

ticulate fraction and using this technique with high specific activity [^3H] N⁶-benzyladenine (BA) revealed at least two types of saturable cytokinin binding sites against a high background of unsaturable binding. The major saturable component had a low affinity for BA ($K_d = 7 \times 10^{-6}$ M) and was resistant to heating. The second site occurred at much lower frequency, could not be detected with radioactive hormone of low specific activity, was heat labile and had a high affinity ($K_d \sim 10^{-7}$ M) for BA. Furthermore, the ability of a series of halogenated analogues of BA to bind to the high affinity site correlated well with the cytokinin activity of these analogues in a number of bioassays including stimulation of cell growth and division in tissue cultures and induction of bud formation. In contrast, there was no correlation between biological activity and binding to the low affinity site. This site shows some of the features of talcum powder, a non-biological material which binds cytokinins in a saturable but non-specific manner. The high background of biologically irrelevant binding together with the lack, until recently, of high specific activity radioactive cytokinins have hampered the search for cytokinin receptor sites, but the high affinity site reported by Sussman and Kende seems to have the required properties.

In contrast, the search for auxin receptors in extracts of maize coleoptiles has borne considerable fruit. Physiological effects of auxins include stimulation of the extension growth of plant cells, induction of vascular differentiation and inhibition of lateral bud development. An important component of auxin action is their preferential movement from the apex towards the base of plant stems. This polar transport is thought to be involved in the regulation of expansion growth of cells below the tip, and in the inhibition of lateral bud development. An initial report of saturable binding of the auxin transport inhibitor 1-naphthylphthalamic acid (NPA) to a particulate fraction in maize coleoptile extracts² has been followed by demonstration of particulate binding sites for the auxins 3-indolylacetic acid (IAA) and 1-naphthylacetic acid (NAA)³. NPA did not compete for the auxin binding sites and *vice versa*. Further study of the NAA binding indicated that a major proportion, but not all of the binding sites were associated with the endoplasmic reticulum (ER)⁴ and that the affinity of a variety of auxin-like molecules for the site correlated reasonably well with auxin activity in a bioassay based on promotion of cell expansion growth⁵. A non-dialysable, heat-stable supernatant factor was found which narrowed the specificity spectrum without

The polyp has no I's

from Jonathan Slack

EVER since Abraham Trembley's work in the eighteenth century, the regenerative powers of *Hydra* have made it a popular organism among experimental biologists. Controversy has raged over the relationship of the various cell types to each other; in particular over the role of the interstitial cells (I cells) which are interspersed between the epitheliomuscular cells of the outer layer and the digestive and gland cells of the inner layer.

Recently some clear results which should dispel the controversy have been obtained by Beverly Marcum and Richard Campbell from the University of California at Irvine (see *J. Cell Sci.* **29**, 17; 1978; *Science* **197**, 771; 1977). Two years ago Campbell succeeded in making *Hydra* which were completely free of I cells by treatment with colchicine (*J. Cell Sci.* **21**, 1; 1976). These animals are rather feeble and have to be fed by hand, but it has proved possible to propagate stocks asexually and some lines have remained completely free of I cells. They also lack nerve cells and nematocytes but have their normal complement of epitheliomuscular and digestive cells, implying that these two tissues are mitotic and need not be continually restocked from the I cells.

Now Campbell and his colleagues have shown that the principal morphogenetic properties of the I cell-free animals are quite normal. When they are cut in two, both halves will regenerate with conservation of polarity. When a head is grafted into a gastric region it will induce a secondary axis from the host tissues. When a segment of the body is inverted between a head and a foot, it becomes repolarised by its surroundings at the same rate as in a normal animal.

The implications of these results are as follows. First, it is now clear

that the differentiated cell layers are capable of regeneration and that the positional information system which controls polarity can reside in these tissues. Second, differentiated nerve cells are not needed for regeneration, in spite of a widely held belief, based partly on data from a variety of other animals such as earthworms, starfish and amphibia, and partly on evidence such as the inhibition of regeneration by neuropharmacological inhibitors.

Third, they seem to cast doubt on one of the most popular candidates for a morphogen, in the form of a peptide isolated from neurosecretory granules and which increases the tentacle number of regenerating animals (see *J. Embryol. exp. Morph.* **29**, 27; 39; 1973; *Cell Diffn.* **5**, 13; 1975). This factor was thought perhaps to be a component in the mechanism proposed by Meinhardt and Gierer (*J. Cell Sci.* **15**, 321; 1974) which has been applied to a number of embryological problems and attracted some support, but it seems that the significance of the head activating substance will now have to be reassessed.

In the same issue of the *Journal of Cell Science* as Campbell's paper, T. Sugiyana and T. Fugisawa report the isolation of an I-cell free mutant which has properties similar to those of the stocks prepared by colchicine treatment. (*J. Cell Sci.* **29**, 35; 1978). They show that I cells will migrate into such a creature from a normal graft, so that it is possible to create chimaeras in which the main tissue layers come from one strain and the I cells and their derivatives from another. This should make a number of questions on cell interactions accessible to experiment.

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altering the number of binding sites⁶.

Meanwhile a second group has provided evidence for two binding sites with different affinities for NAA that could be partially resolved by sucrose density gradient centrifugation⁷. In a major advance these sites were solubilised by acetone treatment and purified 100-fold⁸. Binding of NAA to solubilised sites gave K_d values of 1.7×10^{-7} M and 1.4×10^{-6} M: in the concentration range for half-saturation of auxin transport and physiological responses. The sites are probably proteins and gel exclusion chromatography gave

an asymmetric profile consistent with two incompletely resolved species of molecular weights 47,300 and 40,300. These values may be underestimates since in the April issue of *Plant Physiology*, Cross *et al.*⁹ report the solubilisation of auxin binding sites by Triton X-100 treatment of particles prepared in the presence of phenylmethylsulphonyl fluoride (PMSF) to give detergent-protein micelles, molecular weight about 90,000. In the absence of the protease inhibitor PMSF, secondary peaks of auxin binding activity with much lower molecular weight were