

and after day 3, it is likely that the concentration of the neurosecretory material in the *in vitro* experiments was great enough only on day 3 to give a marked result. It is clear that maximum synthesis and maximum release of leucine aminopeptidase occur at the same time.

We are therefore persuaded that ecdysis in the larval-adult moult of *Phocanema* directly parallels exsheathment in the infective larvae of trichostrongyles, and that the process in *Phocanema* is under neurosecretory control. Our standard culture medium, and presumably the intestine of seals, contain the necessary elements in the stimulus leading to the release of neurosecretion, the consequent synthesis of leucine aminopeptidase, and the eventual emergence of the adult worm from the fourth stage cuticle. Withholding this stimulus by culturing the worms in saline prevents the activity of the neurosecretory cells, the synthesis of leucine aminopeptidase by the excretory gland is abolished, and the worms fail to ecdyse.

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### Heterotrophic Nitrogen Fixation by the Blue-Green Alga *Anabaenopsis circularis*

It has been shown that the blue-green alga *Tolypothrix tenuis* can be grown heterotrophically in complete darkness if, as well as mineral nutrients, appropriate organic substances such as casamino-acid are supplied as sources of nitrogen and carbon<sup>1</sup>. The maximum values for the growth rate and final growth yield obtained in heterotrophic conditions, however, were found to be far less than those attained in the usual autotrophic cultures.

Fay<sup>2</sup> has found that a blue-green alga, *Chlorogloea fritschii*, can fix molecular nitrogen in the dark in a nitrogen-free medium at the expense of organic nutrients. Best growth occurred on sucrose, and in the dark nitrogen fixation was greatest when this substrate was supplied. Some growth occurred on maltose, whereas no growth took place with, for example, glucose, fructose, mannitol, acetate, pyruvate or  $\alpha$ -ketoglutarate.

We have found that another blue-green alga, *Anabaenopsis circularis*<sup>3,4</sup>, has the same ability to grow in the dark. Greatest growth and greatest nitrogen fixation in the dark occurred on glucose in the absence of combined nitrogen. Next to glucose, fructose, sucrose and maltose were most effective, whereas no growth or nitrogen fixation occurred on the following compounds: xylose, ribose, arabinose, rhamnose, mannose, galactose, sorbose, glucose-1-phosphate, trehalose, cellobiose, lactose, melibiose, raffinose, stachyose, inulin, dextrin, glycogen, ethanol, glycerol,

erythritol, mannitol, formate, acetate, propionate, butyrate, lactate, pyruvate, caproate, laurate, myristate, oxalate, malonate, succinate, malate, tartarate, citrate, fumarate, gluconate, oxalacetate, acetoacetate, aconitate, or glutarate.

It is noteworthy that the two species of algae mentioned here can utilize, so far as is known, no organic substance other than sugar in nitrogen-free medium in the dark, and that they utilize different kinds of sugars. For the growth of *Anabaenopsis* in the dark, the optimum concentration was 0.5 per cent for glucose and 0.3 per cent for fructose and sucrose (Table 1).

Table 1. EFFECTS OF SUGARS ON THE GROWTH OF *Anabaenopsis circularis* IN THE DARK IN A NITROGEN-FREE MEDIUM\*

| Concentrations (per cent) | Dry weight of algal cells (mg)† |          |         |
|---------------------------|---------------------------------|----------|---------|
|                           | Glucose                         | Fructose | Sucrose |
| 0.1                       | 42                              | 23       | 19      |
| 0.3                       | 53                              | 74       | 45      |
| 0.5                       | 90                              | 63       | 35      |
| 0.7                       | 46                              | 52       | 13      |

\* Nitrogen-free media contained, in 1,000 ml., 0.25 g of  $K_2HPO_4$ , 0.25 g of  $MgSO_4 \cdot 7H_2O$ , 0.05 g of calcium chloride, 0.05 g of sodium chloride, 2 mg of  $FeSO_4$ , 1 ml. of Arnon's  $A_1$ -solution and the amounts indicated of sugars.

† The weight of cells obtained from 100 ml. culture which were grown (without shaking) in the dark for 2 months at 32° C.

Table 2. EFFECT OF LIGHT ON THE GROWTH OF *Anabaenopsis circularis* IN NITROGEN-FREE MEDIUM\*

| Light    |          | Dark     |          |
|----------|----------|----------|----------|
| No sugar | Fructose | No sugar | Fructose |
| 0.13     | 0.24     | 0        | 0.10     |

\* Figures refer to relative growth constant ( $K_g$ ) estimated from the increase of packed cell volumes and expressed in  $\log_{10}$  day<sup>-1</sup> units.

The effect of light on the growth of this alga was examined using 0.3 per cent fructose as the source of carbon (Table 2). The heterotrophic growth of the alga is accelerated by light. Microscopic observations showed that a fairly large number of akinetes were formed in the heterotrophic culture in the dark.

The fact that these nitrogen-fixing algae can be grown heterotrophically in the dark may be of practical interest, because it suggests the feasibility of growing them on a large scale in tanks to produce large quantities of algal materials to be used for agricultural purposes<sup>5</sup>.

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### Varietal Differences of Groundnut in the Production of Aflatoxin

Toxic substances, known as aflatoxins, shown to cause acute liver damage in different species of animals, have been demonstrated in mouldy groundnuts and groundnut meals<sup>1,2</sup>. These toxic metabolites are elaborated by certain strains of *Aspergillus flavus* which infest the groundnut crop in unfavourable conditions of collection, storage and transport. There have been several reports<sup>3-5</sup> of the conditions of growth of the fungus and production of aflatoxin in relation to agricultural practices involved in growing the groundnut crop. Surveys conducted in the coastal districts of Andhra Pradesh, India<sup>3</sup>, and in the Riverain Provinces of Northern Nigeria<sup>4,6</sup> have indicated that, with the agricultural practices prevalent in the