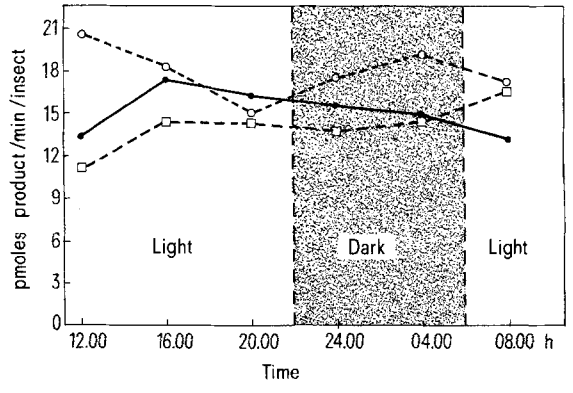
fluctuation in NAT activity is seen over a 24-h period using a similar assay[1,4].

While these results do not support the hypothesis that NAT is involved in the circadian clock of *O. nubilalis*, they do not eliminate the possibility that NAT may act as a pacemaker only in specialized neural cells. Such specialized activity might be masked in whole brain homogenates by larger amounts of non-fluctuating NAT involved in amine regulation and metabolism. Other pathways of amine metabolism may also be involved in circadian regulation. Insects apparently lack monoamine oxidase activity[13,15,16] but a recent report has tentatively identified 2 metabolites of tyramine from the brain of *Manduca sexta* pharate adults, a conjugate of the amine with β-alanine and an O-glycoside of a tyramine derivative, probably the N-acetate[17]. The relative importance and specific roles of N-acetylation and these conjugation reactions in the regulation of endogenous amine levels remains to be determined.

Monitoring the levels of endogenous biogenic amines and their metabolites in the insect brain using new, more sensitive techniques may lead to the discovery of a light-cued biochemical pacemaker analogous to the NAT activity of the vertebrate pineal. An enzymatic clock cued by light would help explain photoperiodic induction of diapause and many other light-controlled processes in insects. Our results suggest that the enzymatic clock in insects may involve a different system than that described for vertebrates.



N-acetyltransferase activity in *O. nubilalis* brain homogenates throughout 24 h using tryptamine (●), dopamine (○), and octopamine (□) as substrates. Activity indicated is radiolabeled acetylated amine. Each point is the mean of 2 assays each of 5 homogenized *O. nubilalis* brains. Details of assay methods were previously described[13].

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Rapid preparation of pure chlorophyll a[1]

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Summary. Very pure chlorophyll a has been obtained from blue-green algae by a simple precipitation procedure. Its purity is at least equal to that of chlorophyll a obtained from spinach by conventional chromatography.

Pure chlorophyll a is usually obtained from extracts of higher plants by multiple chromatography[2]. This method is time-consuming and requires large amounts of mild adsorbents (sugar, cellulose). Recently, one of us described a simple precipitation procedure to obtain pure chlorophyll a from the blue-green algae *Anacystis nidulans*[3], which does not contain chlorophyll b[4]. Now we present an improved version of this procedure and the results of an analysis of the chlorophyll a by high-pressure liquid chromatography (HPLC). HPLC was recently applied to obtaining very pure chlorophyll a for electrochemical studies[5].

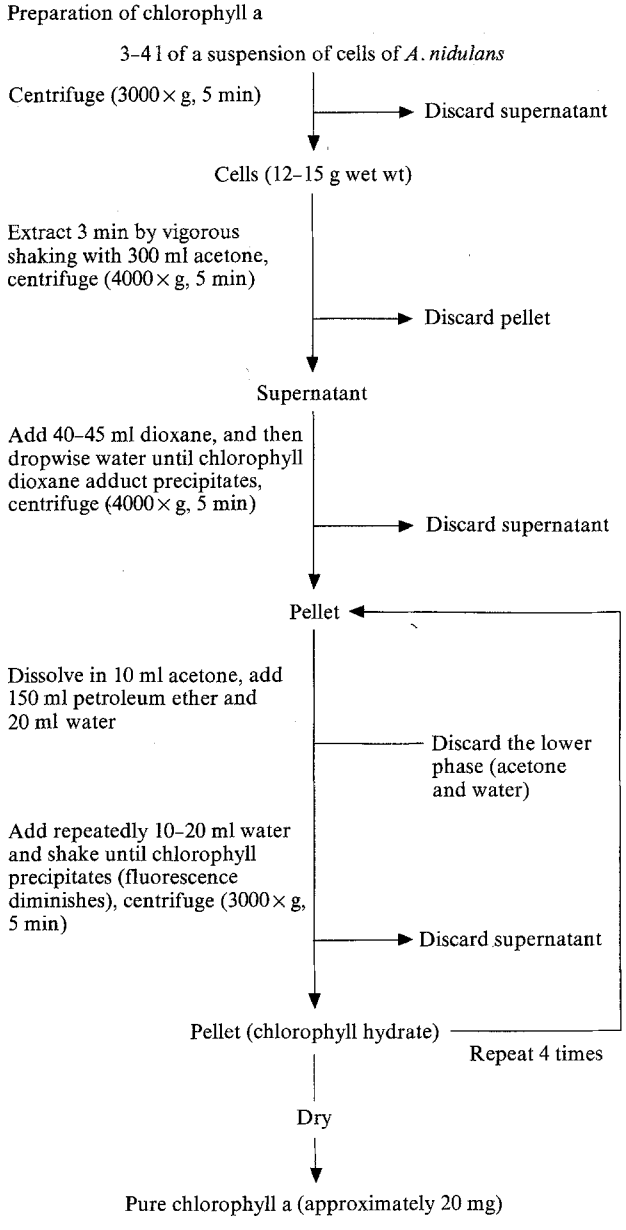
Materials and methods. *A. nidulans* (Culture Collection of Algae, Göttingen, FRG) was cultured as reported[2]. Methyl chlorophyllide a and pheophytin a were prepared according to standard methods of preparation[6,7]. Chlorophyll a' was obtained by conversion of chlorophyll a in heated pyridine[8]. Chlorophyll a from spinach was purchased from Sigma Chem., USA. All organic solvents used were reagents of Merck, FRG.

HPLC was carried out with a μBondapak C_{18} column (300×3.9 mm) from Waters Associates, Inc., USA. The pigments were eluted with acetonitrile:tetrahydrofurane (95:5) at a flow rate of 1 ml · min^{-1} and detected by their OD at 380 nm.

Results. Our method is based on the facts that chlorophyll a

HPLC retention times of chlorophyll and some derivatives

Pigments	Retention times/min
Methyl chlorophyllide a	5.3
Chlorophyll a	20.0
Chlorophyll a'	21.8
Pheophytin a	27.2



is the only chlorophyll in blue-green algae, and that it can be precipitated as chlorophyll dioxane adduct[9] and as chlorophyll hydrate[3]. The chlorophyll hydrate, also termed polycrystalline chlorophyll a, is characterized by its broad absorption peak around 740 nm[3]. A flow sheet of the preparation of chlorophyll a is given in the figure. All work was done under a dim light and with cooled solvents (0–5 °C). The final precipitation of chlorophyll hydrate was carried out from 2-methylbutane, which has a low boiling-point (28 °C) and is therefore easy to remove during drying. The purity of chlorophyll a was checked by elemental analysis, determination of the absorption coefficient, thin layer chromatography and spectrography in the UV, visible and IR regions. All data were in excellent accordance with those known for the purest chlorophyll a, isolated from spinach[2,3,10].

Analysis by HPLC of a routine sample shows 1 peak of chlorophyll a and small amounts (< 1%) of impurities (xanthophyll?, pheophytin a). The fact that chlorophyll a from spinach and *A. nidulans* have the same retention time in HPLC gives - in addition to the other analytical data - further evidence for the identity of the 2 chlorophylls. Mixtures of chlorophyll a with derivatives (methyl chlorophyllide a, pheophytin a, chlorophyll a') are resolved excellently by HPLC. Especially the completeness of the separation of chlorophyll a' (a stereoisomeric chlorophyll a) from the parent chlorophyll a demonstrates the superiority of HPLC to conventional sugar chromatography. The table shows the retention times of the pigments.

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Comparison of chemiluminescence and absorptiometry in enzyme immunoassays for protein quantification[1]

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Summary. The use of chemiluminescence in competitive binding assays for human serum albumin, human alpha-fetoprotein and human immunoglobulin G and in double antibody sandwich enzyme immunoassays for cytomegalovirus and herpes simplex virus increased the sensitivity of the detection of antigen or antibody 16- to 95-fold above that obtained by conventional absorptiometric methods.

Absorptiometry and luminometry have been applied clinically in enzyme-linked immunosorbent assay (ELISA) for the quantification of a variety of medically important substances[3–5]. ELISA which uses an enzyme as the immunoglobulin marker instead of a γ-emitting isotope, was developed as a non-isotopic alternative to radioimmunoassay (RIA)[6]. The assay uses an enzyme-antibody conjugate which becomes bound to a solid-phase support through a series of antigen-antibody reactions and is subsequently detected by the addition of an appropriate substrate. A