fact that the technique has not been used in the past). This is not the case, however. The measure is responsive to a variety of changes in the motivational states of the organism. Changes in alertness as a function of adaptation, sleep, and stressful stimulation markedly alter the resistance level. Changes in the stimulus configuration (opening the cage door, for example), as well as the effect of a specific reward are also readily seen on the BRL record. Figure 1 shows the drastic change in BRL in response to a shock of 5-second duration (at 5 ma, constant current, d-c square wave, 120 pulses per second). The post-shock resistance level, 60 kohm, represents a drop of about 90 percent.

The effect of the rat's movement is a surmountable problem. In some situations, such as straight alley mazes, the grid can be replaced by two parallel stainless-steel plates with a narrow gap, thus reducing the number of times contact with the electrodes is disturbed. Generally, in mazes where the animal's movement is restricted to a single direction, as opposed to a cage or open field, this effect is also much diminished.

The technique has several advantages. It is relatively simple to obtain; it can be used in any situation in which a grid floor can be placed in the maze or cage; it can be obtained concurrently with other measures. Furthermore, the procedure, because the rat is not restrained and does not have electrodes attached, does not alter the organism's level of motivation. Preliminary findings suggest that in the rat—as in humans—skin resistance may be considered an index of the arousal or alertness dimension (*3*).

STEPHEN KAPLAN
RACHEL KAPLAN
Department of Psychology, University of Michigan, Ann Arbor

References and Notes

1. E. Z. Levy, V. H. Thaler, G. E. Ruff, *Science* **128**, 33 (1958).
2. D. T. Lykken and R. Rose, *Am. J. Psychol.* **72**, 621 (1959).
3. This work was supported in part by research grant No. M4239 from the National Institutes of Health, U.S. Public Health Service, to Dr. E. L. Walker. We wish to thank J. L. Hobart of the Physics Department for development of the circuitry used here.

4 September 1962

Mechanism for Plant Cellular Morphogenesis

Abstract. The control of the cylindrical cell form in plants appears to reside in the orientation of the reinforcing cellulose microfibrils in the side walls. In elongating cells the fibrils are typically transverse. Control of new synthesis of oriented wall texture is shown to be in turn related to the orientation of cytoplasmic elements in the cell periphery. Three properties of these cytoplasmic elements have been deduced from polarization optical properties of treated and normal cell walls. These deduced properties—namely, possession of a long axis and the ability to build microfibrils perpendicular to it, a tendency to cross-bond to make a parallel array, and a sensitivity of this alignment to colchicine—are all well-known properties of mitotic spindle and phragmoplast fibers which form the cross-wall after mitosis. It is proposed that proteins of spindle fiber nature exist in cortical cytoplasm of plant cells and are active in the control of wall texture and cell form.

The growth of the plant cell may be viewed as the yielding of the cell wall to the turgor pressure of the cell vacuole. Because the pressure is the same in all directions, the direction of growth taken by the cell must reside in the anisotropic (unequal) yielding of the wall. In elongating cylindrical cells which have expansion taking place all along the cell axis the anisotropy of structure is typically of the expected sort. There is strong transverse reinforcement due to the presence of cellulose microfibrils arranged in this direction (as hoops on a barrel). This transverse texture is maintained during prolonged extension of the cell by the active cytoplasmic synthesis of cellulose in the transverse direction (*1*). The control of cell form thus appears to reside in cytoplasmic elements near the growing side wall. Polarized light studies with growing cells of *Nitella* and *Bryopsis*, described below, indicate that the cytoplasmic elements (i) have a long axis and form cellulose microfibrils at right angles to their length, (ii) have a tendency to be bonded laterally to make a parallel array, and (iii) can be disorganized by colchicine. These properties, deduced here indirectly for the cytoplasm adjacent to growing side walls of plant cells, are well established for the spindle and phragmoplast fibers involved in cross-wall synthesis.

That the cytoplasmic elements involved in side wall synthesis are long is indicated by two experiments where the orientation of synthesis can be made to vary in relation to the direction of maximum strain, or deformation, of the cell. Long cytoplasmic elements would be expected to become aligned in the direction of maximum strain. When a lateral is induced to form by the mechanical constraint of the cell with a perforated jacket, as in Fig. 1*a*, synthesis in the lateral is perpendicular to the direction of maximum strain. A similar perpendicular relation is found when a free-growing cell is treated with 0.2-percent colchicine (Fig. 1*b*). Here the cell rotates rapidly as it elongates. The lines of maximum strain are therefore oblique, reaching 30° off-axial. The synthesis at the inner surface of the wall is also oblique, being perpendicular to the twisted lines of strain (*2*). The orientation of new microfibrils is ascertained with polarized light microscopy. The microfibrils are positively birefringent.

These results suggest that long elements in the cytoplasm adjacent to the wall can become aligned into the direction of maximum strain and then cause microfibrillar synthesis at right angles to their alignment. This alignment would occur in the cortical (gelled) cytoplasm, not in the protoplasmic stream which is considerably more remote from the wall.

There is also evidence that similar strain orientation of long cytoplasmic elements plays a role in the change of form of certain cells. The marine alga *Bryopsis* is a coenocyte whose tip produces not only a main axis but also lateral axes at regular intervals (Fig. 1*e*). Both the main and lateral axes have a transverse pattern of microfibrils. The origin of the lateral axis is seen, in polarized light studies of single layers of wall, to involve the simultaneous production of a small bulge and a concentric arrangement of microfibrils (concentric high indices of refraction). This observation, made by looking for small bulges on living cells before examining their wall, supersedes my earlier report (*3*). The simultaneity of the bulging and the appearance of the concentrically arranged microfibrils again suggest that microfibrillar synthesis can be oriented perpendicular to the direction of maximum strain. The lateral presumably starts as a local softening of the wall.

In the light of this wall behavior, that is, microfibrillar synthesis at right angles to apparent strain-oriented long elements in the cytoplasm, it is significant that a right-angle relation also obtains between the long axis of cytoplasmic fibers and the microfibrils of the developing cross-wall in many

plants. (This was drawn to my attention by Shinya Inoué of the Dartmouth Medical School.) The fibers involved in building the cross-wall could not be distinguished from proteinaceous spindle fibers in the in vivo polarized light studies of Inoué (*4*). The presence of such fibers in the peripheral cytoplasm would account for the induced strain sensitivity in the types of wall development described above.

During normal growth and under one experimental condition with *Nitella*, the above-mentioned potential for strain orientation is not realized. During normal growth the cell shows "spiral" (helical) growth with the lines of the twist in the wall also reflected in the twisting files of chloroplasts (*5*). The orientation of synthesis is *not* perpendicular to these twisted lines of maximum strain but remains very nearly (within 4°) transverse to the cell axis despite some 15° variation in the lines of twists during growth as in Fig. 1*c* (*2, 5*). Also, when the cell is growing solely in circumference, as in Fig. 1*d*, the synthesis remains transverse despite the fact the direction of maximum strain has shifted from the near-axial to the transverse. The failure of the synthesis to be influenced by the direction of maximum strain in these cases can be explained on the assumption that the long elements are normally laterally bonded so as to have a ring-like texture (Fig. 1*f*). These rings would be kept taut by the increase in cell diameter in the two cases described (Fig. 1, *c*, *d*). Under the experimental conditions where strain alignment is realized, the lateral bonds are presumably broken either by the unusual strains of the experiment (Fig. 1*a*) or by colchicine (Fig. 1*b*). A tendency for lateral bonding is an obvious property of spindle fibers (*6*).

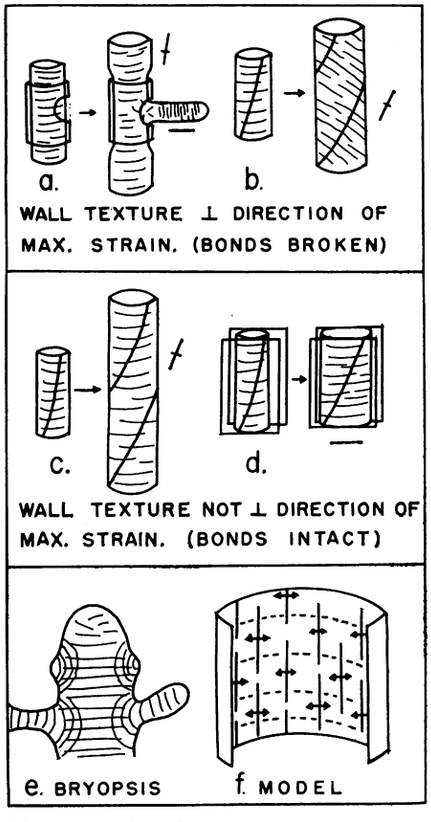

Fig. 1. (*a–d*) Microfibrils of the cell wall of *Nitella* are shown as many fine lines. The ascending helical line shows the direction of the chloroplast files. The cross or bar beside the figure shows the relative magnitude of strain in the indicated direction (qualitative). (*e*) The microfibrillar pattern in *Bryopsis* is shown as deduced from polarized light study. (*f*) Schematic view of the peripheral cytoplasm is given. The vertical lines are cytoplasmic elements of spindle-fiber character. The double-arrows show the nucleation or guidance of microfibrillar growth. The dashed lines show the lateral bonding sensitive to colchicine and abnormal strains.

A third observation linking orientation of synthesis in side walls to spindle-like protein is the action of stronger solutions of colchicine (0.4 percent) on young internode cells. These cells grow into spheres which can be shown in vivo to have essentially random wall texture (Fig. 2). The brightness at the periphery and darkness at the cell center in Fig. 2 is a necessary consequence of the microfibrils being present in the wall but dispensed at random in the plane of the cell surface. At the periphery the polarized light "sees" the wall "end-on" and thus reveals order. Slightly older internodes which are physically jacketed to prevent change in shape show, when treated with colchicine, the deposition of random wall to an optical thickness almost twice that present before the drug was given (*2*). The disorganization of aligned spindle fibers is the best known action of colchicine (*7*).

The control of oriented wall synthesis in the peripheral cytoplasm, and thereby the control of cell form, appears to reside in the orientation of long cytoplasmic elements which have several properties of plant spindle fibers involved in cross-wall formation. A most pertinent property is that of bringing on microfibrillar synthesis perpendicular to their long axis (Fig. 1*f*). The orientation of these cytoplasmic elements can be influenced by strain (flow alignment) when the cross-binding between elements is ineffective. Lateral bonding between elements is proposed to account for those cases where the elements resist alignment by strain (normal growth in *Nitella*). The bonding would unite the elements side by side into loops so that increase in cell diameter would maintain longitudinal alignment of the elements. With such bonding, transverse synthesis could persist in spite of variation in the direction of maximum strain (Fig. 1*f*). These lateral bonds would be broken by colchicine and severe strain. This model does not account for microfibrillar textures in secondary walls or crossed-fibrillar algae (see *8, 9*).

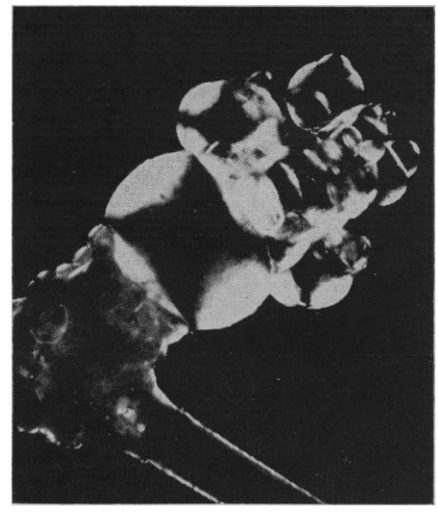

Fig. 2. A living small shoot of *Nitella* grown in 0.4-percent colchicine. The photograph was taken in polarized light, prisms crossed. The large cell's axis lies at 45° to the prism transmission planes. The dark cross reveals a random pattern of microfibrils in the plane of the wall. This pattern is also seen in the small leaf cells above. Untreated cells are cylindrical and show, when photographed this way, two dark bands parallel to the cell axis, revealing negative birefringence (transverse texture).

PAUL B. GREEN

Division of Biology, University of Pennsylvania, Philadelphia 4

References and Notes

1. P. A. Roelofsen and A. L. Houwink, *Acta Bot. Neerl.* **2**, 218 (1953); A. L. Houwink and P. A. Roelofsen, *ibid.* **3**, 387 (1954).
2. P. B. Green, in *21st Growth Symposium* (Academic Press, New York, in press).
3. ———, *Am. J. Botany* **47**, 476 (1960).
4. S. Inoué, *Chromosoma* **5**, 487 (1953); — and A. Bajer, *ibid.* **12**, 48 (1961).
5. P. B. Green, *Am. J. Botany* **41**, 403 (1954).
6. D. Mazia, in *Cell: Biochemistry, Physiology, Morphology*, J. Brachet and A. Mirsky, Eds. (Academic Press, New York, vol. 3, 1961).
7. O. J. Eigsti and P. Dustin, Jr., *Colchicine in Agriculture, Medicine, Biology and Chemistry* (Iowa State College Press, Ames, 1955).
8. R. D. Preston, *Intern. Rev. Cytol.* **8**, 33 (1959).

9. Supported by the National Science Foundation. The technical assistance of Mary Eager is acknowledged. More detailed information is in press (*2*).

26 October 1962