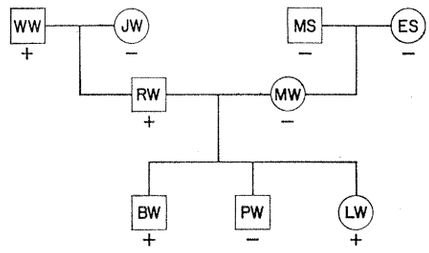

Fig. 1. Family relationships between the lymphocyte donors of Table 2. Reactions of lymphocytes of each individual with serum from Ma (Table 2) are indicated here as positive (+) or negative (−).

The four most strongly reactive immune human serums were tested for cytotoxic activity against lymphocytes of the two fourth-set skin donors as well as those of a panel of 12 individuals selected at random (Table 1). In none of the four immune serums was the presence of antibodies to erythrocytes demonstrated by direct agglutination or by methods in which antibodies to human globulin were used. Reactions with human lymphocytes were clear-cut, however (Table 1). Uptake of trypan blue dye by less than 10 percent of the lymphocytes of a suspension was regarded as a negative cytotoxic reaction; by more than 20 percent, as positive. Most results were well below or above these respective limits. The several values in the low 10 to 20 percent range are probably negative.

The antibody could be adsorbed from positive serums by previous exposure to a reactive lymphocyte suspension. It was present in the γ-globulin fraction of the serum. The unadsorbed serums obtained after grafting yielded positive reactions with lymphocytes from the specific donors, while serum specimens obtained before grafting gave negative reactions. The reaction of the immune serums appeared to differentiate between at least four distinct antigenic factors present on lymphocytes of various donors (Table 1). To date no correlation has been found between the lymphocyte reactivity and the ABO, Rh, MN, Kell, or Duffy blood group types of the lymphocyte donors. No agglutinins for the leukocytes (leukoagglutinins, which would react against neutrophils) were detected in the immune serums.

The results of a study of the lymphocytes of nine individuals, representing three generations of a single family, are presented in Table 2. The ways in which these family members are related are shown in Fig. 1. Serum from only one of the members reacted with the antiserum of Mo or To, all serums reacted with Bu, and four reacted with Ma. Results with Ma are also indicated in Fig. 1 and are consistent with a simple Mendelian hereditary pattern.

These results indicate that antibodies reactive with human lymphocytes, and demonstrable by means of a simple technique, can be produced by skin homografting of humans or by multiple intradermal injections of leukocytes into humans. Evidence from previous work with other animal species and from various laboratories, as reviewed elsewhere (*3*), suggests that such lymphocyte reactions reflect histocompatibility antigens. It is entirely possible that transplantation antigens are also present on other blood cells. Indeed, Payne showed that at least some antigens are shared by neutrophils and lymphocytes (*10*) and a correlation between leukoagglutinin tests and renal homograft survival in humans was claimed by Hamburger *et al.* (*11*). Nevertheless, neutrophil agglutination has been studied intensively since 1952 and has so far failed to define any histocompatibility systems in the human. It is our view that serologic analysis of human transplantation antigens may be accomplished by lymphocyte typing as here described.

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25 February 1964

Knots in Leucothrix mucor

Abstract. *The filaments of* Leucothrix mucor *are able to form true knots under certain cultural conditions. Such structures have apparently not been previously seen in filamentous organisms. As the culture ages, the knots become tighter and eventually the cells in the knot region fuse and form a large bulb. The filament breaks on each side of the bulb, and two shorter filaments and a free bulb are produced. The free bulbs have not been observed to grow into new filaments.*

Leucothrix mucor is a large colorless marine bacterium related to the blue-green algae, and it was first studied in detail by Harold and Stanier (*1*) and Pringsheim (*2*). This organism shows several growth habits: multicellular filaments, unicellular gonidia, and rosettes, the latter being formed by aggregation of the gonidia. As shown by Pringsheim, the filamentous habit is more common in a relatively rich culture medium, and gonidia form by fragmentation when filaments are transferred to a medium more dilute in the organic constituents.

I have found this organism to be a common epiphyte of macroscopic algae in the Friday Harbor, Washington, area, and I was able to confirm the observations of Harold and Stanier and Pringsheim with the strain which I isolated in pure culture. In addition, I have discovered a structural feature previously undescribed by these authors, namely, knots (*3*).

I have found knots when the organism was grown in a variety of simple and complex culture media, the only requirements apparently being good aeration and a medium which induces heavy growth of long filaments. Figure 1 shows organisms grown in the following medium: NaCl, 11.75 g; $MgCl_2$, 2.5 g; Na_2SO_4, 2.0 g; $CaCl_2 \cdot 2\ H_2O$, 0.75 g; KCl, 0.35 g; $NaHCO_3$, 0.1 g; yeast extract, 1.0 g; tryptone, 1.0 g; water, 1000 ml. The yeast extract and tryptone can be replaced with monosodium glutamate (10 g/liter) and Na_2HPO_4 (50 mg/liter) with essentially the same results. In these media gonidial production and rosette formation are infrequent, and the organism grows mostly as long filaments. If the organism is transferred from an agar slant into 1 to 2 ml of this medium and incubated at 25°C in a 16-mm tube shaken with a wrist-action shaker, growth is very pro-

fuse within 12 to 18 hours of incubation. Within the dense tangle of filaments, knots can commonly be seen.

Because of their three-dimensional nature, only rarely can photographs be obtained which convincingly show that these are true knots, but a large number of these structures have been examined individually and carefully drawn (see cover of this issue), and in each case the structure was a knot. Several independent observers also have confirmed the knot-like nature of these structures. In a culture grown as described above, almost every microscopic field revealed at least one knot, and frequently three or four.

Topologically, knots can be characterized by counting the number of apparent crossing points, where one branch of the filament is seen to pass in front of another, after the knot has been deformed so that it has its minimum number of crossing points. In a true knot, there must be at least three crossing points. A large number of *Leucothrix* knots have been carefully drawn and then reproduced in rope. A simple overhand knot (three crossing points) occurs most frequently, but figure-8 knots (four crossing points), timber hitches (five crossing points), and granny knots (six crossing points) have also been seen. A variety of different forms are shown in the drawings on the cover. A single filament may have only a single knot, or it may have five or six. Considering the large number of knots seen, such an unusual event could hardly occur by chance.

Leucothrix mucor does not grow only from the tip, as do filamentous fungi and algae, but throughout the length of the filament, as shown by observations on single filaments in microculture. Thus knot formation is taking place in a "rope" which continues to lengthen while it is being tied. To obtain a knot, we need a mechanism for forming a loop, and then a mechanism for passing the free end through the loop. A search of knot-forming cultures revealed many forms which seemed to be early stages of knot formation, and drawings of some of these are shown on the cover.

When the organism grows in the described medium at low cell densities, it grows as straight filaments, with only slight bends or curves. When it grows in the same medium at high cell densities, it forms many sharp curves, bends, and tight loops. Some sort of interaction must occur at high cell densities

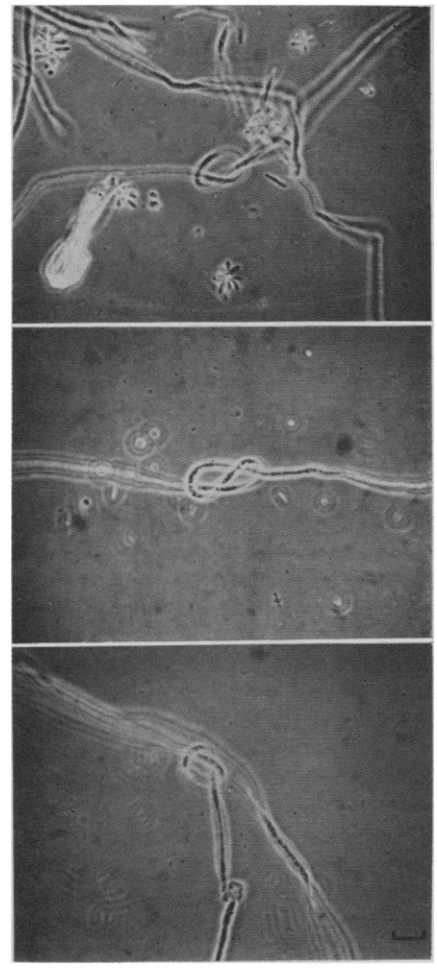

Fig. 1. Photographs of representative knots in *Leucothrix mucor* cultures (see also cover photograph). Phase contrast; horizontal diameter of knot in bottom photograph about 15μ.

which induces the filaments to bend as they grow. A good example of this type of bending can be seen when a colony is formed from a single gonidium on agar: the single filament of which the colony is composed grows in a tight whorl in an almost fingerprint pattern (*1*). In the colony on agar a very high filament density exists, and it is here that the filaments show the tightest bends and loops, although knots, of course, cannot form on the two dimensional surface of the agar plate. A filament would grow in this fashion if the cell wall on the outer circumference of the loop grew faster than the cell wall on the inner circumference, but I have no explanation of how crowding could induce this differential growth.

However, the formation of loops would not lead to the formation of knots unless there were a mechanism for the insertion of the growing free end through the loop. It is thus necessary to postulate that the free end grows toward the loop because it is attracted (in the broadest sense of this word) by the loop.

What is the fate of a knot? Although it has not been possible to follow a single knot to its ultimate destiny, an examination of cultures rich in knots has revealed a variety of forms which seem to be derived from knots and which can be placed into a hypothetical scheme. As the culture ages, the knots appear progressively tighter, until the cells in the knot region are in close contact. Cell fusion then appears to occur and a large, presumably coenocytic bulb is formed. An example of a bulb is shown in the lower photograph of Fig. 1. Later the filament on each side of the bulb separates and the bulb is set free. Thus knot formation leads to the fragmentation of long filaments into several shorter filaments, and is therefore a method of reproduction. The bulbs have not been seen to grow into new filaments.

Bulbs were seen by Pringsheim (*2*), although not by Harold and Stanier (*1*), possibly because the culture medium they used was too dilute in the organic ingredients and gonidial formation was more predominant. Pringsheim did not comment on the method of bulb formation. Bulbs can form in the absence of knot formation when filaments containing tight bends are lifted from agar and placed in liquid medium. Fusion occurs between the cells in the region of the tight bend. It is also possible that a bulb may form by the simple enlargement of a single cell of a filament, but because bulb formation is much more common in cultures rich in knots and tight bends, it is possible that cell fusion is an obligatory feature of bulb formation.

So far as I am aware, knot formation has not been reported in other filamentous organisms. Because of the ease with which *L. mucor* can be grown and studied, and because of the unusual character of the knot, this organism seems to be admirably suited for studies on morphogenesis.

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3. For simplicity I have chosen the Anglo-Saxon word *knot* instead of either of the Latin-based words *nodule* or *nodus*, because neither

of these latter words as employed in current usage explicitly describes the structure with which we are dealing.

4. Research career development awardee, USPHS, grant No. AI-K3-18,403. The isolation of the culture and preliminary observations were done while I was a visiting investigator at the Friday Harbor Laboratories, University of Washington.

5 February 1964

Morphology of Microtubules of Plant Cells

Abstract. *The microtubules present in the cortices of plant cells are found to have a wall made up of slender filamentous subunits, probably 13 in number, which have a center-to-center spacing of about 45 angstrom units. Thus the micromorphology of these tubules is similar to that reported for the 9 + 2 fibrils of rat sperm flagella. This structure can be used to account for a primary function of motion attributable to the microtubules.*

In a recent paper we reported the existence of microtubular elements in the cytoplasm of cells of a few higher plants (*1*). These structures as seen in thin sections by electron microscopy have an outside diameter of 230 to 270 Å and are especially prominent in the cortices of meristematic cells (that portion subjacent to the plasma membrane) during interphase (Fig. 1). They are similar in size and appearance to the so-called fibrils common in the mitotic spindle and also resemble the filaments or fibrils (or microtubules) which make up the 9 + 2 complex of flagella and cilia. In the same report we mentioned several other locations where apparently homologous structures are found and suggested that microtubules thus defined are cytoplasmic structures of wide occurrence in the cells of both plants and animals. In order to provide further support for such a generalization, we have now sought to characterize them in greater structural detail.

It was noted in the earlier report that the limiting dense wall of the plant cell microtubule seems to be made up of subunits which appear circular in transverse section. The evidence, however, was admittedly poor, and whether this substructure existed or not remained a question which became especially important when, within the same year (1963), Pease (*2*) and André and Thiéry (*3*) reported that the "fibrils" or microtubules of the 9 + 2 complex are constructed of 10 or 11 filamentous subunits, bundled together to form the tubular structure. Here it seemed was a detail of fine structure that could be used to define these and other cytoplasmic tubules of this size as probably homologous and as possessing similar functions.

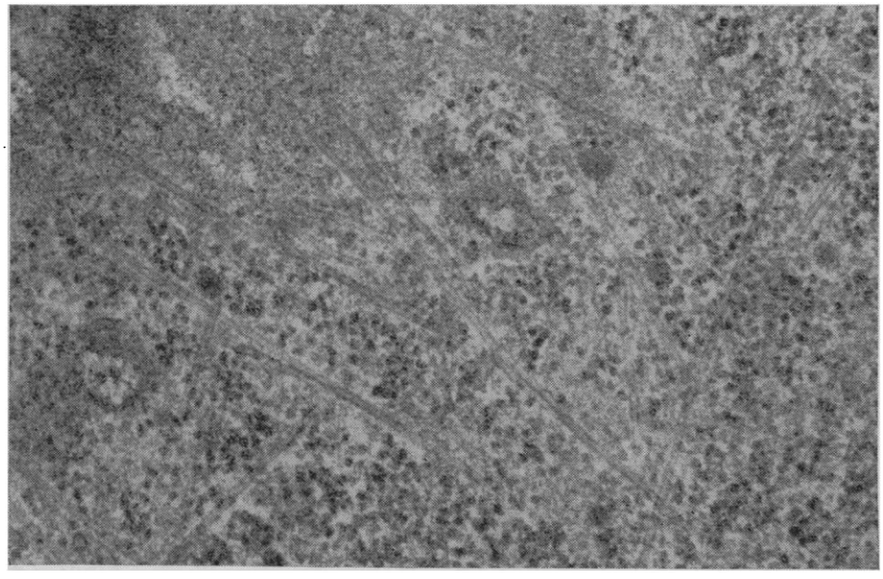

Fig. 1. Electron micrograph of section cut nearly parallel to and including a portion of the end wall (gray area at upper left) and adjacent cell cortex from root tip of *Phleum pratense*. The long, slender microtubules, unlike those found in parallel array along the side walls, here show an apparently random orientation within the cell cortex adjacent to the end wall (× 64,000).

For a further investigation of the problem it was found that certain meristematic cells in the root tips of *Juniperus chinensis* L. and cells from the nectaries of *Euphorbia Milii*, Ch. de Moulins, offered some advantages over materials previously used. Thin sections of these cells were prepared by techniques described earlier (*1*). These were stained only lightly for 10 minutes in uranyl acetate (*4*), washed briefly and then placed in basic lead citrate for 5 minutes (*5*). Longer periods of staining obscured some of the fine structure.

A single microtubule as it appears in this material is shown in cross section in Fig. 2. It is circular in form and about 230 Å in diameter, and has the characteristic "hollow" or low-density center. The wall, which is about 70 Å thick, is apparently constructed of a number of subunits also circular in outline. The center-to-center spacing of these subunits is about 45 Å. This structure, and most especially the clearly demonstrated subunits, have been encountered so far only in particular cells of *Juniperus* and *Euphorbia* which display after staining an overall greater density in many components of the cytoplasm. It appears that some substance in the protein-containing structures of these dark-staining cells greatly increases their affinity for uranyl and lead salts and therefore increases their density as seen in the electron microscope. The ribosomes, for instance, are extraordinarily prominent and the cytoplasmic membranes in which the three-layered structure is usually hard to resolve here show this structure with outstanding clarity. This unusual staining extends as well to the wall of the microtubules and introduces a problem of interpretation. We decided tentatively that the distinctly clear halo, which was noted earlier (*1*) as surrounding the microtubule, had in these cells stained to give the darker, more prominent image of the tubule. The effect of this, according to the interpretation, is to provide a natural "negative staining" of the structural elements in the wall, elements which now appear as circles of low density.

To test this interpretation we have compared images of transverse sections of ordinarily and "negatively" stained tubules as encountered in light- and dark-staining cells of *Euphorbia*, separated by only a cell wall. The dimensions and relative densities of these