surfaces of ponds and ditches, and the larvae descend to the mud where the pupae later develop. In both species, two pointed and retractable spiracles can be pushed through the wall of a nearby root to reach the large gas spaces characteristic of aquatic plants.

N. riparia pierces a root while it is in the larval stage, and oxygen passes through the two spiracles into the tracheal system of tubes which distributes the gas throughout the larva. The spiracles are pushed out of the body and into the root by means of an increase in blood pressure. Houlihan finds that freshly collected larvae will evert and retract their spiracles up to forty times a minute in anoxic water, but more slowly when oxygen is available.

While the larva of *N. riparia* develops into a pupa, the spiracles remain in the plant and become hardened by layers of sclerotin. The spiracles are then continuous with the gas spaces which surround the pupa. The position of the spiracles embedded in the roots is shown in the diagram. In the case of *E. squalida*, larva and pupa can both pierce the plant root, doing so by contracting a special set of muscles on the prothorax behind the spiracles. When the adult *E. squalida* is ready to emerge from the pupa, it pulls the spiracles out of the root and moves up to the surface. But adult *N. riparia* emerge while the pupa is still attached to the root, and crawl up the plant stem to the surface or rise up in a bubble of air.

The plants which provide such convenient stores of oxygen for these mud-dwelling insects are chiefly *Glyceria maxima* or reed grass, *Typha latifolia* or great reedmace, also known as cat's tail, and *Juncus effusus* or soft rush. They all have large spaces between radiating rows of cells joining the epidermis and the central part of the root. *T. latifolia* seems to be the least popular source of oxygen for *N. riparia*, perhaps because the epidermis may be too thick to be pierced completely. Nevertheless, this ingenious relationship seems to be essential to the wellbeing of the insects when they are living in the mud.

AUXINS

Softening Cell Walls

from our Plant Physiology Correspondent

THOSE who maintained that auxins cause plant cell walls to grow simply by inducing hydrolytic enzymes that break up cell wall materials seem to have been wrong after all. A. W. Ruesink (*Planta*, **89**, 95; 1969) has found recently that hydrolytic enzymes cannot substitute for the auxin indolyl-3-acetic acid (IAA) in causing extension growth of plant cells.

This hormone has long been known to stimulate nucleic acid metabolism and to induce enzyme synthesis, which has led to the idea that IAA controls growth by stimulating the production of enzymes which can "soften" the firm cellulose wall surrounding the cell. After the wall has been softened, the theory goes, the cell can begin to take up water and so increase in length. Plant physiologists insist, however, that this increase can only be called growth if the turgor pressure of the cell—the pressure exerted on the restraining wall by water within the cell—remains constant. Because cell walls are elastic, changes in cell shape resulting from variations in turgor pressure could give rise to spurious results.

It is true that the cell wall must be softened before growth, and there are two theories to explain how this is regulated. The simplest idea is that hydrolytic enzymes are manufactured somewhere in the cell and transported to the walls, where they break the bonds between structural carbohydrates, and so bring about an increase in extensibility. In this case the rate of growth would be a function only of the extensibility of the walls. The balance of evidence, however, is against the idea of growth control through enzyme synthesis, at least in this simple form.

The alternative, and more complex, theory is that active cell metabolism controls the rate of extension by incorporating and rearranging macromolecules, such as proteins or carbohydrates, in the extending wall. In other words, much more than simple enzyme loosening of the wall may be required for growth.

Ruesink has tested the two theories by treating sections of oat coleoptile with an extract from the fungus *Myrothecium verrucaria* containing a mixture of two potent enzymes, β -1,3 and β -1,4 glucanase, which can hydrolyse wall polysaccharides to simpler units, and so effectively soften cell walls. The oat coleoptile, the thin cylindrical sheath which surrounds and protects the first leaf, has long been a favourite in plant growth studies. If the first theory were correct, treating the coleoptile walls with the fungal extract should increase the rate of growth of the coleoptile sections. But Ruesink found no difference between the growth rates of treated and untreated sections, whether IAA was added to the growth medium or not. Turgor pressure of the cells did not alter during his experiments, and there was no doubt that the fungal enzymes had effectively softened the wall of the coleoptile.

This increased extensibility was measured on an 'Instron' extension tester, which pulls materials between two clamps. It is interesting that sections treated with IAA alone underwent a far greater increase in extensibility than those treated with fungal extract. It seems clear that cell growth is regulated by a much more complex mechanism than the activity of hydrolytic enzymes alone. Ruesink suggests as a possible alternative the insertion of hemicellulose into the growing wall as Ray proposed (*J. Cell. Biol.*, **35**, 659; 1967). The role of IAA in the process remains obscure.

PROTEINS

Varieties of Histones

from our Molecular Biology Correspondent

SEVERAL laboratories are now grappling with the fractionation and sequencing of various of the numerous histones. Last year the first complete sequences were reported by Smith and his group, which showed the almost complete identity of histone IV, which is rich in arginine and glycine, from two widely different sources. An independent determination of the same fraction from calf thymus has now appeared (Ogawa *et al.*, *J. Biol. Chem.*, **244**, 4387; 1969) and agrees with the sequence of Smith and his colleagues, except for one residue, an arginine, which the latter workers placed at position 44 and which Ogawa *et al.* place at 40. In a chain of 102 residues this extent of agreement is gratifying, particularly because different sequencing strategies were used in the two laboratories.