A major plasma glycoprotein, α_2M, binds trypsin-like enzymes and prevents them from digesting large proteins. α_2M has been found to be in higher concentrations in human diabetics[14]. Brownlee[15] hypothesizes that in humans this increased α_2M inhibits the activity of leucocyte neutral proteases which aid in the digestion of basement membrane. This could result in the increased basement membrane thickness and microangiopathy of diabetics. Our data did not show a positive correlation of α_2M levels with diabetes (glucosuria or FBG) but α_2M levels were positively correlated with hexosamine and lipid levels (table 2).

We have, however, found that diabetes causes an increase in the charge and molecular weight of α_2M in hamsters[5], both of which could affect the binding and action of neutral protease and lead to the microvascular changes seen in diabetics[16].

Our data support the supposition that diabetes is a disorder of many etiologies. The great variety and diversity in glycoprotein observed in the highly inbred hamster lines emphasizes the complexity of establishing meaningful parameters in a heterogenous human population. We hope that by studying changes in the glycoproteins and lipoproteins in one of the diabetic lines we may be able to correlate the changes in plasma glycoproteins with a specific complication of diabetes.

1 Acknowledgments. For the support which made this project possible, we wish to thank the Michigan Heart Association and The Upjohn Co.
2 D.K. Yue, K. Morris, S. McLennan and J.R. Turtle, Diabetes *29*, 296 (1980).
3 H.F. Bunn, K.H. Gabbrey and P.M. Gollop, Science *200*, 21 (1978).
4 G.C. Gerritsen and W.E. Dulin, Diabetalogia *3*, 74 (1967).
5 E.J. Copeland and L.C. Ginsberg, J. Hered., in press (1982).
6 G.C. Gerritsen and W.E. Dulin, Diabetes *15*, 331 (1966).
7 F.L. Schmidt, L.G. Leslie, J.R. Schultz and G.C. Gerritsen, Diabetalogia *6*, 1456 (1970).
8 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.L. Randall, J. biol. Chem. *193*, 265 (1951).
9 A.L. Kennedy, T.W. Kendall and T.J. Marimee, Diabetes *28*, 1006 (1979).
10 R. Gatt and E.R. Berman, Analyt. Biochem. *15*, 167 (1966).
11 R. Saunders, B. Ryce, W.E. Vannier and B.J. Haverback, J. clin. Invest. *50*, 2376 (1971).
12 N.H. Nie, C.H. Hull, J.G. Jenkins, K. Steinbrenner and D.H. Bent, Statistical Package for the Social Sciences, 2nd edn. McGraw-Hill, New York 1975.
13 A.Y. Chang, Gen. Pharmac. *9*, 447 (1978).
14 H.G. Schwich, K. Heide and Haupt, in: The Glycoconjugates. vol. 1, sec. 1, p. 261. Ed. M.I. Horowitz and W. Pigman. Academic Press, New York 1977.
15 M. Brownlee, Lancet *2*, 779 (1976).
16 W. Hollander, M.A. Colombo, B. Kirkpatric and J. Paddock, Atherosclerosis *34*, 391 (1979).

Biogenic amine acetylation: No detectable circadian rhythm in whole brain homogenates of the insect *Ostrinia nubilalis*

P.H. Evans and D.M. Soderlund

Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva (New York 14456, USA), 15 July 1981

Summary. With the biogenic amines tryptamine, dopamine, and octopamine as substrates, N-acetyltransferase activity shows no detectable circadian rhythm in homogenates of whole brains of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). The circadian clock of this insect may be fundamentally different from the N-acetyltransferase pacemaker in the pineal gland of vertebrates.

N-acetyltransferase (NAT) functions as a biological clock in the pineal gland of vertebrates[1]. NAT activity oscillates in a circadian rhythm, peaking at night in both diurnal and nocturnal mammals and in diurnal birds, and its activity controls the biosynthesis of pineal melatonin from the biogenic amine serotonin. NAT levels are also correlated with circadian activity of locomotion in many vertebrates[2]. NAT shows an endogenous circadian rhythm in vitro and responds to environmental light in cultured chicken pineal glands[3,4] and even in dispersed cell culture of the chick pineal[5]. In insects, circadian rhythms of locomotor activity, cuticle deposition, and various physiological processes are well established[6,7]. Evidence from transplantation and ablation experiments on the moths *Antheraea pernyi*[8,9], *Hyalophora cecropia*[9], and the cockroach *Leucophaea maderae*[10] suggest that the brain is the location of the photoperiodic clock. There is some evidence of circadian fluctuation of serotonin levels in *Drosophila melanogaster*[11], and Houk and Beck[12] have suggested that dopamine or a metabolite 'may be involved in an endogenous time measuring system and/or diapause induction-termination' in *Ostrinia nubilalis*. The biogenic amine dopamine is present in *O. nubilalis* brain[12] as is an NAT capable of acetylating dopamine and other amines[13]. This neural NAT is distinct from cuticular NAT participating in sclerotization on the basis of substrate specificities for a series of 8 amines[13]. Since a distinct neural NAT and amine substrate are present in an anatomical location which may contain the circadian center, we wished to determine if there was a rhythm to *O. nubilalis* brain NAT activity similar to that found in the pineal gland of vertebrates.

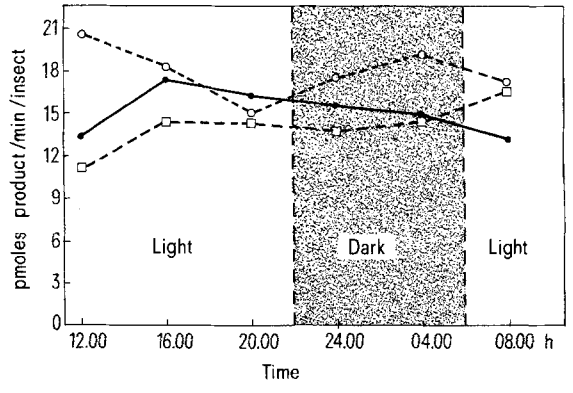
2 colonies of *O. nubilalis* were maintained on artificial diet in a long day, non-diapausing (LD 16:8) photoperiodic regime. One colony was 14 h out of synchronization to the other in its LD cycle to allow data for all time points to be obtained within a 12-h time span. Brains were dissected from cold-anesthetized live insects, homogenized, and assayed as previously described[13] within 15 min of the indicated clock time. All NAT assays were by a modification[13] of the radiochemical method of Deguchi and Axelrod[14] using ^{14}C-acetyl CoA and the unlabeled amines tryptamine, dopamine, and octopamine. Incubation times and tissue concentrations were chosen to ensure linear rates of product formation.

We were unable to demonstrate any circadian rhythm in NAT activity using either the phenylethylamines dopamine and octopamine or the indolealkylamine tryptamine as substrates (fig.). In the chick pineal, a 5–7-fold rhythmic

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].

1 S.A. Binkley, J.B. Riebman and K.B. Reilly, Science *202*, 1198 (1978).
2 J.L. Stephens and S. Binkley, Experientia *34*, 1523 (1978).
3 T. Deguchi, Science *203*, 1245 (1979).
4 C.A. Kasal, M. Menaker and J.R. Perez-Polo, Science *203*, 656 (1979).
5 T. Deguchi, Nature *282*, 94 (1979).
6 S.D. Beck, Insect Photoperiodism. Academic Press, New York and London 1968.
7 D.S. Saunders, Insect Clocks. Pergamon Press, Oxford 1976.
8 C.M. Williams, Symp. Soc. exp. Biol. *23*, 285 (1969).
9 J.W. Truman and L.M. Riddiford, Science *167*, 1624 (1970).
10 J. Nishiitsutsuji-Uwo and C.S. Pittendrigh, Z. vergl. Physiol. *58*, 14 (1968).
11 D.J. Fowler, C.J. Goodnight and M.M. LaBrie, Ann. ent. Soc. Am. *65*, 138 (1972).
12 E.J. Houk and S.D. Beck, J. Insect Physiol. *23*, 1209 (1977).
13 P.H. Evans, D.M. Soderlund and J.R. Aldrich, Insect Biochem. *10*, 375 (1980).
14 T. Deguchi and J. Axelrod, Analyt. Biochem. *50*, 174 (1972).
15 S.A. Dewhurst, S.G. Croker, K. Ikeda and R.E. McCaman, Comp. Biochem. Physiol. *43B*, 975 (1972).
16 P.H. Evans and P.M. Fox, J. Insect Physiol. *21*, 343 (1975).
17 G.D. Maxwell, M.M. Moore and J.G. Hildebrand, Insect Biochem. *10*, 657 (1980).

Rapid preparation of pure chlorophyll a[1]

G. Gleixner, V. Karg and P. Kis

Institute of Physical Chemistry, University of Vienna, Währingerstr. 42, A-1090 Vienna (Austria), 12 June 1981

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