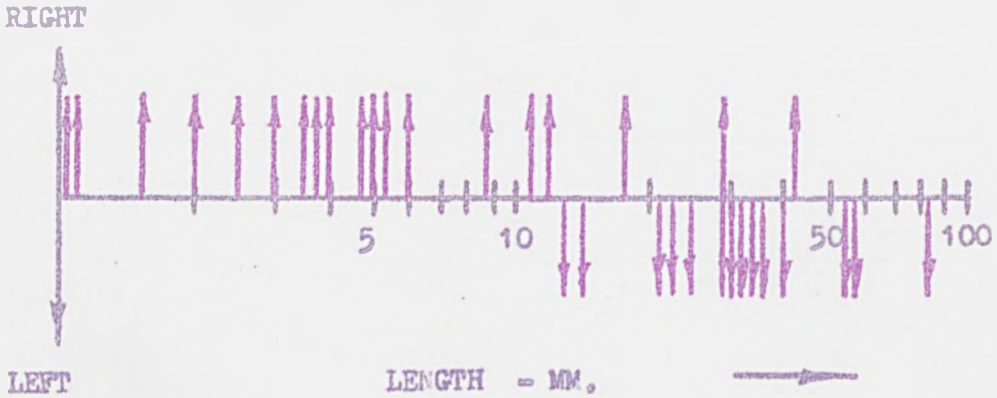


FIG. 79.

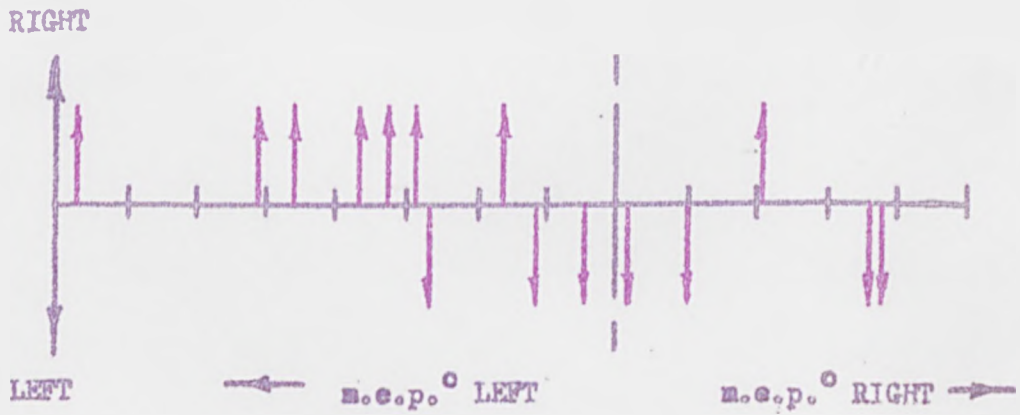


FIG. 80.

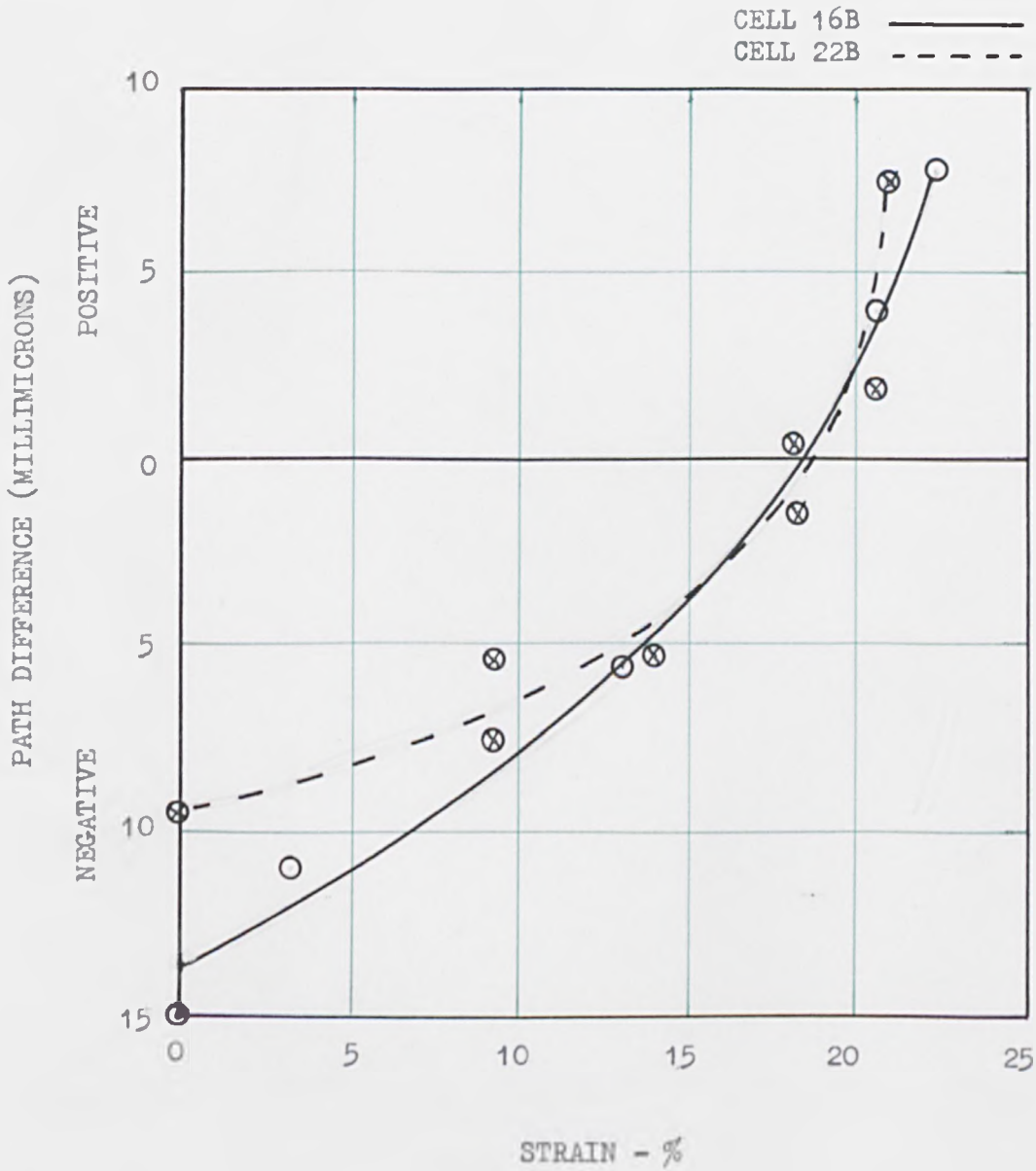

CELL 16B
CELL 22B
PATH DIFFERENCE (MILLIMICRONS)
POSITIVE
NEGATIVE
10
5
0
5
10
15
0
5
10
15
20
25
STRAIN - %

FIG. 81.