Journal of the Mississippi Academy of Sciences

Volume 44 July 1999 Number 3

Journal of the Mississippi
Academy of Sciences

Editor
Kenneth J. Curry
University of Southern Mississippi

Associate Editor
Maureen Corcoran
Corps of Engineers
Waterways Experiment Station

Abstracts Editor
John Boyle
Mississippi State University

The Journal of the Mississippi Academy of Sciences (ISSN 0076-9436) is published in January (annual meeting abstracts), April, July, and October, by the Mississippi Academy of Sciences. Members of the Academy receive the journal as part of their regular (non-student) membership. Information regarding subscriptions, availability of back issues, and address changes is available from the Mississippi Academy of Sciences, 405 Briarwood Drive, Suite 107E, Jackson, MS 39206-3032; telephone: 601-977-0627.

The *Mississippi Academy of Sciences* maintains a website for Academy announcements, the latest issue of this journal, and general information about the Academy. The address is http://www.msstate.edu/Org/MAS/MAS. HTML

Editorial policy is located on the inside front cover. *Information for contributors* is located on the inside back cover. Manuscripts and inquiries about publication and information about advertising should be sent to the editor: Kenneth J. Curry, University of Southern Mississippi, Post Office Box 5018, Hattiesburg, MS 39406-5018.

Contents

General Articles

Research Articles

Departments

OFFICERS OF THE
MISSISSIPPI ACADEMY OF SCIENCES

President . . . . . Robin Rockhold
President-Elect . . . . . Susan Nodruft
Immediate Past-President . . . . . Catherine Perry Cotten
Executive Officer . . . . . John A. Boyle
Journal Editor . . . . . Kenneth J. Curry
Junior Academy Director . . . . . Joan Messer
Directors . . . . . Maureen Corcoran
Portia Harris
Charles Swann
Administrative Assistant . . . . . Cynthia Huff

Editorial

Election Results

Congratulations to **William B. Lushbaugh** who becomes *President-elect* and to **Johnnie-Marie Whitfield** who joins the *Board of Directors.*

Another Call for Volunteers

I put out a call for help in the April issue of this journal from which I received no response. I know that some of you would like to get involved. If I could ask you face to face, you would gladly agree to help. Unfortunately I do not know who you are to ask you to join us. Read the call below. If you have any interest, but hesitate because you are not sure what each of the described postions entails, give us a call—no obligation. The Mississippi Academy of Sciences is a growing organization, and we need your help to keep growing.

Poster Sessions. When I first affiliated with the Mississippi Academy of Sciences, the annual meeting was about a day and half long with talks starting on Thursday and ending Friday around noon. Now talks continue through Friday late afternoon. We are still growing and accommodating that growth by encouraging more poster sessions. This is important, since we cannot find a suitable meeting place with more breakout rooms than the hotel/convention centers we have been using and we are not quite big enough for a three day meeting. The importance of poster sessions to the Academy was felt strongly at our meeting in February when we had the largest demand for posters ever (over 70 on Friday). We need somebody to help with this important aspect of the Academy annual meeting. The person would be responsible for getting the backboards and easels to the meeting, setting them up both on Wednesday night and Thursday night (for the Thursday and Friday sessions respectively), and taking them down for return to their storage facility in Jackson. The person would also be responsible for maintenance of the easels, i.e., we need somebody that can repair the wooden easels. Interested in getting involved? Contact John Boyle, Rob Rockhold, or me.

Associate Editor: Photographer, etc. Each year this April issue of the journal contains some photographs of the annual meeting. The job of photography has been passed around among various volunteers with various levels of success. I am looking for an Associate Editor to be responsible for taking photographs at the annual meeting. I would also like some help in identifying and securing advertising for the journal to help offset printing costs. Interested in getting involved? Contact me.—Ken Curry

[insert Ohaus advertisement here]

The Face of HIV/AIDS in Mississippi: 1999

1999 Dodgen Lecture

Harold M. Henderson
University of Mississippi Medical Center, Jackson, MS 39216

The human immunodeficiency virus (HIV) is the causative agent of AIDS, the acquired immunodeficiency syndrome. About 700,000 cases of AIDS have been reported in the United States since the start of the epidemic in the early 1980's. According to data gathered by the Centers for Disease Control (CDC) most of these cases have occurred in large urban centers, especially in the early years of the epidemic (Figure 1). These cases primarily involved gay white males and intravenous drug users.

As the epidemiology of HIV infection has evolved not only have the total numbers of AIDS cases increased dramatically but cases in rural areas, especially in the southeastern U.S., have been occurring with increasing frequency (Figures 2 and 3). These trends are reflected in Mississippi HIV statistics, which show a large increase in infected persons since the early 1990's, particularly in African-Americans. Combined with the poverty and the lack of access to adequate health care prevalent in many parts of the state, the increasing numbers of HIV infected individuals in Mississippi represent a public health emergency, and are taxing the ability of the state's health care system to respond in an effective way.

HIV is a retrovirus: its genetic material is RNA, and it possesses an enzyme-reverse transcriptase-which encodes DNA following viral infection of a host cell. This viral DNA may integrate into the host cell's DNA and remain in a "latent" or quiescent form, or it may replicate itself and ultimately form new viral organisms which can then infect other host cells, where the cycle is repeated again and again. The primary cell types affected are cells of the immune system, which bear a molecule known as CD4 on their surface. CD4-bearing lymphocytes are highly critical components of a mature, healthy immune system. HIV infection of these cells results in their dysfunction and then their destruction. This process ultimately results in a devastated immune system, and thus a dangerous susceptibility to the unusual infec-

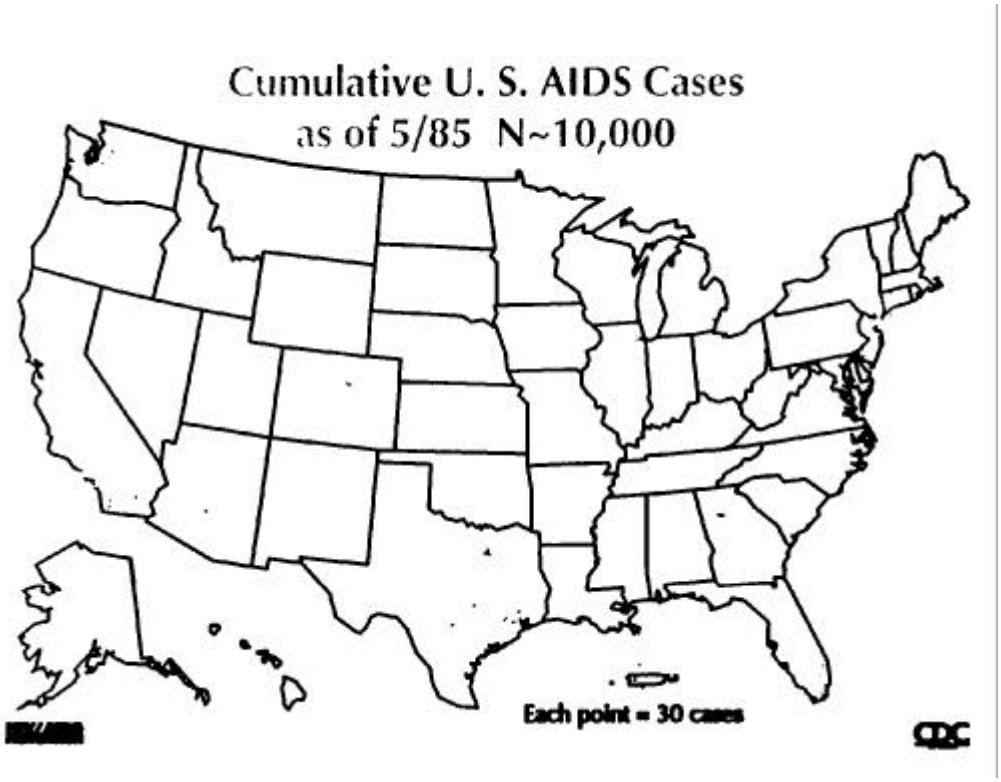

Figure 1

tions, such as Pneumocystis pneumonia, which are the hallmarks of AIDS.

The natural history of untreated HIV infection is a long, slow one. Several years generally elapse from the time of initial, established infection until the development of full-blown AIDS. On average, HIV-infected persons will progress to AIDS in about 8-10 years. During most of these years, while the infection slowly progresses, the large majority of infected persons are completely asymptomatic. There are no outward signs or symptoms of infection with a virus that is destroying their immune system. Despite the absence of symptoms and the outward appearance of normalcy, the virus can be transmitted to others throughout this time via sex or exposure to blood or other infected body fluids. A positive blood test for HIV antibodies is the usual way that a diagnosis of HIV infection is made.

According to statistics from the State Department of Health, as of late 1997 more than 2,500 cases of AIDS and more than 4,000 cases of HIV infection had been reported in Mississippi. Many others in the state can be assumed to be infected but not yet diagnosed, so these numbers are an underestimation. The annual rate of HIV infection per population rose steadily in Mississippi throughout the late 1980's and the 1990's. This rising rate has been due primarily to the high incidence of infection among African-American men and women. During the 1980's gay men represented the primary risk group for HIV infection in Mississippi, but the rates of heterosexual transmission have progressively risen during the present decade, and heterosexual intercourse is currently the most common mode of HIV transmission in the state. The majority of infected persons are young, between the ages of 20 and 35. Early age of sexual involvement, the practice of trading sex for drugs (often crack cocaine), and the common occurrence of other sexually transmitted diseases such as syphilis and Herpes simplex all contribute to the rising incidence of HIV infection in Mississippi.

Despite this rising incidence of HIV infection the number of AIDS cases reported to the Mississippi State Health Department in 1997 were less than in 1996. This is a reflection of the success of current anti-HIV drug therapy. Today, persons infected with HIV are typically treated with a combination of at least three drugs. These drugs inhibit the function of one of two critical viral enzymes-the reverse transcriptase and a protease-and thus prevent HIV from reproducing itself and infecting other cells. In order to prevent HIV from developing drug resistance, combinations must be potent enough to completely suppress measurable viral burden in the blood to "below the level of detection." With the right drug combination this is achievable in about 70-80% of HIV-infected persons who have not previously been treated with anti-HIV agents and who are fully adherent with their medications. The use of such combinations in Mississippi has resulted in the de-

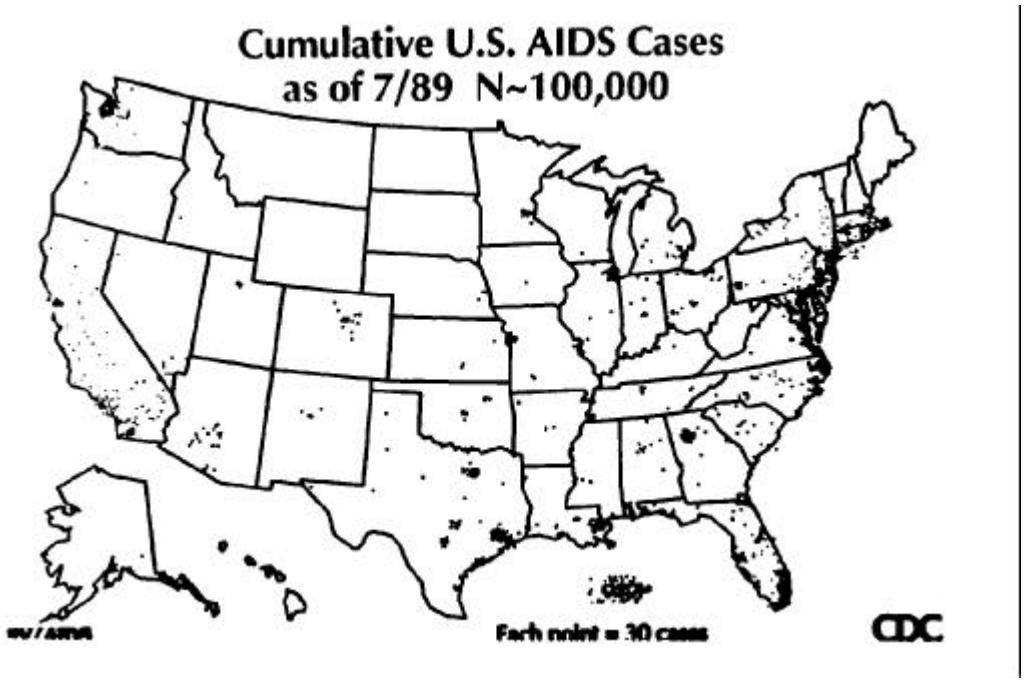

Figure 2

creased numbers of reported AIDS cases noted above, decreased hospitalization rates for AIDS-related complications, and a decrease in the death rate due to AIDS. In addition, the widespread use of the anti-HIV agent zidovudine during pregnancy has resulted in a drastic decrease in the numbers of HIV-infected infants born to mothers infected with HIV.

Unfortunately the success of anti-HIV drug therapy has not come without significant problems. A typical combination of three anti-HIV drugs costs about $1,000 per month. The expense of the drugs poses a severe problem particularly in Mississippi, which is the poorest state in the United States and has high numbers of individuals without medical insurance. Despite the availability of drug assistance programs designed to furnish HIV-infected persons with their medications if they cannot obtain the drugs by themselves, the cost of the drugs does prevent some people from receiving them on a consistent basis. In addition there is a shortage of experienced HIV caregivers in Mississippi. Many of the drugs have side effects and are not always tolerated well. Drug combinations are often complex and inconvenient, and many persons are unable to take these demanding regimens on a consistent basis, day after day after day. These problems-drug cost and complexity, lack of access to care, drug side effects-all contribute to intermittent and suboptimal drug adherence, which can lead to the emergence of resistant viral strains, followed by drug failure.

The problems associated with anti-HIV drug therapy are indeed daunting, and have prevented many HIV-infected Mississippians from achieving the promise offered by drug therapy. Furthermore, the incidence of new HIV infections in Mississippi remains high. Much work thus remains to be done in both the areas of prevention and treatment. However, the success of modern anti-HIV drug therapy has been nothing short of remarkable, and with hope for even better treatments to come, offers unprecedented hope for infected individuals to live healthy, productive lives. We must first continue to work and educate our young people to avoid those behaviors which place them at risk for HIV infection, and then to provide access to effective therapy for those already infected. I am optimistic that with continued hard work our state will see further decreases in the numbers of AIDS cases reported annually, and begin to see a downturn in the numbers of new HIV infections, as well.

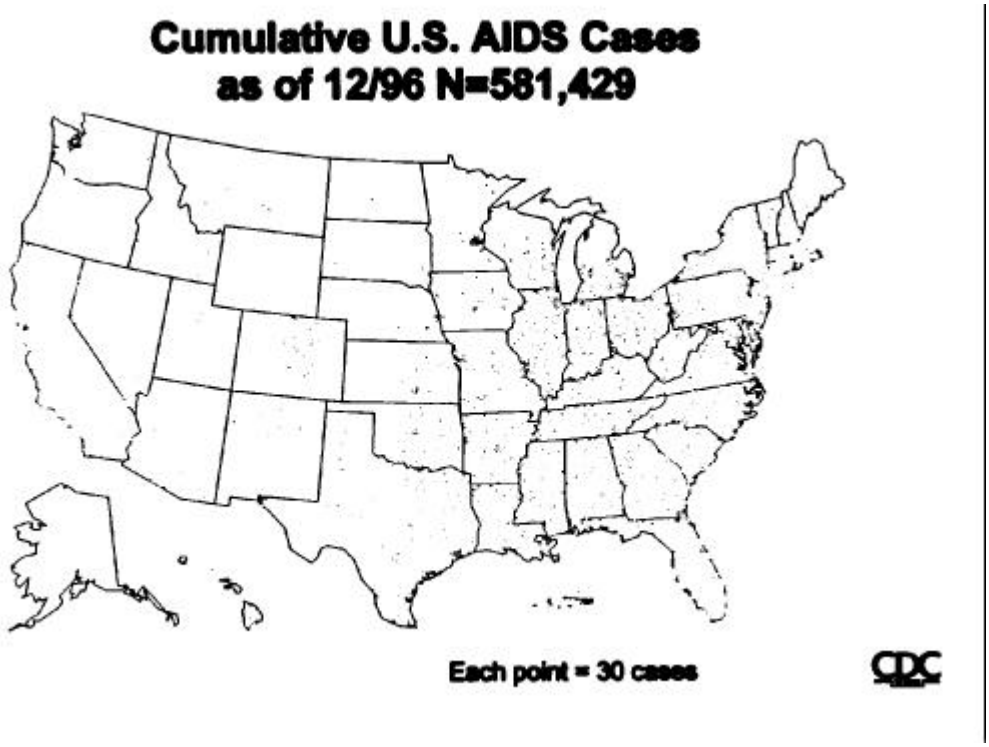

Figure 3

Imidazoline Receptors: A Platelet Marker for Depression?

John E. Piletz[1,2], Angelos Halaris[3], Sudhakar Madakasira[4], Nancy Goldman[4], He Zhu[4], Warren May[5], and Amelia Nasrallah[4]

[2]Department of Physiology, [3]Department of Pharmacology, [4]Department of Psychiatry and Human Behavior, and [5]Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, MS 39216-4505

Ongoing research at the University of Mississippi Medical Center suggests that higher numbers of imidazoline receptors on platelets might characterize patients with affective disorders. A group of 11 unipolar depressives was studied before and after eight weeks of treatment with bupropion (Wellbutrin). We measured platelet receptor binding parameters and the density of a specific protein band on Western blots related to the I_1-imidazoline (I_1) receptor. We were particularly interested in detecting associations between clusters of depressive symptoms and these platelet parameters. We succeeded in determining associations between the retardation and endogenomorphic clusters extracted from the Hamilton Rating Scale for Depression and these platelet parameters. If confirmed, these findings could establish the platelet I_1 receptor as a useful blood marker for depression.

Depression is a very treatable disease. But, depression can present with many faces and therefore it is not always easily diagnosed. Furthermore, uncertainties abound regarding the type of therapy, the course of treatment, and the risk of relapse. Some patients will respond to one antidepressant medication and not another, while milder cases may be better treated with counseling. Pharmacological therapy can take up to 8 weeks to establish efficacy, and during this lengthy period the physician often has to titrate the dose to avoid unwanted side effects. Thereafter, patients are typically maintained on the antidepressant they responded to in order to avoid relapsing. Such clinical decisions rely on the experience of well-trained physicians, but sometimes even the best informed decisions can be flawed. A simple and reliable blood test for depression would therefore be useful as an adjunct for any health care professional treating depression. To date, no reliable and specific test is available.

Platelets are a readily obtainable blood element sharing a number of properties in common with brain serotonergic neurons. For instance, platelets possess the apparatus for serotonin release and reuptake similar to serotonergic neurons. These properties are of particular interest because most antidepressants affect serotonergic neurons directly or indirectly. In as much as platelets are a model of serotonergic neurons, these blood elements have led to a number of investigations in depressed patients (Piletz et al., 1995). Human platelets also possess I_1 and I_2 subtypes of imidazoline binding sites (Piletz and Slettern 1993; Ernsberger et al., 1995), as well as immuno-related 33 kD and 45 kD proteins (Garcia-Sevilla et al., 1996; Ivanov et al., 1998). These binding sites are believed to reside on imidazoline receptors (IR), which, in some tissues, modulate neurotransmitter release (Molderings et al., 1991).

The density of I_1 binding sites on platelets of depressed patients has consistently been shown to be elevated compared with healthy control subjects (Piletz et al., 1995, 1996). This up-regulation is normalized (i.e., down-regulated) following antidepressant treatments with desipramine (Norpramin) or fluoxetine (Prozac) (Piletz et al., 1995, 1996). An elevation in I_1 binding sites has also been found in correlation with the degree of severity of dysphoria in women with premenstrual syndrome (Halbreich et al., 1993), but not in patients with generalized anxiety disorder (Piletz et al., 1996). Treatments with other antidepressants (citalopram, clomipramine, or imipramine) also down-regulated the immuno-related 45 kD protein on platelet membranes of depressed patients (Garcia-Sevilla et al., 1996).

An I_2 binding site has been identified to reside within an amino acid domain of monoamine oxidases

[1]Author for correspondence. jpiletz@psychiatry.umsmed.edu, Tel. (601) 984-5898

(Raddatz et al., 1997). Because monoamine oxidase inhibitors are established antidepressants, the presence of this I_2 site on monoamine oxidases is suggestive of a possible target for future antidepressant drug development. Increased I_2 binding sites have been suggested in the frontal cortex of depressed suicide victims (Garcia-Sevilla et al., 1996). Thus, considerable evidence now links an elevation in one or another subtype of imidazoline binding sites with the state of depression. There is no information whether the reported changes in these sites are specific for a particular subtype of affective disorders, or for that matter, for one or more symptoms of the affective symptomatology spectrum.

The present study was designed to determine whether the down-regulatory effects on I_1 imidazoline binding sites and immuno-related proteins, as previously observed with other antidepressants in unipolar depressives, could be related to the chemical structure of the antidepressant used. We chose bupropion (BUP), because it is a monocyclic compound which is structurally and pharmacodynamically different from previously studied antidepressants; its antidepressant efficacy is well established (Lineberry et al., 1990; Feighner et al., 1991). However, the precise mechanism of action of BUP remains unclear (Ascher et al., 1995).

One of the specific aims of the present study was to determine whether correlations exist between severity of depressive symptomatology and platelet I_1 radioligand binding parameters and related immunodensities. An ancillary aim was to detect possible correlations between clusters of depressive symptomatology extracted from the Hamilton Depression Rating Scale (Bech et al., 1988). The following clusters were selected: anxiety (A), cognition (C), retardation (R), somatization (S) and melancholic/endogenomorphic (E). Based on previous studies of depression, we hypothesized that the major IRBP-immunoreactive protein on platelets (the 33 kD protein) would be increased on plasma membranes of depressed patients at pretreatment, and that the increase would normalize upon successful antidepressant treatment with BUP. The present results demonstrate a high level of platelet IR-related immunodensity and I_1 binding density (B_{MAX}) associated with moderate to severe depressive symptomatology. The retardation and endogenomorphic clusters of symptoms (Bech et al., 1988) may have predictive value in combination with platelet immunodensities.

METHODS

The data for this report comes from a large multivariate study. Other variables have been reported elsewhere (Zhu et al., 1999). Subjects were recruited through hospital referrals and treated as outpatients. Diagnoses were made by two experienced psychiatrists, and independently validated using the SCID-P (Structured Clinical Interview to Diagnose DSM-IIIR Disorders–Patient Edition). Depressed patients met DSM-IV criteria for Primary Major Depressive Disorder (MDD), unipolar type. The severity of depression was evaluated using the 21-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1967). Eleven depressed patients were entered into the study. All patients were free of psychoactive medications for at least one month prior to entry into the protocol. We also included seventeen healthy control subjects. The female: male ratios and mean ages (± SD) were as follows: MDD = 8 females/ 3 males (overall, 39 years ± 10, SD) and healthy control subjects = 12 females/ 5 males (overall, 36 years ± 8 SD).

Subjects underwent the same dietary and activity restrictions as in our previous studies. None of the women reported taking contraceptive agents. Patients were declared responders to treatment if their post-treatment HAM-D scores were less than 10 or at least 50 % reduced from their baseline score. By these criteria, two patients failed to respond after 8 weeks of BUP treatment. Patients were started at 75 mg/day of BUP and the dose was increased up to a maximum 450 mg/day (in divided doses not exceeding 150 mg per dose) based on patient tolerance and therapeutic response. Patients were monitored for plasma BUP concentrations, beginning at the 4th week of treatment (at least two weeks after the final dose adjustment). Therapeutic plasma BUP concentrations were obtained for all subjects except one; that patient had low levels (~150 ng/ml) at week 6, but responded to treatment nonetheless.

Platelets were isolated from 100 ml of blood that had been collected as previously described (Piletz et al., 1996). Blood for the analysis of plasma BUP was also collected in a citrate-dextrose vacutainer and centrifuged at room temp at 3,500 rpm for 10 minutes. Plasma was frozen at -80°C and transferred to the laboratory of Lindsay De Vane (Department of Psychiatry, Medical University of South Carolina) where the BUP assays were performed. BUP and its metabolites were extracted and assayed by HPLC as

previously described (Sweet et al., 1995). Radioligand binding to purified platelet membranes was performed using [^{125}I]-p-iodoclonidine as described (Piletz et al., 1996), except extended to 15 nM concentration of radioligand to better assess the K_D. Gel electrophoresis and Western blotting have also been described previously (Ivanov et al., 1998). Imidazoline Receptor Binding Protein (IRBP) antiserum was kindly provided by Professor Donald Reis (Cornell Medical College, NY) (Wang et al., 1993). After developing the films with IRBP antiserum, the Western blots were stripped and the integrin β3 chain subunit was assessed on the same blots using anti-CD 61 antibody (Becton Dickinson, San Jose, CA), a platelet membrane protein used for reference.

Statistical analysis was performed as part of the larger study having 11 target variables in the experimental design (Zhu et al., 1999). Four dependent variables are reported herein (HAM-D scores before and after treatment, HAM-D subscore clusters, radioligand binding data, 33 kD/CD 61 O.D.); the other 7 dependent variables will be reported elsewhere (e.g., CD 61 and CD 62 platelet markers, plasma BUP and amine and metabolite levels). Analysis of variance (ANOVA) was used to compare group means, which were supplemented by Student t tests. Correlational analyses were performed to estimate any significant relationships between the target variables (as well as their change scores). Spearman's correlation values ("rho values") were also calculated, with statistical significance for rho assigned at 0.65 or greater. Otherwise data are expressed as the mean ± standard errors (S.E.M.).

RESULTS AND DISCUSSION

Scores of depression severity at pretreatment were HAM-D = 26 ± 1.1 for the entire patient cohort (i.e., moderately severe), while the post-treatment HAM-D scores were 7.4 ± 2.0 (n = 11). Responders to BUP treatment had HAM-Dpre = 26 ± 1.2 and HAM-Dpost = 5.0 ± 1.3 (n = 9). These differences were highly significant ($p < 0.001$). The healthy control subjects had HAM-D scores < 2.0.

Western blots displayed a major immunoreactive band of approximately 33 kD size with IRBP antiserum. Depressed patients averaged a 26% increase ($p = 0.05$) in the immunodensity of the 33 kD IR-related protein (0.036 ± 0.004; expressed in ratio with CD 61 membrane marker protein), compared with healthy control subjects (0.027 ± 0.002). After 6–8 weeks of treatment with BUP, the immunodensity of the 33 kD band from the same group of patients was downregulated to a level (0.029 ± 0.003) comparable to that of controls ($p = 0.003$; Wilcoxon signed ranks test). Depressed patients also displayed a 23 % higher mean platelet I_1 B_{MAX} value at pretreatment (96 ± 10 fmoles/mg protein) compared to controls (79 ± 9 fmoles/mg), but this difference was not statistically significant ($p = 0.18$, t test). Furthermore, no significant downregulation in the B_{MAX} of the I_1 binding sites was observed after treatment (98 ± 6 fmoles/mg). The radioligand binding results *at pre-treatment* agreed with previous reports of elevations in the B_{MAX} values of platelet I_1 sites in depression (Piletz et al., 1995, 1996). This rise also agreed with the 26% elevation observed in the 33 kD protein band at pretreatment revealed on Western blots in this study.

No significant correlation was obtained between the total HAM-D scores from the entire cohort when comparing pre- to post-treatment (Spearman's rho = 0.10). This indicated that total HAM-D pre-treatment scores did not predict the degree of depressive symptomatology experienced after treatment with BUP. Furthermore, none of the target variables in this study, or their change scores, were directly correlated with *total* HAM-D scores at either pre- or post-treatment. However, the platelet immunoreactive IR band (33 kD band per CD 61 band) showed a significant correlation between pre- and post-treatment (Spearman's rho = 0.89; slope = 0.6) for the entire cohort (responders and non-responders), indicating that the extent of upregulation of this band at pre-treatment predicted the extent of down-regulation at post-treatment (Fig. 1).

All five HAM-D clusters were significantly reduced at post-treatment, as predicted (Bech et al., 1988). Any statistically significant associations with the platelet parameters were obtained for only two of the HAM-D cluster subscores, namely, the endogenomorphic (E) and the retardation (R) dimensions. The endogenomorphic cluster showed a significant correlation with I_1 B_{MAX} before treatment whether or not the two non-responders were included in the analysis (Spearman's rho = 0.77) (Fig. 2). Similarly, a correlation existed between the retardation subscore and platelet I_1 B_{MAX} before treatment, whether or not the two non-responders were included in the pre-treatment analysis (Spearman's rho = 0.57). Furthermore, the retardation cluster showed a significant correlation between the change score (before to after treatment) and the decrease in the 33 kD band (per

CD 61) after treatment (Fig. 3). In this analysis the Spearman's rho value was 0.84 for responders but only 0.52 (not significant) when the two non-responders were included (Fig. 3).

Of what potential importance are these data? As an example, let us look at the correlation between platelet I_1 B_{MAX} values and the endogenomorphic symptom cluster of depression (Fig. 2). The endogenomorphic symptoms are characteristics of "melancholic depression." They include psychomotor agitation as well as the typical vegetative symptoms of weight loss, mid-nocturnal and/or early morning awakening, retardation (of movement, thought and speech), and depressed mood. The correlation of these symptoms with platelet I_1 B_{MAX} values was 0.77 for all subjects. A rho value of 0.77 is highly significant ($p < 0.05$) and is relatively close to unity. If there were a perfect correlation (i.e., rho = 1.0), the "dots" would fall on a straight line; that is, the platelet I_1 B_{MAX} value would perfectly predict the severity of the endogenomorphic symptoms of depression. Since the rho value is less than 1.0, this means there is some "imprecision" in our ability to predict the severity of these symptoms from the platelet I_1 B_{MAX}. But, this correlation quantifies the variability, and, in this case, rho = 0.77 means that a straight line is a decent fit.

In summary, we have observed elevations in two platelet parameters during a moderately symptomatic phase of unipolar depression: platelet 33 kD IRBP immunodensities, and less significantly, platelet I_1 B_{MAX} values. After eight weeks of treatment with BUP, a significant reduction was observed in the immunodensity of the 33 kD protein. Of potential diagnostic interest is the observation that both of these biochemical parameters on the platelet (immunodensity and radioligand binding) correlated with two important dimensions of the depressed state of the illness; namely, the retardation and the endogenomorphic (or melancholic) clusters of symptoms. These clusters of symptoms are hallmarks of depression. In spite of the small sample size in this study, these two parameters showed significant correlations. If these preliminary findings can be confirmed in larger ongoing studies at the University of Mississippi Medical Center, a reliable biological marker for two of the hallmark features of affective disturbances may be forthcoming.

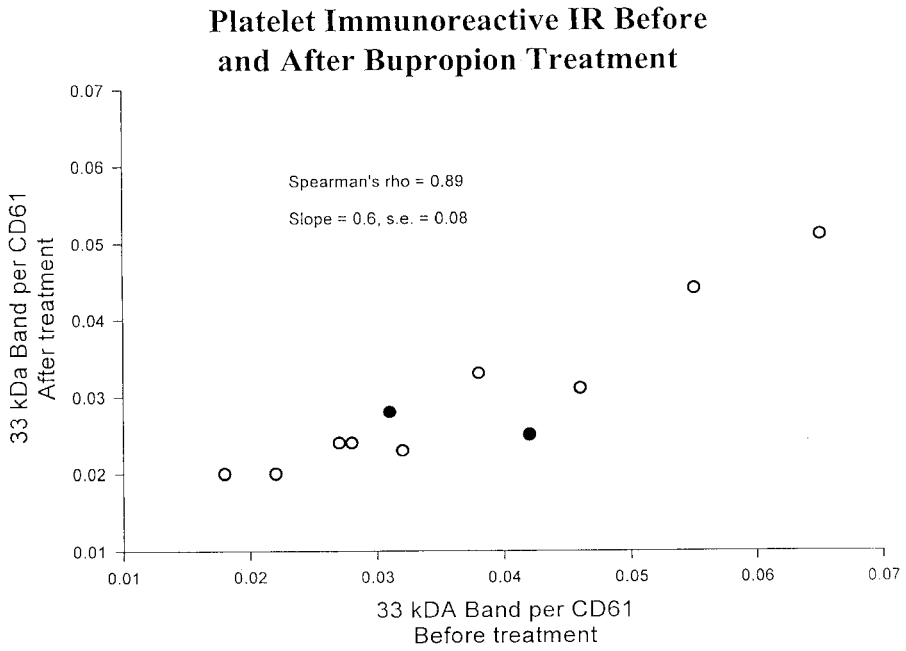

Figure 1. Correlation of the immunodensities of the 33 kDa band per CD 61 before and after treatment with bupropion.

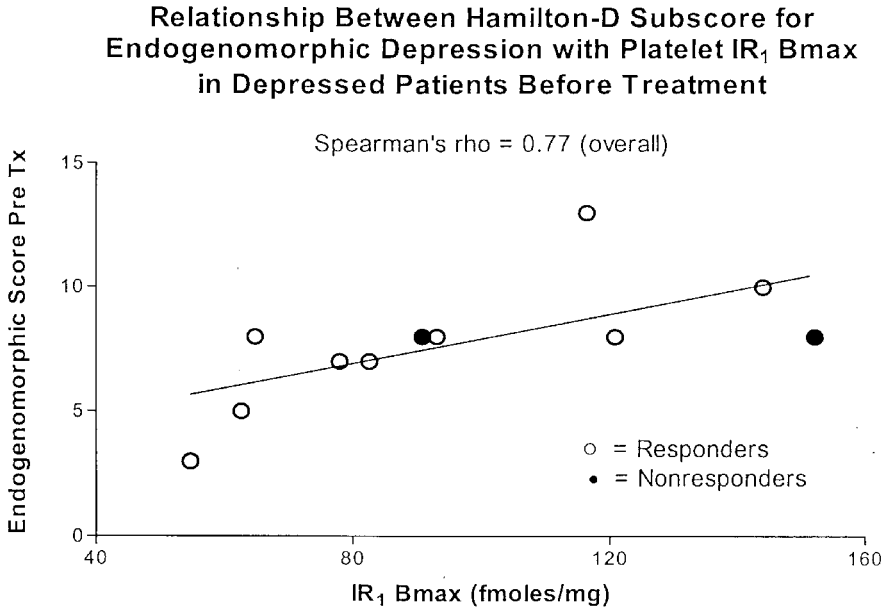

Figure 2. The endogenomorphic cluster score correlates significantly with the platelet I_1 B_{MAX} before treatment.

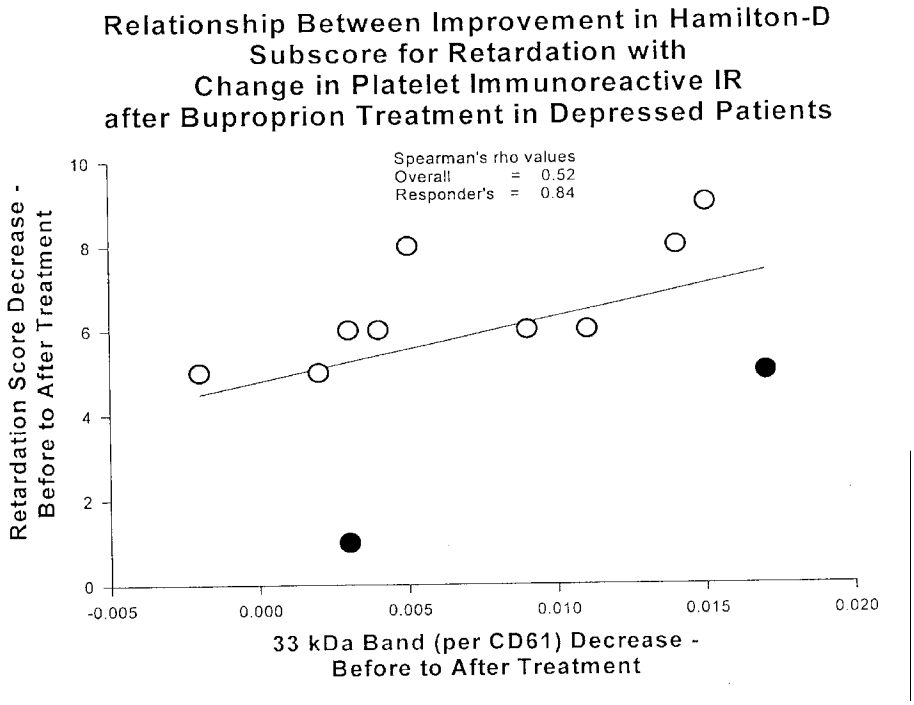

Figure 3. Correlation between improvement in the retardation subscore and the change in the platelet 33 kDa immunodensity from before to after treatment with bupropion.

ACKNOWLEDGMENTS

This project was supported by grants MH 57601-01A1 and MH 42859-06A1 from the National Institute of Mental Health. Dr. Lindsay DeVane kindly performed the BUP assays at the Medical University of South Carolina.

LITERATURE CITED

Ascher, J.A., J.O. Cole, J.-N. Colin, J.P. Feighner, R.M. Ferris, H.C. Fibiger, R.N. Golden, P. Martin, W.Z. Potter, E. Richelson, and F. Sulser. 1995. Bupropion: a review of its mechanism of antidepressant activity. J. Clin. Psychiatry 56:395–401.

Bech, P., P. Allerup, L.F. Gram, P. Kragh-Sorensen, O.J. Rafaelsen, N. Reisby, and P. Vestergaard. 1988. The diagnostic melancholia scale (DMS): dimensions of endogenous and reactive depression with relationship to the Newcastle scales. J. Affective Disord. 14:161–170.

Ernsberger, P., M.E. Graves, L.M. Graff, N. Zakieh, P. Nguyen, L.A. Collins, K.L. Westbrooks, and G.G. Johnson. 1995. I_1-imidazoline receptors: definition, characterization, distribution and transmembrane signalling. Annals of NY Acad. Sci. 763:22–42

Feighner, J.P., E.A. Gardner, J.A. Johnston, S.R. Batey, M.A. Khayrallah, J.A. Ascher, and C.G. Lineberry. 1991. Double-blind comparison of bupropion and fluoxetine in depressed outpatients. J. Clin. Psychiatry 52:329–335.

Garcia-Sevilla, J.A., P.V. Escriba, M. Sastre, C. Walzer, X. Busquet, G. Jaquet, D.J. Reis, and J. Guimon. 1996. Immunodetection and quantitation of imidazoline receptor proteins in platelets of patients with major depression and in brains of suicide victims. Arch. Gen. Psychiatry 53:803–810.

Halbreich, U., J.E. Piletz, S. Carson, A. Halaris, and N. Rojansky. 1993. Increased imidazoline and α_2 adrenergic binding in platelets of women with dysphoric premenstrual syndromes. Biol. Psychiatry 34:676–686.

Hamilton, M. 1967. Development of a rating scale for primary depression. Brit. J. Social and Clin. Psychology 6:278–296

Ivanov, T., Y. Feng, H. Wang, S. Regunathan, D.J. Reis, D.N. Chikkala, P. Gupta, J.C. Jones, and J.E. Piletz. 1998. Imidazoline receptor proteins are regulated in platelet-precursor MEG-01 cells by agonists and antagonists. J. Psychiatric Res. 32:65–79.

Lineberry, C.G., J.A. Johnston, R.N. Raymond, B. Samara, J.P. Feighner, N.E. Harto, R.P.J. Granacher, R.H. Weisler, J.S. Carman, and W.F. Boyers. 1990. A fixed-dose (300 mg) efficacy study of bupropion and placebo in depressed outpatients. J. Clin. Psychiatry 51:194–199.

Molderings,G.J., F. Hentrich, and M. Gothert. 1991. Pharmacological characterization of the imidazoline receptor which mediates inhibition of noradrenaline release in the rabbit pulmonary artery. Naunyn-Schmiedebergs Arch. Pharmacol. 344:630–638.

Piletz, J.E., P.R. Ernsberger, and A. Halaris. 1995. Psychopharmacology of imidazoline and α_2-adrenergic receptors: implications for depression. CRC Critical Reviews in Neurobiology 9:29–66.

Piletz, J.E., A. Halaris, J. Nelson, Y. Qu, and M. Bari. 1996. Platelet I_1-imidazoline binding sites are elevated in depression but not generalized anxiety disorder. J. Psychiatric Res. 30:147–168.

Piletz, J.E., and K. Sletten. 1993. Nonadrenergic imidazoline binding sites on human platelets. J Pharmacol Exp Ther. 267:1493–502.

Raddatz, R., A. Parini, and S.M. Lanier. 1997. Localization of the imidazoline binding domain on monoamine oxidase B. Mol Pharmacol. 52:549–53.

Wang, H., S. Regunathan, D.A. Ruggiero,and D.J. Reis. 1993. Production and characterization of antibodies specific for the imidazoline receptor protein. Mol. Pharmacol. 43:509–515.

Zhu, H., A. Halaris, S. Madakasira, P. Pazzaglia, N. Goldman, L. DeVane, M. Andrew, D. Reis, and J.E. Piletz. 1999. Effect of bupropion on immunodensity of putative imidazoline receptors on platelets of depressed patients. J. Psychiatric Res. (in press).

Current Status of Neuropathologic Findings and Treatment Strategies in Alzheimer's Disease

Garth Bissette[1]
Departments of Psychiatry and Human Behavior and Pharmacology and Toxicology,
University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505

The incidence of Alzheimer's disease is increasing at alarming rates and recent evidence indicates the neuropathology associated with AD may be present 10 or more years before diagnosis is possible with currently available techniques. Longitudinal studies of at-risk subjects are the only hope for discovery of biological markers for AD in the early stages of the disease. Such markers would allow treatments for AD to begin before neurodegeneration produces irreversible deficits and patients become disabled. The suitablility of several potential biological markers of brain systems affected by AD neuropathology are reviewed and methods to determine their usefulness in early diagnosis of AD are discussed.

Alzheimer's disease (AD) affects 40% of the United States population reaching 80 years of age. This neurodegenerative disease is characterized by progressive dementia and increases in incidence with age, doubling every 4.9 years after the age of 65. Two-thirds of the cases of dementia are due to AD and it is the fourth leading cause of death in the U.S. population. In 1997 there were an estimated 2.32 millions people already suffering with Alzheimer's disease in the United States and an additional 360,000 people are expected to develop the disease each year. Half the nursing home beds in the U.S. contain AD patients and the time course of the disease ranges from two to twenty years with an average of eight years survival after clinical diagnosis. Projections are for the numbers of new and existing cases of AD to quadruple in the next 50 years (Brookmeyer et al., 1998). This dire forecast represents an enormous burden for public health resources, yet delaying onset of the disease by only two years would reduce the numbers expected in 50 years by some two millions in this country alone. The presence of AD can only be inferred from clinical symptoms at present and requires a brain sample to be definitively diagnosed. Such samples are almost always obtained after death and this is where most of the tissue used for research has necessarily been procured. Thus our knowledge of what changes in the brain during AD is largely based upon a picture obtained at death with all it's attendant complications. This is analogous to knowing the final score of a sports match without knowing what transpired during the game; was the lead exchanged, who were the major players, etc. In the face of the looming catastrophic health problem of AD as foreseen by epidemiologists, this state of affairs is entirely unsatisfactory.

The first priority at present must be establishing who is at risk for AD so that treatment can begin before the effects of neurodegeneration becomes intolerable. It is not even necessary to be able to stop the neurodegeneration, although that would be preferable and is the ultimate goal. Treatments for Parkinson's disease currently give it's victims an average of eight years of symptom control in the face of the continuing neurodegeneration associated with this disease. Unfortunately, the most recent neuroimaging and neuropsychiatric data indicates that in AD the associated pathology may begin as much as 10 years before the disease can be accurately diagnosed with current clinical techniques. These two studies were both elegantly conducted but differ in that one (Fox et al., 1998) found that memory disturbances were detectable 10 years before a clinical diagnosis of AD was possible and preceded decreases in regional cerebral blood flow detected by magnetic resonance imaging (MRI), while the other detected temporal and parietal cortex blood flow decreases in individuals with increased genetic risk for AD before symptoms of memory disturbance were observed (Reiman et al., 1996). The measurement of cerebral blood flow with

[1]Tel: (601) 984-6675; email: gbissette@psychiatry.umsmed.edu

MRI is a proxy measure of neuronal metabolic activity as blood flow is increased in regions of activity relative to other brain regions. A decrease in blood flow in a particular brain region indicates possible areas of neurodegeneration or neuronal hypoactivity in AD patients and the areas showing these changes get larger as the disease progresses (Brown et al., 1996). With current technology, the presence of regions with reduced blood flow cannot be used to definitively diagnose AD because other non-AD conditions can also cause similar changes. Thus, without improved diagnostic ability, it will not be possible to find individuals developing AD until the cognitive deficits become established and the disease will remain untreated when treatment would be most advantageous and effective.

At present, a definitive diagnosis of AD can only be made after a microscopic evaluation of brain tissue has established increased numbers of two neuropathological structures, neuritic amyloid plaques (NAP) and neurofibrillary tangles (NFT), in a patient with a clinical history of progressive dementia. As some areas have more of these structures than others, recommendations have been made as to which brain regions should be examined. These brain structures must also be histologically stained to contrast them from other brain structures and allow accurate counting. Depending upon the success of the staining procedure, more or less structures will be visible. Additionally, NAP are often present in more than one type, necessitating training to identify all forms that should be counted. The greatest problem, however, is that some of these structures are present in normal brain and increase with increasing age. This requires relating the number of structures in the suspected AD brain to the numbers encountered in normal subjects of the same age. In reality, this means that most of the AD cases can be identified, but those without much neuropathology, either because AD has not been present long enough or who may be resistant to the pathology, are in an intermediate category that is not easily assigned. Some cases of aged individuals with abundant AD-like neuropathology are found who were not demented during life and therefore cannot be said to have had AD. Other individuals may be at increased risk of AD because of genetic changes in biochemical pathways affecting the production or destruction of NAP and NFT.

Genetic transmission of increased risk for AD does occur, but thus far only account for about 10% of all AD cases. In the known forms of genetic predisposition for developing AD, we may know the identity of the protein that is genetically changed, but we often do not know how that protein contributes to the disease process and in no case do we know how the genetic change has changed the shape of the protein and thus altered the protein's function. The precursor protein for the β-amyloid protein found in NAP, amyloid precursor protein (APP), is encoded on chromosome 21 and people with Down's Syndrome have an extra copy of this chromosome (trisomy 21). Such individuals develop all the pathological changes of AD when they live to the age of 40 years indicating that overproduction of APP can essentially produce early-onset AD. Developing AD before the age of 55 is considered to be the early onset form relative to the more common late onset variety which usually affects individuals after the age of 70 years. Two other proteins, presenilin 1 (chromosome 14) and presenilin 2 (chromosome 1) are implicated in other forms of early onset AD. These proteins are known to function in the intracellular machinery that packages protein in membranes for internal and external distribution, but it is not known how a dysfunctional presenilin would cause early-onset AD, although several theories are being actively pursued. An increased risk for developing late-onset AD (Corder et al., 1993) has been associated with the epsilon 4 allele of apolipoprotein E (chromosome 19). This protein is found in three forms termed epsilon 2, 3, or 4 (roughly 20, 70, and 10%, respectively, of the U.S. population has one of these alleles) and one allele is inherited from each parent. Those individuals with both parents contributing a copy of epsilon 4 (roughly 3% of the U.S. population) have approximately 80% chance of developing the disease by the age of 80 years, about double the risk for those without a known risk factor. It is important to recognize that not everyone with a genetic risk gets AD at the predicted age (incomplete penetrance) nor does an increased risk predict what will happen to an individual. For this reason, genetic tests cannot be used to diagnose the disease but may add confidence to a clinical diagnosis of possible AD when other criteria are not yet definitive.

There have been numerous studies that sought to establish a laboratory diagnostic test for AD based upon biochemical and neurotransmitter changes in the brain. The presence of the blood-brain-barrier (BBB) has hindered this process as many substances that are present in brain are not able to be measured in blood and if they are, non-brain sources often contribute to blood levels and complicate interpretation of the

brain's contribution to any apparent changes in blood levels. Thus at present, the only diagnostic tests for AD are based upon changes in the concentration of biochemicals in the cerebrospinal fluid (CSF) or in brain tissue (Bissette et al., 1991). Because each individual's amounts of these biochemicals are different, these diagnostic tests have a range of values for normal and AD patients and these ranges overlap to different extents depending upon the biochemical agent being measured. This situation prevents one from being able to assign about 10–15% of people to either the normal range or the AD range and thus has about an 85–90% of correctly assigning a patient to the AD group based upon a single CSF sample. A single sample is also unable to provide information about how much change in a particular biochemical is occurring in an individual patient, only serial samples over time can do this. There have been embarrassingly few such serial sample studies to date and, for most of the biochemicals associated with AD, the time of initial involvement and the rate of change over the course of the disease remain unknown.

The current diagnostic tests being marketed for improving clinical diagnosis of AD are based upon proteins associated with the two types of neuropathologic markers. β-amyloid is a 42 or 43 amino acid protein fragment of APP that is deposited in the extracellular space as insoluble fibers within a NAP, appearing as a diffuse region containing neuritic endings of nerve processes (diffuse plaque) or as a central core surrounded by neuritic processes (mature plaque) after staining under light microscopy. The application of aged solutions of synthetic β-amyloid to nerve cells growing in culture or directly into the brains of laboratory animals causes neuronal death and degeneration, but this process has not been demonstrated directly for endogenous β-amyloid in AD brain tissue. β-amyloid can be measured in CSF and, according to most reports, decreases in groups of patients with a clinical diagnosis of AD compared to groups of normal controls (Motter et al., 1995). The rate of β-amyloid decrease over time in CSF of an individual with AD is not known but it is thought that the soluble form detected in CSF decreases as a result of being deposited in plaques.

The NFT is an intraneuronal structure caused by pathological hyperphosphorylation of several amino acid positions in the tau protein. Tau protein is found in the intracellular microtubule structure that comprises part of the interior nerve cell scaffolding and, upon phosphorylation of these several sites, forms paired helical filaments which together make a twisted and dysfunctional neuronal process visible under light microscopy after staining as a NFT. Tau and it's pathologic phosphorylated form are present in CSF and increases in group mean CSF concentrations of tau are seen in AD patients compared to non-AD patients (Vigo-Pelfrey et al., 1995). Changes in CSF concentrations of tau and β-amyloid are the basis of a diagnostic test marketed by Athena Diagnostics in Worchester, MA and a 90% accuracy is claimed in diagnosing AD if β-amyloid is below the normal range and tau is above the normal range.

Neuropil threads (NPT) are intraneuronal proteins also associated with the internal nerve cell scaffolding. As neurons die and are disrupted in AD, the concentrations of these proteins increase in the extracelluar fluid and CSF concentrations are increased (de la Monte et al., 1992). This is the basis of a diagnostic test marketed by Nymox, Inc. of Rockville, MD and also claims a 90% accuracy in confirming a clinical diagnosis of AD when elevated beyond the range found in non-AD controls. This test has recently also become available for urine samples where the specificity is reported to be 85%. Like β-amyloid, neither tau or NTP has been measured in the same individual with AD over time and rate of change during the disease process is unknown.

While many different neurotransmitter systems have been shown to be altered in post-mortem AD brain tissue relative to non-AD brain, the contribution of only a few of these have been associated with cognitive state during life and many of the reported changes are only seen when the patient had an extensive history of disease. Three neurotransmitter systems that have been found to be reliably and reproducibly decreased in AD are acetylcholine (ACh), corticotropin-releasing factor (CRF) and somatostatin (somatotropin-release inhibiting factor, SRIF). The ACh deficits were the first to be described and are almost exclusively limited to a population of nerve cells in the forebrain region of the nucleus basalis. These neurons provide almost all of the ACh to the cerebral cortex and their loss is associated with memory impairments and cognitive deficits. The original studies found 60–80% of these ACh neurons in the nucleus basalis were gone at death in AD when stained for the biochemical enzyme that synthesizes ACh, choline acetyltransferase (CAT). In these studies the average time from death to tissue collection was above 24 hours. A few studies using biopsy samples of cerebral cortex obtained from living

patients that were an average of 3.5 years from initial clinical diagnosis of AD also found similar amounts of CAT decrease and these tissues also had decreases in the high-affinity transport of choline into the remaining ACh terminals in cerebral cortical tissue (Palmer, 1996). However, more recent work using imaging techniques and rapidly obtained autopsy tissue has called the conclusions of this prior work into question. The high-affinity choline transporter (HACT) actually controls the rate of ACh production as it's delivery of choline across the neuronal membrane is rate-limiting, energy-dependent and stimulus-regulated. In a normal response to decreased synaptic availability of ACh, the remaining ACh neurons would increase their release of ACh to compensate, causing increased firing rates and increased amounts of choline delivered by HACT to replace the released ACh. This is precisely what was found in freshly obtained AD cortical tissue within two hours of death compared to non-AD control tissue obtained under similar conditions (Bissette et al., 1995). Additionally, the amount of CAT decrease in this rapid autopsy AD tissue was under 40% of normal or about half of what had been previously reported in post-mortem and ante-mortem studies. Interestingly, the two receptor types associated with cholinergic neurons, muscarinic and nicotinic, are only decreased where the pre-synaptic neuron degenerates and apparently remain unchanged in the post-synaptic neuronal populations. Further evidence for more modest cholinergic changes are found in recent reports using imaging techniques to quantify ACh vesicular transporter (provides uptake of ACh into synaptic vesicles), where mid-stage disease AD patients were shown to have about a 30% decrease in the activity of this ACh transporter in brain (Kuhl et al., 1996). Only a relatively small proportion of AD patients treated with inhibitors of the enzyme that degrades ACh, acetylcholinesterase (AChE), show measurable or sustained improvements in cognitive function. These recent data and the marginal treatment response are consistent with either a more modest cholinergic involvement during AD and at death than was previously believed or the rapid degradation of these markers in tissue removed more than two hours after death or removal from the living brain. Because ACh is broken down very rapidly by acetylcholinesterase (AChE), it cannot usually be measured directly in either brain or CSF without rapid denaturing of AChE by microwave irradiation. This effectively prevents measuring ACh directly in human post-mortem tissue. However AChE can be measured in brain and CSF and the ability of AChE to break down ACh increases with increasing age. However, in AD, AChE decreases in activity and amount in the CSF (Urakami et al., 1989), although the rate at which this occurs in individuals with the disease is not well known.

Somatostatin (SRIF) was the second neurotransmitter substance to be reported to be decreased in the brains of AD patients. Somatostatin is a neuropeptide containing either 14 or 28 amino acids and plays the inhibitory role in the dual regulation of pituitary hormone secretion. Outside the hypothalamus, SRIF is found in cortical interneurons and is often co-localized with inhibitory amino acid transmitters. The cortical layers where these interneurons reside provide communication between the various regions of the cerebral cortex and their loss would interrupt this communication. Actual loss of SRIF-containing neurons in AD brain have been demonstrated in only a few studies (Davies et al., 1990) and some AD researchers believe SRIF systems are targets of disregulation rather than neurodegeneration. Many studies have reproduced the finding of decreased amounts of SRIF in brain tissue from the cerebral cortex and in CSF (Bissette et al., 1986) of AD patients, but decreased SRIF is also found in the CSF in many diseases that affect cognitive function (Bissette and Myers, 1992). This prevents using decreased SRIF in CSF as a diagnostic marker for AD. The degree of cognitive deficit in AD is correlated with the amount of SRIF deficit in both CSF and post-mortem brain (Tamminga et al., 1987). Three studies have reported reversal or slowing of SRIF decreases in CSF of AD patients after treatment. One of the studies (Alhainen et al., 1991) used the AChE inhibitor, Tacrine, but the other two (Karlsson et al., 1986 and Widerlov et al., 1989) used environmental stimulation where the hospital nursing staff made extra efforts to involve the patient in daily activities and orient them in space and time. Two CSF samples from each individual were compared in these studies, making it possible to demonstrate that the AD patients in the treated groups had less change in the declining amounts of SRIF measured than was present in the untreated AD patients. Because the receptor populations that recognize SRIF are also decreased in AD, it may not be possible to use drugs that activate SRIF receptors effectively in attempts to compensate for the decreased SRIF in AD brain. However, the evidence that behavioral treatment can slow or

reverse the CSF deficits in SRIF offers encouragement that these changes may not be completely irreversible in AD.

Corticotropin-releasing factor is a 41 amino acid neuropeptide neurotransmitter found mainly in the hypothalamus and regulates the pituitary-adrenal axis. CRF is also found within cerebral cortex interneurons that also often contain the inhibitory chemical neurotransmitter, gamma amino butyric acid (GABA). CRF is depleted from cortical regions in AD (Bissette et al., 1985) due to degeneration of these interneurons and recent reports indicate that the CRF deficits are more widespread throughout the cortex (Bissette et al., 1998) and are even more associated with cognitive decline than can be demonstrated for SRIF (K.Davis, personal communication). Unlike SRIF, however, CSF levels of CRF are not reliably found to be decreased in AD (Nemeroff et al., 1984 and Martignoni et al., 1990) and receptors for CRF increase rather than decreasing as for SRIF. The increase in regional CRF receptor number is inversely proportional to the regional decrease in CRF content (DeSouza et al., 1986) and such "up-regulated" numbers of CRF receptors indicates that treatment to increase synaptic availability of CRF would have an effective target to act upon. Drugs that release bound CRF from the CRF-binding protein are being developed for use in AD, but clinical trials first must demonstrate safety and lack of toxicity.

Many investigators have wondered which of these various neurotransmitter and neuropathologic marker changes occur first in AD and how quickly they change over the course of the disease. One hypothesis is that the degeneration of cholinergic neurons causes further degeneration of the cortical interneurons containing CRF and SRIF. However, when laboratory animals are given lesions in the nucleus basalis that destroy the cholinergic neurons affected in AD and are allowed to recover for nine months (Millard et al., 1990), the cortical concentrations of CRF and SRIF are greatly increased rather than decreased as this hypothesis would predict. Evidence from post-mortem tissue where both CRF and SRIF were measured in the same sample in various brain regions demonstrates that the largest deficits in both neuropeptides occurs in the entorhinal and parietal cortex. This is the area where imaging techniques demonstrate decreased blood flow during the development of AD. Thus, an alternative hypothesis would be that the neuropeptide changes occur either before or in concert with cholinergic neuron loss and that these changes would occur first in the entorhinal/parietal cortex regions. It is possible that such changes in CRF and SRIF might underlie the personality and emotional changes often reported to precede memory loss by the caregivers of AD patients and to be the first indication to the caregiver that there was something wrong. As it usually takes several years for the progressive dementia in AD to develop after these first behavioral changes occur, this would be an ideal time to begin treatment if our diagnostic ability could be extended to this early stage of the disease process. The test of this alternative hypothesis awaits a few courageous patients and their families.

If a small group of people who were at increased risk for AD from a family history (anecdotal reports of senility or dementia in the two preceding generations) or newly diagnosed with possible AD (due to either mild memory or emotional change) would consent to participate in a research study underway at the University of Mississippi Medical Center this idea could be tested. The person would not enter the study until they reached the age-appropriate range where pathological changes might first be detected. This would typically be at or after the age of 60 years unless the family history indicated an earlier onset form. The person would be cognitively normal except for the mild memory or emotional change and would be able to understand the reasons for the project and what would be required of them, i.e. informed consent. The person would agree to allow a sample of CSF to be collected and a regional MRI image of cerebral blood flow would be generated. The person would be tested for cognitive ability before these procedures and would be given the tests again each year or up to twice per year. Another CSF sample and MRI scan would be performed at three year intervals if no cognitive changes occur or at two year intervals if progressive cognitive decline is detected. If a large change in cognitive status is detected between two test periods, another CSF sample and MRI scan will be requested. Patients are allowed to withdraw from the study at any time and if cognitive decline proceeds to the point where the patient is no longer able to give informed consent themselves, their legal guardian would decide whether they should continue in the study. It is important that the person at risk for AD tell their families about their decision and wishes for the future in regard to continuing in the study so that the families will proceed as the person desires. By

comparing the amounts of AChE, SRIF and CRF in two or more CSF samples from the same person at-risk for AD, we can test when and which of these transmitter systems are first affected. The amounts of the three diagnostic neuropathology marker proteins will also be measured, providing a continuing assessment of changes in these disease-related proteins and the related neurodegeneration. The cerebral blood flow changes will be compared to the biochemical changes in an attempt to localize the brain region(s) likely contributing to the biochemical changes. All of these measurements are made at no cost to the patient. Thirty or forty people are followed until they have a high-probability of AD and have provided three, four or more CSF samples and MRI images, it will be possible to determine if one or another of these known biochemical changes occurs in that individual. If most people show the same order of biochemical changes, it would provide good evidence for which therapeutic target should be selected at a particular stage of AD. This knowledge should produce better treatment responses than are presently seen in the majority of patients treated with the currently available drugs. Unless some courageous and altruistic people are willing to provide such biological samples and imaging data, our ability to treat the appropriate biochemical target of AD changes will only occur by accident if the treatment and change happen to coincide in time. To paraphrase a popular song by David Bowie: We can be heroes just for one day (every two or three years).

LITERATURE CITED

Alhainen, K., J. Sirvio, E.-L. Helkala, K. Renikainen, and P. Riekkinen. 1991. Somatostatin and cognitive functions in Alzheimer's disease—the relationship of cerebrospinal fluid somatostatin increase with clincial response to tetrahydroaminoacridine. Neurosci. Lett. 130:46–48.

Bissette, G., G.P. Reynolds, C.D. Kilts, E. Widerlov, and C.B. Nemeroff. 1985. Corticotropin-releasing factor-like immunoreactivity in senile dementia of the Alzheimer type: reduced cortical and striatal concentrations. J. Am. Med. Assn. 254:3067–3069.

Bissette, G., E.Widerlov, H. Walleus, I. Karlsson, A. Forsman, and C.B.Nemeroff. 1986. Alterations in cerebrospinal fluid concentrations of somatostatin-like immunoreactivity in neuropsychiatric disorders. Arch. Gen. Psychiat. 43:1148–1154.

Bissette, G., W.H. Smith, K.C. Dole, B.J. Crain, H. Ghanbari, B. Miller, and C.B. Nemeroff. 1991. Alterations in Alzheimer's disease-associated protein in Alzheimer's disease frontal and temporal cortex. Arch. Gen. Psychiat. 48:1009–1012.

Bissette, G., F.J. Seidler, C.B. Nemeroff, and T.A. Slotkin. 1995. High-affinity choline transporter status in Alzheimer's disease tissue from rapid autopsy. Pages 223–230 *in* JH Growdon, RM Nitsch, S Corkin and RJ Wurtman, eds., The Neurobiology of Alzheimer's Disease, Center for Brain Sciences and Metabolism Charitable Trust, Cambridge, MA.

Bissette, G., and B. Myers. 1992. Minireview: Somatostatin in Alzheimer's disease and depression. Life Sci. 51:1389–1410.

Bissette, G., L.Cook, W. Smith, K.C. Dole, B. Crain, and C.B. Nemeroff. 1998. Regional neuropeptide pathology in Alzheimer's disease: Corticotropin-releasing factor and Somatostatin. J. Alzheimer's Disease 1:1–15.

Brookmeyer R., S. Gray, and C. Kawas. 1998. Projections of Alzheimer's disease in the U.S. and the public health impact of delaying disease onset. Am. J. Pub. Health, 88:1337–1342.

Brown, D.R.P., R. Hunter, D.J. Wyper, J. Patterson, R.C. Kelly, D. Montaldi, and J. McCulloch. 1996. Longitudinal changes in cognitive function and regional cerebral function in Alzheimer's disease: A SPECT blood flow study. J. Psychiat. Res. 30:109–126.

Corder E.H., A.M. Saunders, W.J. Strittmatter, D.E. Scmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines, and M.A. Pericak-Vance. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease. Science 261:921–923.

Davies, C.A., D.R. Morroll, D. Prinja, D.M.A. Mann, and A. Gibbs. 1990. A quantitative assessment of somatostatin-like and neuropeptide Y-like immunostained cells in the frontal and temporal cortex of patients with Alzheimer's disease. J. Neurologic. Sci. 96:59–73.

de la Monte, S.M., L. Volicer, S.L. Hauser, and J. Wands. 1992. Increased levels of neuronal thread protein in cerebrospinal fluid of patients with Alzheimer's disease. Ann. Neurol. 32:733–742.

DeSouza, E.B., P.J. Whitehouse, M.J. Kuhar, D.L. Price, and W.W. Vale. 1986. Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. Nature 319:593–595.

Fox, N.C., E.K. Warrington., A.L. Seiffer, S.K. Agnew, and M.N. Rossor. 1998. Presymptomatic cognitive deficits in individuals at risk of familial Alzheimer's disease. Brain 121: 1631–1639.

Karlsson, I., E. Widerlov, E.V. Melin, A.-L. Nyth, G.A.M. Brane, E. Rybo, J.F. Rehfield, G. Bissette, and C.B. Nemeroff. 1985. Changes in CSF neuropeptides after environmental stimulation in dementia. Nord. Psychiatry J. 39:75–81.

Kuhl, D.E., S. Minoshima, J.A. Fessler, K.A. Frey, N.L. Foster, E.P. Ficaro, D.M. Wieland, and R.A. Koeppe. 1996. In vivo mapping of cholinergic terminals in normal aging, Alzheimer's disease and Parkinson's disease. Ann. Neurol. 40:399–410.

Martignoni, E., F. Petraglia, A. Costa, A. Monzani, A.R. Genazzani, and G. Nappi. 1990. Cerebrospinal fluid corticotropin-releasing factor levels and stimulation test in dementia of the Alzheimer type. J. Clin. Lab. Anal. 4:5–8.

Millard, W.J., G.W. Arendash, A.J. Dunn, and E.M. Meyer.

1990. Effects of nucleus basalis lesions on cerebral cortical concentrations of corticotropin-releasing hormone (CRH)-like immunoreactivity in the rat. Neurosci. Lett. 113:233–239.

Motter, R., C. Vigo-Pelfrey, D. Kholodenko, R. Barbour, K. Johnson-Wood, D. Galasko, L. Chang, B. Miller, C. Clark, R. Green, D. Olson, P. Southwick, R. Wolfert, B.Munroe, I. Lieberburg, P. Seubert, and D. Schenk. 1995. Reduction of ß-amyloid peptide$_{42}$ in the cerebrospinal fluid of patients with Alzheimer's disease. Ann. Neurol. 38:643–648.

Nemeroff, C.B., E. Widerlov, G. Bissette, H. Walleus, I. Karlsson, K. Eklund, C.D. Kilts, P.T .Loosen, and W.W. Vale. 1984. Elevated concentrations of CSF corticotropin-releasing factor like-immunoreactivity in depressed patients. Science 226:1342–1344.

Palmer, A.P. 1996. Neurochemical studies of Alzheimer’s disease. Neurodegeneration 5:381–391.

Reiman, E. M., R.J. Caselli, L.S. Yun, K. Chen, D. Bandy, S. Minoshima, S.N. Thibodeau, and D. Osborne. 1996. Preclinical evidence of Alzheimer's disease in persons homozygous for the e4 allele for apolipoprotein E. N. Eng. J. Med. 334:752–758.

Tamminga, C.A., N.L. Foster, P. Fedio, E.B. Bird, and T.N. Chase. 1987. Alzheimer's disease: low cerebral somatostatin levels corelate with impaired cognitive function and cortical metabolism. Neurology 37:161–165.

Urakami, K., Y. Adachi, E. Awaki, and K. Takahashi. 1989. Characterization of the course of senile dementia of the Alzheimer type using cerebrospinal fluid levels of acetylcholinesterase and somatostatin. Acta. Neurol. Scand. 80:232–237.

Vigo-Pelfrey, C., P. Seubert, R. Barbour, C. Blomquist, M. Lee, D. Lee, F. Coria, L. Chang, B. Miller, I. Lieberburg, and D. Schenk. 1995. Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. Neurology 45:788–793.

Widerlöv, E., G. Brane, R. Ekman, M. Kihlgren, A. Norberg, and I. Karlsson. 1989. Elevated CSF somatostatin concentrations in demented patients parallel improved psychomotor functions induced by integrity-promoting care. Acta Psychiatr. Scand. 79:41–47.

The Neurobiology and Psychopharmacology of Schizophrenia: An Update

Henry A. Nasrallah[1]
University of Mississippi Medical School, Jackson, MS 39216

Schizophrenia is a serious neuropsychiatric brain disorder characterized by many disabling clinical manifestations. Many lines of evidence point to a neurodevelopmental pathogenesis in schizophrenia, although recent research points to a possible neurodegenerative component as well, especially in the first few years after the initial psychiatric hospitalization. The major treatment of schizophrenia is pharmacological, but psychosocial and rehabilitative therapies are also vital after stabilizing the acute psychotic symptoms. The first generation of antipsychotics which became available in the mid-1950s were limited in their efficacy and associated with many neurologic and medical side effects which engender poor compliance and frequent relapses. The new generation of antipsychotics, also called serotonin-dopamine antagonists, became available in the mid-1990s and have proven to have broader efficacy, more tolerable side-effect profile, and better outcomes. Neuroscience research into the neurobiology and psychopharmacology of schizophrenia will continue to elucidate the pathophysiology and treatment of this disabling brain disease.

Schizophrenia is arguably the most severe and disabling psychiatric brain disorder (Shriqui and Nasrallah, 1995). Its onset is usually in the late tens to mid twenties, but its course lasts a lifetime, often reducing a previously functioning and promising young person to a vocationally disabled, frequently hospitalized and socially isolated individual who is almost totally dependent on others for sustenance and care. It is a cruel disease that robs its victims of the core of what makes them human: to love and to work.

CLINICAL FEATURES

The clinical manifestations of schizophrenia are numerous, indicating that many brain regions are involved. Many, but not all of these clinical symptoms are included in the official diagnostic criteria for schizophrenia (The American Psychiatric Association, 1994). The clinical features are generally categorized into four domains:

***(1) Positive symptoms*—**These include several psychotic symptoms that almost invariably lead to psychiatric hospitalization. The most common positive symptoms include hallucinations (auditory, visual, somatosensory), delusions (paranoid or non-paranoid), bizarre behavior, disorganized speech and thought disorder.

***(2) Negative symptoms*—**Also called deficit symptoms, they include the inability to express appropriate emotions or gestures, failure to express complex conceptual thoughts, social withdrawal, apathy and lack of motivation, and inability to derive pleasure from any activity, and impaired attention. These symptoms may manifest slowly and insidiously long before the appearance of the acute psychotic symptoms that prompt hospitalization, and they became more clinically prominent after the remission of the positive symptoms (Andreasen, 1982).

***(3) Cognitive dysfunction*—**Several key main functions are impaired n schizophrenia, including memory, learning, attention and executive functions. These deficits are often the reason for the inability of a person with schizophrenia to return to school or to hold a job, and they seem to deteriorate following the initial psychotic episode before which the cognitive functioning of patients may be quite better than average.

***(4) Comorbid psychiatric disorders*—**Patients with schizophrenia often manifest one or more additional psychiatric syndromes, most commonly

[1]Tel: (601) 368-4159; email: hnasra2905@aol.com

depression, anxiety, aggression, and substance abuse. There comorbidities often complicate the course and outcome of treated schizophrenia.

NEUROANATOMIC ABNORMALITIES

The advent of brain imaging techniques over the past two decades have led to the detection of many structural brain abnormalities in schizophrenia using computerized tomography (CT) and magnetic resonance imaging (MRI) scans (Krishnan and Doraiswamy, 1997). These finding include dilated lateral and third cerebral ventricles, overall cerebral hypoplasia as well as regional hypoplasia in the volumes of frontal, temporal limbic structures, thalamic nuclei and corpus callosum. Clinical significance of these neuroanatomic findings is the subject of ongoing research. However, certain clinical correlates have been reported such as an association between temporal lobe abnormalities (hypoplasia of the superior temporal gyrus and the hippocampus) with positive symptoms and between frontal lobe pathology with negatives symptoms. Cognitive deficits are also associated with fronto-temporal dysfunction.

PATHOGENESIS

Many possible etiologies for schizophrenia have been proposed. However two overarching hypotheses have recently emerged; the neurodevelopmental and neurodegenerative models.

The neurodevelopmental hypothesis of schizophrenia (Nasrallah, 1993) is based on multiple live of evidence which point to a disruption of brain development during fetal life which may include both genetic and environmental factors. The evidence includes 1) excess of perinatal and obstetric complications in schizophrenia, 2) neurophysiological and behavioral pathology during infancy and childhood of adult onset schizophrenia, 3) presence of hypoplastic brain changes at the onset of the illness in adulthood, 4) postmortem histopathological evidence suggestive of impaired neuronal cell migration, a second trimester process, 5) absence of gliosis in postmortem samples of schizophrenia.

The neurodegenerative hypothesis on the other hand, is supported by the observed clinical deterioration, cognitive decline, and reports of progressive cerebral tissue loss after psychotic relapse early in the course of schizophrenia (Knoll et al., 1998). While the putative processes that result in cerebral atrophy following the onset of psychosis are still unknown, possible mechanisms may include 1) neurotoxicity (secondary to glutamate), 2) free-radical damage due to impaired antioxidants, 3) hypercortisolemia-induced atrophy and 4) apoptosis secondary to increased brain catecholamines. Many researchers agree that both neurodevelopmental and neurodegenerative factors are probably involved in schizophrenia.

PSYCHOPHARMACOLOGY

The start of pharmacologic treatment of schizophrenia occurred in the mid 1950s with the serendipitous discovery that chlorpromazine suppressed psychotic symptoms in many patients institutionalized for chronic schizophrenia (DiMascio and Shader, 1970). This momentous discovery launched the psychopharmacologic era of antipsychotic treatments which ended the practice of "warehousing" schizophrenic patients in state hospital often for a lifetime due to the lack of any effective treatment for psychosis. Between 1955 and 1975, dozens of antipsychotic compounds were synthesized to treat schizophrenia, while all of them were efficacious in treating delusions, hallucinations and agitation in many psychotic patients, it became clear that these drugs had significant limitations including: 1) about a third of the patients failed to derive any benefit from them, 2) only positive symptoms, but not negative, cognitive or depression symptoms, responded to these medications, 3) serious side-effects were associated with these first generation antipsychotics (also called neuroleptics) especially extrapyramidal side effects (EPS) such as dystonia, akathisia, dyskinesia and parkinsonism, and the often irreversible oro-buccal movement disorder known as tardive dyskinesia which emerges in more than half the patients, 4) patient compliance was very low, leading to frequent relapses, increased mortality and poor social and vocational outcomes.

Much was learned about the neurochemistry of mental disorders such as schizophrenia with the discovery of the first generation of antipsychotics (which also led to the discovery of tricyclic antidepressant drugs). The EPS motoric side effects of the neuroleptics strongly suggested that blockade of dopamine receptors was one of the major mechanisms of action of those drugs, and implicated dopamine and other catecholamine pathways in the

neurochemistry of psychosis (Seeman et al., 1976). However, two dopamine tracts in the brain are associated with psychosis (meso-cortical and meso-limbic) while others like the nigrostriatal and tobero-infundibular were associated with the side effects (EPS and hyperprolactinesia) of the D_2 blockade action of the first generation antipsychotics.

It was clear that a better class of antipsychotics was needed to address the clinical needs of schizophrenia. However, it took twenty years from the launch of the last "conventional" neuroleptic (molindone) in 1975 to the introduction of the new class of novel or second-generation antipsychotics (Kane and McGlashan, 1995). The new class of antipsychotics are their broader efficacy (on positive, negative, cognitive and mood symptoms) and better safety and tolerability (such as far less EPS). This improved profile may be due to the fact that the novel antipsychotics are selective to the limbic and frontal dopamine receptors (D_3, D_4, D_5, and D_6 receptors), thus causing far less EPS by avoiding the D_2 receptors in the striatum. Further, the newer antipsychotics also block serotonin $5HT_2$ receptors in addition to dopamine receptors (which is why they are also called serotonin-dopamine antagonists or SDAs), and that encloses them with efficacy on some negative and mood symptoms and probably the lower tendency for EPS as well.

The outcomes of pharmacological treatment of schizophrenia have improved significantly with the new generation antipsychotics, although it is hoped that even more efficacious medications may be developed over the next few years. Perhaps antipsychotics with non-dopamine mechanisms such as serotonin or glutamate pathways may be developed to treat the symptoms of this serious brain disorder. In fact, considerable research is focusing on the NMDA receptor in schizophrenia because phencyclidine (PCP) psychosis is the closest mg-induced model of schizophrenia (Andersson et al., 1998). Amphetamine, a dopamine agonist, produces a paranoid psychosis which is comprised predominantly of positive symptoms, in contrast to PCP, an NMDA antagonist, which produces positive, negative and cognitive features.

PSYCHOSOCIAL ASPECTS

Schizophrenia cannot be managed with a pharmacologic agent alone. This is a complex biopsychosocial disorder that necessitates individual and family psychotherapy as well as social and vocational rehabilitation (Herz et al., 1990). This is a very costly disease (about $60 billion total annual cost, direct and indirect) and most schizophrenic individuals depend on public support for lifetime medical care, housing and subsistence. Significant more research is needed to identify the specific causes, better treatments and prevention of schizophrenia.

CONCLUSIONS

Despite many recent advances in he neurobiology and psychopharmacology of schizophrenia, much more needs to be learned about this severe and heterogeneous psychiatric brain disease. The knowledge generated by research into schizophrenia will elucidate not only the pathogenesis and treatment of psychosis but will also improve the understanding of brain mechanisms underlying behavior, thoughts, emotions and cognition in many related psychiatric disorders. The significant recent advances in the pharmacology of schizophrenia are likely to be superceded by even more impressive advances over the next decade.

ACKNOWLEDGMENTS

Supported in part by VISN16 Mental Illness Research, Education and Clinical Center (MIRECC).

LITERATURE CITED

American Psychiatric Association. 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Washington, D.C. APPI. 886 pp.

Andersson, C., M. Chakos, R. Mailman, and J. Lieberman. 1998. Emerging roles for novel antipsychotic medications in the treatment of schizophrenia. Psychiatric Clinics of North America 21:151–179.

Andreasen, NC. 1982. Negative symptoms in schizophrenia: Definition and reliability. Archives of General Psychiatry 39:784–788.

DiMascio, A., and R.I. Shader RI (eds). 1970. Clinical Handbook of Psychopharmacology, Science House, New York. 395 pp.

Herz, M.I., S.J. Keith, and J.P. Ducherty (eds). 1990. Handbook of Schizophrenia (H.A. Nasrallah, series ed.) Volume 4: Psychosocial Treatment of Schizophrenia, Elsevier, Amsterdam. 418 pp.

Kane, J., and T.H. McGlashan. 1995. Treatment of Schizophrenia. Lancet 346:820–825.

Knoll, J.L., D.L. Garver, J.E. Rambug, S.J. Kingsbury, D. Croissant, and B. McDermott. 1998. Heterogeneity of the psychosis: Is there a neurodegenerative psychosis? Schizophrenia Bulletin 24:365–379.

Krishnan, K.R.R., and P.M. Doraiswamy (eds). 1997. Brain Imaging In Clinical Psychiatry, Marul Dekker, New York. 652 pp.

Nasrallah, H.A. 1993. Neurodevelopmental pathogenesis of schizophrenia. Psychiatric Clinics of North America 16: 269–280.

Seeman, P., T. Lee, Chau-Wong, and K. Wong. 1976. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature 26:717–719.

Shriqui, C.L., H.A. Nasrallah (eds). 1995. Contemporary Issues in the Treatment of Schizophrenia, American Psychiatric Press Inc., Washington, D.C. 863 pp.

Silica-scaled Chrysophyceae and Synurophyceae (Chrysophyta) from Mississippi

Daniel E. Wujek
Department of Biology, Central Michigan University, Mt. Pleasant, MI 48859

A total of 36 silica-scaled chrysophytes (Chrysophyceae: 4 *Paraphysomonas* spp., 2 *Spinifero-monas* spp., and 2 *Chrysosphaerella* spp.; Synurophyceae: 17 *Mallomonas* spp., and 11 *Synura* spp.) was recorded from 39 water bodies in 16 Mississippi counties using transmission electron microscopy. The number of taxa per location varied from 0 to 12. One new record for North America was observed, *Paraphysomonas punctata* Zimmermann ssp. *punctata*. The non-siliceous scaled *Pseudodendromonas vlkii* Bourrelly was also collected, hitherto known only in North America from Alabama.

The Mississippi freshwater algal flora, and silica-scaled chrysophytes in particular (classes Chrysophyceae and Synurophyceae), are known only from studies based on light microscopy (Prescott and Scott, 1942; Goodwin, 1953; O'Quinn and Sullivan, 1983; Maddox et al., 1997), with only studies by Goodwin (1940), Whitford (1950) and Williams et al. (1977) having reported silica-scaled chrysophytes. The flora of neighboring states based on electron microscopy (EM) is becoming well documented: Florida (see Wujek and Siver, 1997 and Siver and Wujek, 1998 for literature), Alabama (Wujek and Asmund, 1979; Wujek and Menapace, 1998), and Louisiana (Wee et al., 1993). Asmund and Kristiansen, (1986) has documented that EM is the only reliable instrument for confirming the identification of silica scaled chrysophytes.

In this study, the silica-scaled algal flora from 39 water bodies in 16 Mississippi counties was examined using transmission electron microscopy (TEM).

MATERIALS AND METHODS

Near shore phytoplankton samples were collected with a plankton net (10 µm mesh size) from 4 to 8 March 1996 from 39 different ponds and lakes (Table 1). Samples were fixed in 2% acid Lugol's iodine. Physicochemical parameters (Table 1) measured in the field at the time of sampling were water temperature, pH (Markson model 85), and conductivity (Oakton WD-60); time of sampling varied during daylight hours. For TEM, subsamples were placed on Formvar-coated, carbon-stabilized grids, air-dried, and examined with a Philips 300 TEM. All identifications were based on TEM.

Table 1. Number of silica-scaled chrysophyte taxa and environmental factors associated with phytoplankton samples collected in 16 Mississippi counties (4–8 March 1996).

Location	Date	No. Taxa	pH	Temp. (°C)	Conduct. µScm^{-1}
Tishomingo County	4 March				
1. Moser Pond (T3S, R11W, Section 19)		4	5.45	12.6	35.2
2. Lake in Tishomingo State Park (T5S, R10E, Section 25)		6	8.85	12.7	31.5
Prentiss County	4 March				
3. Dam Pond (T5S, R7E, Section 10)		1	7.30	14.7	115.6
4. Dam Pond (T5S, R7E, Section 20)		5	8.15	13.7	49.2
5. Anderson's Pond (T6S, R6E, Section 1)		6	8.45	14.8	66.4
6. Lake in Baldwyn (T6S, R6E, Section 26)		2	8.85	16.5	115.1
Lee County	4 March				
7. Five County Sportsman Lake (T7S, R6E, Section 26)		1	8.45	13.5	190.9

8. Lake Lamar Bruce (T8S, R6E, Section 16)38.3514.547.7				
9. Barley Lake (T10S, R5E, Section 24)	1	8.55	15.1	45.7
Monroe County 4 March				
10. Lake Monroe (T13S, R7E, Section 16)	1	8.25	14.8	37.0
11. Treas Lake (T14S, R7E, Section 29)	3	6.25	14.3	36.7
Oktibbeha County 5 March				
12. Impoundment (T18N, R15E, Section 6)	1	8.78	15.8	217.0
13. M.S.U. Golf Course (T19N, R15E, Section 28)	1	7.85	15.1	228.0
Lowndes County 4 March				
14. Lake Catherine (T18S, R18W, Section 28)	1	7.20	15.7	40.4
15. Lake Lowndes (T19S, R17W, Section 3)	1	7.30	17.4	24.0
16. Front Yard Pond (T19S, R17W, Section 6)	3	8.35	19.4	23.9
Noxubee County 5 March				
17. Land of Lakes (T16N, R16E, Section 15)	1	8.65	16.2	125.3
18. Howard Lake (T15N, R17E, Section 32)	2	6.85	18.1	130.7
19. Impoundment (T15N, R16E, Section 31)	1	6.95	15.7	47.5
Winston County 5 March				
20. Lake Tiak-O'Khata (T14N, R12E, Section 5)	1	8.35	17.5	32.8
21. Taylor Lake (T15N, R12E, Section 32)	4	6.40	16.5	36.6
Choctaw County 5 March				
22. Choctaw Lake (T16N, R11E, Section 2)	3	6.55	15.9	39.8
Oktibbeha County 5 March				
23. Moon Lake (T18N, R13E, Section 12)	12	6.35	18.4	63.8
24. Impoundment (T18N, R13E, Section 12)	3	6.55	18.0	38.7
Oktibbeha County 6 March				
25. Oktibbeha County Lake (T19N, R13E, Section 21)	1	8.80	20.0	98.7
Webster County 6 March				
26. White Creek Lake (T20N, R10E, Section 31)	4	8.45	21.8	46.9
27. American Legion Lake (T20N, R10E, Section 6)	10	8.85	23.1	31.9
28. Dorroh Lake (T21N, R9E, Section 10)	2	8.35	23.1	31.9
Calhoun County 6 March				
29. Pond (T22N, R9E, Section 4)	6	8.25	24.1	36.8
30. Caviness' Ponds (T23N, R9E, Section 14)	0	6.40	23.5	138.0
Chickasaw County 6 March				
31. Tindall Drive (T14S, R3E, Section 4)	1	8.55	25.4	128.8
32. Linn Lake (T14S, R4E, Section 22)	3	8.30	17.9	36.0
Clay County 6 March				
33. Dean's Lake (T16S, R4E, Section 24)	4	8.35	24.5	57.5
34. Hurst (Catfish) Ponds (T17S, R15E, Section 10)	1	7.30	20.4	49.1
35. Walker Lake (T20N, R5E, Section 10)	0	6.25	19.5	42.9
Leake County 7 March				
36. Impoundment (T10N, R6E, Section 25)	6	6.45	6.50	23.6
Rankin County 7 March				
37. Goshen Springs Boat Launch (T8N, R4E, Section 30)	4	7.05	20.5	44.9
38. Spring Lake (T5N, R2E, Section 15)	2	6.70	15.3	35.3
Pearl River County 8 March				
39. Tate's Pond (T6S, R16W, Section 20)	2	7.60	13.6	22.4

Table 2. Distribution of silica-scaled chrysophytes from 16 Mississippi counties; see Table 1 for descriptions of sampling localities. Bold type indicates complete cells or colonies were observed.

Taxon	Sampling Site
Synurophyceae	
**Mallomonas acaroides* Perty emended Ivanov	29
M. akrokomos Ruttner	2, 3, 4, 5, 8, 15, 17, 24, 27, 29, 33, 36, 39
M. annulata (Bradley) Harris	29
**M. caudata* Kreiger	2, 5, 8, 9, 10, 12, 13, 27, 28, **32**, 33, 37, 38
M. crassisquama (Asmund) Fott	4, 8, **23**, 29, 33, 34
M. cristata Dürrschmidt	11, 23, 31
M. cyathellata Wujek & Asmund	28
***M. quttata* Wujek	1, 2, 4
M. hamata Asmund	1, 23, **27**, 29, 33
M. heterospina Lund	23
M. mangofera Harris & Bradley f. *mangofera*	**22**, 23, 36
M. mangofera f. *foyeata* Dürrschmidt	**23**
M. multiunca Asmund	16
M. papillosa Harris & Bradley	1, 28
M. pumilio Harris & Bradley	1
M. tonsurata Teiling emended Kreiger	17, 23
M. transsylvanica Peterfi & Momeu	6, 7, 27
***Synura australiensis* Playfair	4, **18**, **27**
S. curtispina (Petersen & Hansen) Asmund	5, 27, 36, 37
S. echinulata Korshikov	5, 21, 23, 27, 36
S. mammillosa Takahashi	21
S. mollisvina (Petersen & Hansen) Peterfi & Momeu	**37**
S. petersenii Korshikov f. *petersenii*	**4**, 16, 21, 23, **27**, 36, 37
S. oetersenii f. *qlabra* Korshikov	**37**
S. petersenii f. *kufferathii* Petersen & Hansen	19, 23, 26, **28**
S. spinosa Petersen & Hansen f. *spinosa*	2, 5
S. spinosa f. *longispina* Petersen & Hansen	1, 15, 37
**S. uvella* Stein	2, **4**, **5**, 6, 7, 8, **11**, 16, 21, 22, 24, 26, 27, **28**, 36, 37
Chrysophyceae	
Chrysosphaerella brevispina Korshikov	25
C. coronacircumspina Wujek & Kristiansen	11
Paraphysomonas gladiata Preisig & Hibberd	23
P. imperforata Lucas	20
P. punctata Zimmermann ssp. *punctata*	29, **34**
P. vestita (Stokes) de Saedeleer	14, **22**, 23, 25, 26, **27**, 29, 36, 38, 39
Spiniferomonas bourrellyi Takahashi	38
S. trioralis Takahashi	2, 11, 18, **23**, 24, 26, 29, 36

* = Taxon previously reported for Mississippi

** = Taxon reported to be tropical (Cronberg, 1996)

RESULTS AND DISCUSSION

Thirty-six silica-scaled chrysophytes and one non-siliceous scaled organism with chrysophyte affinities were found during this investigation. The list of taxa (Table 2) includes 17 *Mallomonas* taxa, 11 *Synura* taxa, including three formae of *S. petersenii*, four *Paraphysomonas* taxa including one new record for North America, *P. punctata* Zimmermann ssp. *punctata* (Fig. 1), 2 *Chrysosphaerella* taxa and 2 *Spiniferomonas* taxa.

The number of scale-bearing chrysophyte taxa observed per sample varied from 0 to 12. Species richness was greatest with a decreased water temperature (Table 2). The most frequently observed *Mallomonas* species were *M. akrokomos* and *M. caudata* (present in 33% of the 39 samples), *M. crassisquama* (15%), and *M. hamata* (13%). Frequently occurring taxa in other genera included *Synura uvella* (41%), *S. petersenii* f. *petersenii* (18%), *S. echinulata* (13%), *Paraphysomonas vestita* (26%), and *Spiniferomonas trioralis* (21%). In contrast, 15 taxa were encountered at only a single locality: *Mallomonas acaroides*, *M. annulata*, *M. cyathellata*, *M. heterospina*, *M. mangofera* var. *foveata*, *M. multiunca*, *M. pumilio*, *Synura mammillosa*, *S. mollispina*, *S. petersenii* f. *glabra*, and *Chrysosphaerella brevispina*, *C. coronacircumspina*, *Paraphysomonas gladiata*, *P. imperforata*, and *Spiniferomonas bourrellyi*.

Two sites, Caviness' Ponds and Walker Lake, apparently lacked scaled chrysophytes. The reason for this is unknown. Moon Lake (12 taxa) and American Legion Lake (10 taxa) had the highest diversity. One-half the sites (19 of 39) yielded only 1 or 2 taxa each.

The range in water temperatures (6.5–25.4°C) and the time of year suggest the collections contained elements of both late spring and summer floras (Table 1, Fig. 3). Species that were observed in this study, such as *Mallomonas akrokomos* and *M. transsylvanica*, have often been observed under the ice in more northern regions (Cronberg and Kristiansen, 1980; Siver, 1991). In contrast, taxa such as *M. crassisquama*, *M. tonsurata* and *Svnura curtispina* are more commonly observed during the summer (Siver, 1991).

The pH of samples ranged from 5.45 to 8.85. Many of the species observed in localities with lower pH values (e.g., *Mallomonas hamata*, *M. transsylvanica*, and *Synura echinulata*) have been previously reported as common in acidic habitats (Siver, 1988, 1989, 1991). Our observations clearly support these earlier findings. The pH ranges for the 11 most frequently observed taxa present in 10% of the samples are shown in Fig. 4. *Mallomonas hamata* showed the largest range (5.45–8.85) followed by *Synura uvella* and *Spiniferomonas trioralis* (6.25–8.5). *Synura petersenii* f. *kufferathii* and *Mallomonas crassisquama* had the narrowest ranges (6.35–8.45). The data for all 11 species are within the values reported in the literature.

Conductivity ranged from 22.4–228 μScm^{-1}. For those taxa observed in 10% or more of the samples (Fig. 5), *Synura petersenii* f. *kufferathii* and *Mallomonas crassisquama* had the narrowest ranges (24–64 and 37–64, μScm^{-1} respectively). The largest ranges were observed for *Mallomonas caudata* (32–228), *M. akrokomos* (22–125), *Synura uvella* (24–191) and *Spiniferomonas trioralis* (24–131). The last two taxa were reported as exhibiting similar ranges in Alabama (Wujek and Menapace, 1998). Siver and Hamer (1989) were the first to demonstrate the usefulness of specific conductivity in regulating the distribution of scaled chrysophytes. Of the three physicochemical parameters measured, only conductivity correlated significantly with taxa number (Pearson Correlation = -0.377). Siver (1993) pointed out that additional studies, particularly those of the effects of specific ions, are needed before conductivity gradients can be established for these organisms.

Non-siliceous scales of the free-swimming biflagellate *Pseudodendromonas vlkii* Bourrelly (Fig.

Figures 1, 2. 1. Cell of *Paraphysomonas punctata* ssp. *punctata* with scales sloughing from cell. 2. Single scale from a cell of *Pseudodendromonas vlkii*. Scale bars = 1 µm.

2), a colorless and typically stalked organism, were observed only in the Treas Lake sample from Monroe County. The taxonomic position of the genus is uncertain. It has been variously described as a colorless member of the algal classes Chrysophyceae (Bourrelly, 1953) or Prymnesiophyceae (=Haptophyceae) (Mignot, 1974). Zoological treatments place the genus in the protozoan class Zoomastigophorea (as characterized by Levine et al., 1980) and in a new order Pseudodendromonadida (Hibberd, 1985), family Pseudodendromonidae (Patterson and Zöffel, 1991) or the phylum Opalozoa, class Proterozoea, order Cyathobodonida (Corliss, 1994). This report is the second TEM observation of this taxon for North America. Other locations for *P. vlkii* are France (Bourrelly, 1953; Mignot, 1974), England (Hibberd, 1975) and the U.S. (Alabama: Wujek and Menapace, 1997).

In conclusion, as has been demonstrated for other regions in the Southeastern U.S., Mississippi supports a diverse flora of silica-scaled chrysophytes. Further collections and observations from other localities in the state as well as sampling over an annual cycle should yield additional taxa. The silica-scaled chrysophytes (Chrysophyceae and Synurophyceae) found in Mississippi now include 36 taxa, 33 of which are new reports (Table 2). This is less than half the 73 taxa reported from Florida (Siver and Wujek, 1998 and literature therein) or 83 in Michigan (Wujek and Igoe, 1989, and literature therein), states in which the silica-scaled chrysophyte floras have been more intensely studied. It does compare favorable with Louisiana and Alabama, neighboring states which have had 43 and 45 taxa reported, respectively (Wee et al., 1993; Wujek and Menapacef 1998).

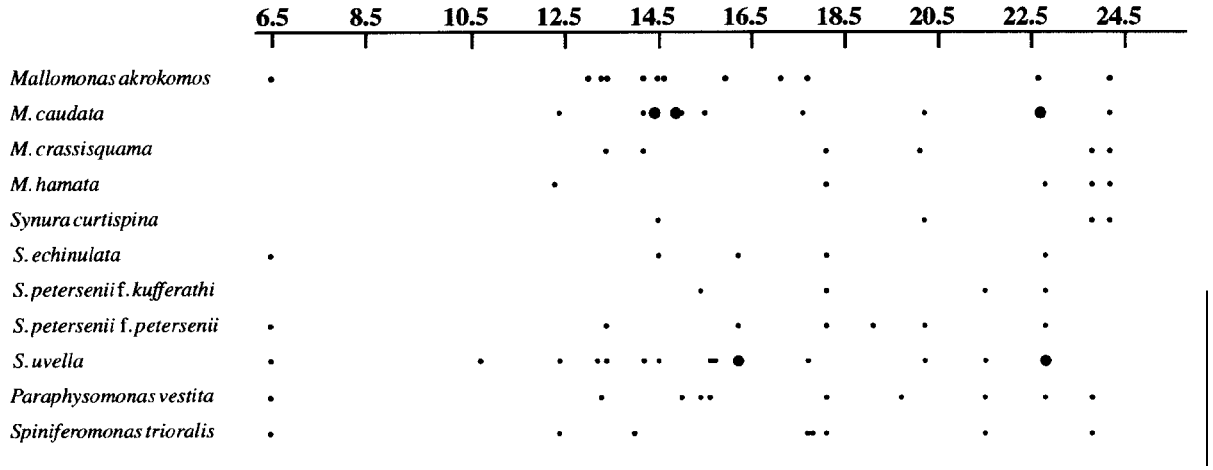

Figure 3. Water temperature ranges for the 11 most frequently observed taxa. Enlarged circles indicate two or more sites with identical temperatures.

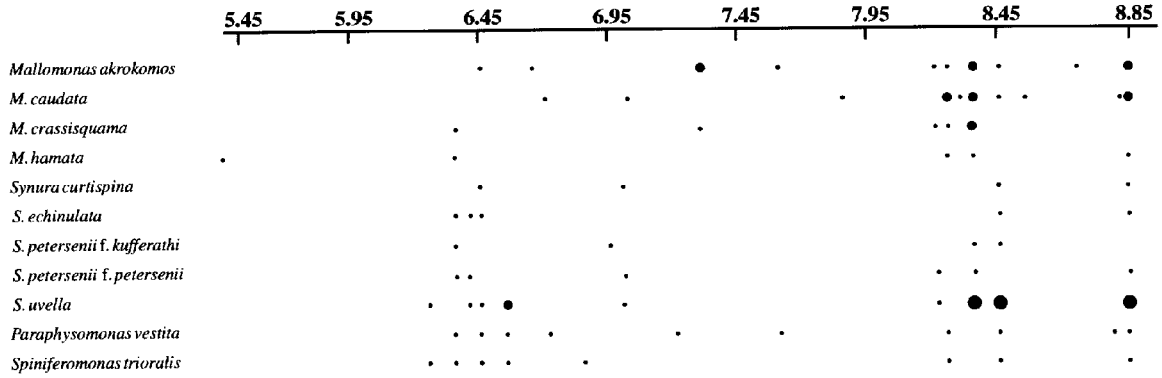

Figure 4. pH ranges for the 11 most frequently observed taxa. Enlarged circles indicate two or more sites with identical pH.

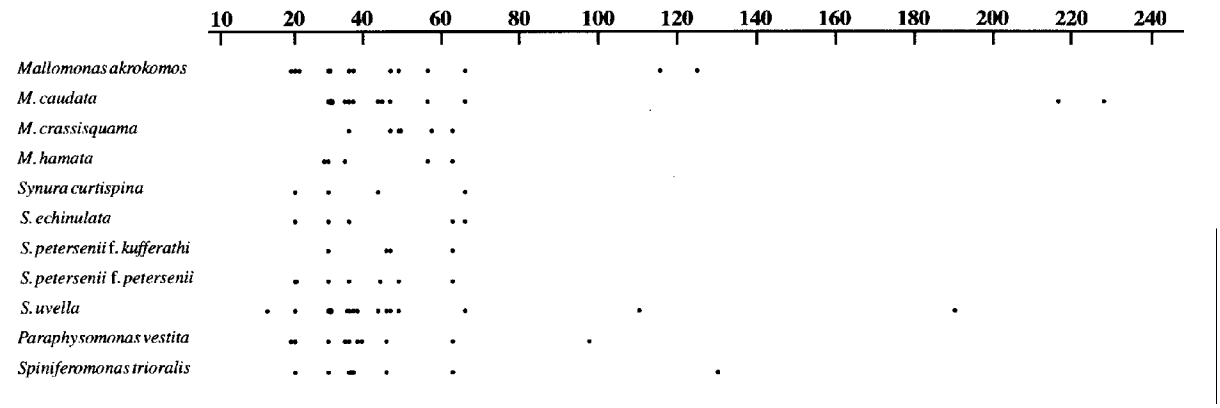

Figure 5. Conductivity ranges for the 11 most frequently observed taxa. Enlarged circles indicate two or more sites with identical conductivities.

ACKNOWLEDGMENTS

I thank M.G. Wujek for assistance in the field, P.R. Elsner for preparing the carbon-coated grids, G.A. Bechtel and Dr. M.J. Sullivan for indicating specific collecting sites and literature, Dr. J. Kristiansen for confirming several of the identifications, and CMU Graphics Production for preparation of the photographic plate. This study was in part funded by a grant from the CMU Faculty Research and Creative Endeavors Committee.

LITERATURE CITED

Asmund, B., and J. Kristiansen. 1986. The genus *Mallomonas* (Chrysophyceae). Opera Botanica 85:1–128.

Bourrelly, P. 1953. Flagellé incolores rares ou nouveaux. Österr. Bot. Zeit. 100:533–539.

Corliss, J.O. 1994. An interim utilitarian ("user-friendly") hierarchal classification and characterization of the protists. Acta Protozool. 33:1–51.

Cronberg, G. 1996. Scaled chrysophytes from the Okavango Delta, Botswana, Africa. Beih. Nova Hedwigia 114: 91–108.

Cronberg, G., and J. Kristiansen. 1980. Synuraceae and other Chrysophyceae from central Småland, Sweden. Bot. Notiser 133:595–618.

Goodwin, G.S. 1940. The plankton algae of Bluff Lake, Mississippi. M.S. Thesis, Mississppi State University, 16 pp.

Goodwin, C.B. 1953. A comparative study of the vernal plankton of three artificial ponds in the vicinity of State College. M.S. Thesis, Mississppi State University, 46 pp.

Hibberd, D.J. 1975. Observations on the ultrastructure of three new species of *Cyathobodo* Petersen and Hansen (*C. salpinx, C. intricatus*, and *C. simplex*) and on the external morphology of *Pseudodendromonas vlkii* Bourrelly. Protistologica 12:249–261.

Hibberd, D.J. 1985. observations on the ultrastructure of new species of *Pseudodendromonas* Bourrelly (*P. operculifera* and *P. insignis*) and *Cyathobodo* Petersen and Hansen (*C. peltatus* and *C. gemmatus*), Pseudodendromonadida ord. nov. Archiv Protistenk. 129:3–11.

Levine, N.D., J.O. Corliss, F.E.G. Cox, G. Deroux, J. Grain, B.M. Honigberg, G.F. Leedale, A.R. Loeblich, III, J. Lom, D. Lynn, E.G. Merinfeld, F.C. Page, G. Poljansky, V. Sprague, J. Vavra, and F.G. Wallace. 1980. A newly revised classification of the Protozoa. J. Protozool. 27:37–58.

Maddox, V.L., J.V. Krans, and M.J. Sullivan. 1997. Survey of algal and cyanobacterial species on golf putting greens in Mississippi, USA. Inter. Turfgrass Soc. Res. J. 8:495–505.

Mignot, J.P. 1974. Étude ultrastructurale d'un protiste flagellé incolore: *Pseudodendromonas vlkii* Bourrelly. Protistologica 10:397–412.

O'Quinn, S.R., and M.J. Sullivan. 1983. Community structure dynamics of epilithic and epiphytic diatoms in a Mississippi stream. J. Phycol. 19:123–128.

Patterson, D.J., and M. Zölffel. 1991. Heterotrophic flagellates of uncertain taxonomic position. Pages 453–476 *in* D.J. Patterson and J. Larsen, eds. The biology of freeliving heterotrophic flagellates. Clarendon Press, Oxford.

Prescott, G.W., and A.M. Scott. 1942. The fresh-water algae of the southern United States. I. Desmids from Mississippi, with descriptions of new species and varieties. Trans. Amer. Micros. Soc. 61:1–19.

Siver, P.A. 1988. Distribution of scaled chrysophytes in 17 Adirondack (New York) lakes with special reference to pH. Can. J. Bot. 66:1391–1403.

Siver, P.A. 1989. The distribution of scaled chrysophytes along a pH gradient. Can. J. Bot. 67:2120–2130.

Siver, P.A. 1991. The biology of *Mallomonas*: Morphology, taxonomy and ecology. Kluwer, The Netherlands, 230 pp.

Siver, P.A. 1993. Inferring the specific conductivity of lake water with scaled chrysophytes. Limnol. Oceangr. 38:1480–1492.

Siver, P.A., and J.S. Hamer. 1989. Multivariate statistical analysis of the factors controlling the distribution of scaled chrysophytes. Limnol. Oceangr. 34:368–381.

Siver, P.A., and D.E. Wujek. 1998. Scaled Chrysophyceae and Synurophyceae from Florida, U.S.A.: VI. Preliminary observations on the flora from waterbodies in the Ocala National Forest. Nova Hedwigia (In press).

Wee, J.L., D.J. Boothand, and M.A. Bossier. 1993. Synurophyceae from the Southern Atlantic Coastal Plain of North America: A preliminary survey in Louisiana, U.S.A. Nord. J. Bot. 13:95–106.

Whitford, L.A. 1950. Some fresh-water algae from Mississippi. Castanea 15:117–123.

Williams, L.R., W.D. Taylor, F.A. Hiatt, S.C. Hern, J.W. Hilgert, V.W. Lambou, F.A. Morris, R.W. Thomas, and M.K. Morris. 1977. Distribution of phytoplankton in Mississippi lakes. Environ. Protect. Agency, Las Vegas, Ecological Research Series EPA600/3-77-101.

Wujek, D.E., and B.C. Asmund. 1979. *Mallomonas cyathellata* sp. nov. and *Mallomonas cyathellata* var. *kenvana* var. nov. (Chrysophyceae) studied by means of scanning and transmission electron microscopy. Phycologia 18:115–119.

Wujek, D.E., and M.J. Igoe. Studies on Michigan Chrysophyceae. VII. Beiheft Nova Hedwigia 95:269–280.

Wujek, D.E., and F.J. Menapace. 1997. The genus *Pseudodendromonas* Bourrelly in North America. J. Alabama Acad. Sci. 68:255–258.

Wujek, D.E., and F.J. Menapace. 1998. Silica-scaled chrysophytes from Alabama. J. Alabama Acad. Sci. 69:33–43.

Wujek, D.E., and P.A. Siver. 1997. Studies on Florida Chrysophyceae (Paraphysomonadaceae) and Synurophyceae (Mallomonadaceae). V. The flora of north-central Florida. Florida Scientist 60:21–27.

The Effect of NaCl and $MgCl_2$ on Algal Physiology and Accumulation of Organic Solutes in *Chlorella pyrenoidosa* L.

Mark G. Hardy, Sophia Sanders-Leggett and Gregorio B. Begonia[1]
Department of Biology Jackson State University Jackson, MS 39217

This study addressed the effect of osmoregulation on physiological and biochemical changes involved in the adaptation of *Chlorella pyrenoidosa* (L.) to changing salinities. *Chlorella pyrenoidosa* grown at high salinities demonstrated increased percent sexuality and a decreased amount of dissolved oxygen. An inverse relationship existed between total growth and increased salinities (NaCl or $MgCl_2$) as indicated by reductions in total growth of *C. pyrenoidosa*. Growth medium pH was not affected by changes in salinity. Nuclear magnetic resonance (NMR) analysis indicated that increasing salinities caused cellular osmoregulation by *C. pyrenoidosa*, resulting in increased production of intracellular ^{13}C-labeled organic solutes.

The use of microalgae as an agricultural fertilizer has been demonstrated by coastal farmers for a number of years (Booth, 1965). As early as 1980, researchers explored developing fertilizers that do not contaminate ground and surface waters or cause erosions (Waaland, 1981). Conventional fertilizers contain multiple nutrients, nitrogen, phosphate, potash materials, and secondary micronutrients that can cause degradation in sub-surface water quality. Fertilizers produced using fossil fuels are expensive and remain a major issue with the agricultural industry because of leachates resulting from such chemicals (Lobban and Wynne, 1981).

According to the Association of American Plant Food Control Officials 1996 Annual Report, the United States consumed a total of 50,744,419 tons of commercial fertilizers in 1995. Furthermore, as of June 1996 the total usage increased to 53,439,634 tons. The prohibitive costs and environmental consequences of synthetic fertilizer use, further necessitate a need to investigate microalgae as a "biofertilizer." A potential organic source of nitrogen, microalgae degrade in the soil of economic crops and drastically reduce the problem of leaching exhibited by other organic and some slow release fertilizers.

Algae are potentially excellent biofertilizers in that they have sufficient amounts of nitrogen and potassium but are low in phosphate. Consequently, if algae are employed as alternative fertilizers, they must be supplemented with phosphate for use with most crops. Nevertheless, the advantages of algae as a biofertilizer are that they: (1) are free of weed seeds and fungal spores which can harm terrestrial crops, (2) are good sources of trace elements, and (3) can regulate crop growth and ripening because of the auxin, cytokinin, and gibberellin associated with them. The use of algae as a biofertilizer may also inhibit certain pathogens and some fungi from causing harm to crops (Waaland, 1981). Some deleterious effects of seaweed manures had been reported which include excess manganese release in low pH soils, water-logging of soils fertilized with *Pachymenia*, inhibitory effects on plant growth, and low availability to plants of some of the nitrogen in seaweeds (Boney, 1965).

The purpose of this research was to investigate the feasibility of improving algae as biofertilizers by increasing their intracellular nitrogen. In aquatic environments, salinity is considered an important ecological variable, particularly in estuarine regions, salt water ponds, and in shore areas where planktonic algae are often subjected to widely fluctuating salt concentrations (Guillard, 1962). The changes in the salinity of seawater usually affect the growth, metabolism, and survival of marine phytoplankton. Guillard (1962) also suggests that the osmoregulatory mechanisms of algae living in marine environments can tolerate a wide range of external salinities. Thus far, salinity changes have been explored in only a few microalgal species. Therefore, *C. pyrenoidosa* was exposed to five concentrations of two salts, NaCl and $MgCl_2$, respectively, to assess the physiological and biochemical changes that are involved in the

[1]Author for correspondence.

adaptation of freshwater microalgae to changing salinities.

MATERIALS AND METHODS

***Microalgae Culture*—**A stock culture of *C. pyrenoidosa* was maintained in 500 ml Erlenmeyer flasks containing 100 ml of a 1.5% semi-solid diatom agar (FW1) medium. Algal cell inoculum was prepared from stock cultures by aseptically washing cells from the semi-solid agar medium using 5 ml of sterile FW1 medium and shaking flasks forcefully (Hardy and O'Kelley, 1986). The cell suspension was decanted into a 25 ml sterile beaker and diluted by adding 20 ml of fresh FW1. Transmittance readings of FW1 medium and inoculum (9:1, v/v) indicated that a 2 ml volume contained approximately 280,000 algal cells needed for optimal growth initiation.

Eighteen 250 ml Erlenmeyer flasks containing 50 ml sterile FW1 medium and isotopically labeled $NaH^{13}CO_3$ (10 µM) were aseptically inoculated with 2 ml of the algal suspension. The initial transmittance of the medium was 100%. The growth medium, initially adjusted to pH 6.8, also contained NaCl or $MgCl_2$ concentrations of 1, 10, 100, 1000, and 10,000 ppm with each concentration prepared in replicates of six. Culture flasks were maintained for 21 days at 25 ± 1 °C in a Mark III Environmental Growth Chamber. Light intensity was maintained at 2.90 Wm^{-2} using Westinghouse cool-white fluorescent tubes. A day and night regime was set at 12:12 hour light/dark cycle. At the end of each culture period in medium allowing optimal growth, the final packed volume of algal cells was approximately 1.0 ml/L of culture.

***Parameter Analysis and Algal Assessment*—**After the 21 day growth period, the following parameters were analyzed: percent sexuality, amount of dissolved oxygen, total growth, pH and cellular osmoregulation by nuclear magnetic resonance (NMR) analysis (General Electric, GN 300 MHz). Photosynthetic activity was determined by measuring dissolved oxygen using a YSI model 51B oxygen meter (YSI Inc., Yellow Springs, OH). An oxygen probe was placed in each algal sample and stirred for approximately 2 to 3 minutes. The O_2 solubility factor was set at 25°C and dissolved oxygen content measured in mg/L. The probe was rinsed in distilled water and recalibrated after each measurement. Medium pH was determined for each culture using an Orion research digital Ionalyzer/501.

Total growth assessments were obtained by using a Bausch and Lomb Spectronic-20D spectrophotometer, 520 nm wavelength. The spectrophotometer was calibrated to a transmittance reading of 100% using a culture tube containing 10 ml of FW1 medium. Medium (10 ml) containing algae from each experimental flask was placed in a culture tube and spectrophotometrically analyzed.

A Bright-Line hemacytometer and a demoscope, consisting of a light microscope connected to a 19" RCA television was used to determine the percent sexuality of each alga. Percent sexuality was calculated by dividing the number of zygotes by the number of zygotes and vegetative cells in a 1 ml sample, counting at least 150 cells per sample. Zygotes were distinguished from vegetative cells in that they contained "spines" on their cell wall or the appearance of two or more cells in a parent cell wall.

After a 21 day growth period, algal cells were collected by centrifugation using a Beckman Centrifuge model J21-B. Algal pellets were obtained after centrifugation at 20,000 rpm for 10 to 15 minutes. Pellets were resuspended in 3–5 ml FW1 medium and dispensed into 10 ml test tubes for NMR analysis of ^{13}C.

***Experimental Design*—**Experimental flasks were arranged in a 1 (inoculation treatment) x 5 (NaCl or $MgCl_2$ concentrations) factorial arrangement in a completely randomized design (CRD) with 6 replications. Each identical 250 ml Erlenmeyer flask containing 2 ml inoculum in 50 ml FW1 medium constituted a treatment replicate. Algal cells were grown in batch culture for 21 days and manually shaken twice a day for adequate dispersal of nutrients.

RESULTS

Cell growth of *C. pyrenoidosa*, measured as transmittance, was an average of 3.35 times greater in NaCl compared to $MgCl_2$ at all concentrations tested (Table 1). The greatest growth inhibition was observed in cultures containing 10,000 ppm $MgCl_2$. As NaCl increased, zygospore production increased. Zygospore production in algal cells grown in NaCl was directly proportional to the amount of NaCl present in each flask (Table 2). There was no significant difference in zygospore production of *Chlorella* at any $MgCl_2$ concentration. An average of 1.69 times more oxygen was measured in *C. pyrenoidosa* cultures when $MgCl_2$ was added to the media compared to NaCl. Oxygen evolution was the same in all cultures of *C. pyrenoidosa* for all

concentrations of $MgCl_2$ tested (Table 3). Medium pH in cultures grown in $MgCl_2$ rose 20% higher than medium containing NaCl (Table 4).

NMR spectra of ^{13}C-labeled *C. pyrenoidosa* indicated that all concentrations of $MgCl_2$ produced significantly higher amounts of organic solutes than controls (Table 5). The highest accumulation of organic solutes was observed in cultures containing 100 ppm $MgCl_2$. There were no significant differences in the accumulation of organic solutes in algal cells grown at 0, 1, and 1000 ppm NaCl. The highest concentration of organic solutes was noted in *C. pyrenoidosa* grown at 10 ppm NaCl.

Table 1. Effect of NaCl and $MgCl_2$ on total growth of *Chlorella pyrenoidosa* after 21 days of growth.

Salt	Concentration (ppm)	Total Growth (% Transmittance)
NaCl	0	4.96 a
	1	4.23 b
	10	4.83 a
	100	5.73 c
	1000	5.56 c
	10000	5.63 c
$MgCl_2$	0	4.96 A
	1	14.80 B
	10	17.33 B
	100	14.80 B
	1000	12.97 B
	10000	26.90 C

Means followed by the same lower case or upper case letters are not significantly different at the 0.05% level (Duncan Multiple Range Test). Transmittance at day 0 was 100%, which was equivalent to 280,000 algal cells/ml.

Table 2. Effect of NaCl and $MgCl_2$ on percent sexuality of *Chlorella pyrenoidosa* after 21 days of growth.

Salt	Concentration (ppm)	% Sexuality
NaCl	0	13.0 a
	1	15.3 a
	10	29.0 b
	100	26.0 b
	1000	30.4 b
	10000	45.3 c
$MgCl_2$	0	13.0 A
	1	33.7 B
	10	31.0 B
	100	43.2 B
	1000	43.6 B
	10000	43.4 B

Means followed by the same lower case or upper case letters are not significantly different at the 0.05% level (Duncan Multiple Range Test). Percent sexuality was expressed as the number of zygotes divided by the number of zygotes plus the number of vegetative cells multiplied by 100.

Table 3. Effect of NaCl and $MgCl_2$ on the amount of dissolved oxygen in cultures of *Chlorella pyrenoidosa* after 21 days of growth.

Salt	Concentration (ppm)	Oxygen evolution (mg/L)
NaCl	0	3.93 a
	1	3.76 b
	10	3.80 b
	100	3.60 c
	1000	3.63 c
	10000	3.60 c
$MgCl_2$	0	3.93 A
	1	6.20 B
	10	6.27 B
	100	6.17 B
	1000	6.27 B
	10000	6.30 B

Means followed by the same lower case or upper case letters are not significantly different at the 0.05% level (Duncan Multiple Range Test).

Table 4. Effect of NaCl and $MgCl_2$ on the pH of *Chlorella pyrenoidosa* after 21 days of growth.

Salt	Concentration (ppm)	Medium (pH)
NaCl	0	8.13 a
	1	8.01 b
	10	7.96 c
	100	7.94 c
	1000	7.95 c
	10000	7.87 d
$MgCl_2$	0	8.13 A
	1	8.27 B
	10	8.42 C
	100	8.26 B
	1000	8.43 C
	10000	8.44 C

Means followed by the same lower case or upper case letters are not significantly different at the 0.05% level (Duncan Multiple Range Test).

Table 5. NMR spectra peak measurements of Carbon-13 labeled organic solutes in *Chlorella pyrenoidosa* after 21 days of growth.

Salt	Concentration (ppm)	NMR peak (mm)
NaCl	0	08 a
	1	16 a
	10	52 c
	100	35 b
	1000	05 a
	10000	20 b
$MgCl_2$	0	08 A
	1	55 B
	10	69 C
	100	75 C
	1000	65 C
	10000	51 B

Means followed by the same lower case or upper case letters are not significantly different at the 0.05% level (Duncan Multiple Range Test).

DISCUSSION

After 21 days of growth, *C. pyrenoidosa* grew significantly better in high NaCl concentrations when compared to $MgCl_2$. Magnesium chloride also had a deleterious effect on algal growth at all concentrations with a six-fold decrease compared to NaCl at 10,000 ppm. Magnesium chloride demonstrated the greatest inhibitory effect on growth when compared to NaCl. While there was no difference in growth in 0 and 10 ppm NaCl, *C. pyrenoidosa* grew better at 1 ppm than in controls. In *C. autotrophica*, the inhibition of growth at high salinities was found to be associated with decline in the cells' turgor pressure (Ahmad and Hellebust, 1984). We observed that the decline in total growth was due mainly to an increase in zygospores with a corresponding decrease in vegetative cells as salinity increased. These findings agree with other observations that sexual reproduction is often induced by stressful conditions (Sze, 1993).

Herbst and Bradley (1989) observed that growth and persistence of algae in a saline environment required physiological tolerance and/or resistance stages in the life cycle. *Chlorella* demonstrated a greater physiological tolerance to NaCl than $MgCl_2$ as indicated by higher growth measurements after 21 days. Increases in the number of zygospores with corresponding reductions of vegetative cells indicated that both salts promoted sexuality and persistence in *C. pyrenoidosa*. Fifteen percent zygospore production in controls was increased to over 40% in cultures grown in NaCl and $MgCl_2$. This demonstrated a dramatic shift in the life cycle of *C. pyrenoidosa*. Previous studies by Setter and Greenway (1983) showed evidence of osmotic regulation control in *C. emersonii* using two endogenous osmotic solute concentrations of proline and sucrose which resulted in large changes in cell life cycle.

We observed a decrease in oxygen evolution in experimental flasks containing NaCl when compared to controls. However, *C. pyrenoidosa* produced 1.7 times more oxygen in medium containing $MgCl_2$ than with NaCl. In studies with *Chlamydomonas eugametos* and *Chlamydomonas moewusii*, Lewin (1956) found that production and maintenance of gametes in the light required the presence of oxygen, and that gametic activity is lost more rapidly in the dark under anaerobic conditions than in the presence of air. Their findings could explain why we observed a two-fold increase in sexuality in cultures containing $MgCl_2$ compared with those grown in NaCl. Stifer (1959) also suggested that the presence of oxygen affected mating in algal cultures. Medium pH of *C. pyrenoidosa* increased from 6.80 to as high as 7.87 at 10,000 ppm NaCl. The pH of *C. pyrenoidosa* cultures grown in $MgCl_2$ rose from 6.80 to as high as 8.44 at 10,000 ppm. Coleman (1962) suggested that within the bounds of physiological neutrality, there were no striking effects of pH in the mating reactions of algal gametes. Mating has been reported in cultures ranging in pH from 4 to 8.5. This corresponds to our findings of zygospore production in cultures containing NaCl or $MgCl_2$.

However, we observed that zygospore production was higher in $MgCl_2$ cultures compared to algae grown in NaCl. Biebi (1962) suggested that an increased zygote yield correlated with a slight rise in pH value of the medium, but the effect was rarely as much as twofold. According to Gerloff et al. (1952) both growth and survival of many algae seemed to be endangered only at pH values exceeding 10.

Microalgae naturally accumulate a variety of intracellular organic compounds. The halotolerant alga *Dunaliella baradawii* grown in NaCl medium contained glycerol, proteins, lipids, carbohydrates, carotene, and chlorophyll (Ben-Amotz and Avron, 1982). NMR spectra peak measurements of *C. pyrenoidosa* indicated that it contained an increase in ^{13}C-labeled organic solutes when grown in medium containing high concentrations of NaCl or $MgCl_2$ when compared to ^{13}C-labeled organic solute production in controls. Ben-Amotz and Avron (1982) suggested that an alga producing massive amounts of useful organic compounds by osmoregulation make it a natural organism for cultivation. It thus provided valuable products and a high protein-containing feed material (Ben-Amotz and Avron, 1983).

Based on the increases in organic compounds we observed, *C. pyrenoidosa* is potentially a very useful alga when employed as a biofertilizer to economic crops. However, further analyses must be done to determine the composition of the organic compounds that *C. pyrenoidosa* produced in high salinities. If high quantities of nitrogenous compounds were accumulated, *Chlorella* will be an excellent alternative to conventional fertilizers. This alga could be grown economically and when applied to economic crops, potential leachates from synthetic fertilizers would be virtually eliminated and eutrophication of lakes and streams could be drastically reduced.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support of the Environmental Science Ph.D. Program in the School of Science and Technology at Jackson State University and the Department of Biology.

LITERATURE CITED

Ahmad, I., and J.A. Hellebust. 1984. Osmoregulation in the extremely euryhaline marine micro-alga *Chlorella autotrophica*. Plant Physiol. 74:1010–1015.

Ben-Amotz, A., and M. Avron. 1983. Accumulation of metabolites by halotolerant algae and its industrial potential. Annu. Rev. Microbiol. 37: 95–119.

Ben-Amotz, A., and M. Avron. 1982. Accumulation of metabolites by halotolerant algae. J. Phycol. 18:529–537.

Biebi, R. 1962. Seaweeds. Pages 711–815 *in* R. Lewin, ed. Physiology and biochemistry of algae. Academic Press, New York.

Boney, A.D. 1965. Aspects of the biology of the seaweeds of economic importance. Adv. Mar. Biol. 3:105–253.

Booth, E. 1965. The manurial value of seaweed. Bot. Mar. 8:138–143.

Coleman, D. 1962. Sexual isolation in *Pandorina morum*. J. Protozool. 6:249–264.

Gerloff, G.C., G.P. Fitzgerald, and F. Skoog. 1952. The mineral nutrition of *Microcystis aeruginosa*. Amer. J. Bot. 39:26–32.

Guillard, R.R.L. 1962. Salt and osmotic balance. Pages 529–540 *in* R. Lewin, ed. Physiology and biochemistry of algae. Academic Press, New York.

Hardy, M.G., and J.C. O'Kelley. 1986. Exogenous steroids and growth of *Neospongiococcum* sp. (Chlorophyceae). J. Phycol. 22:564–566

Herbst, D.B., and T.J. Bradley. 1989. Salinity and nutrient limitations on growth of benthic algae from two alkaline salt lakes of the western great basin (USA). J. Phycol. 25:673–678.

Lewin, R.A. 1956. Control of sexuality in *Chlamydomonas* by light. J. Gen. Microbiol. 15:170–185.

Lobban, C.S., and M.J. Wynne. 1981. The biology of seaweeds. University of California Press, Berkeley, CA. 786 pp.

Setter, T.L., and H. Greenway. 1983. Changes in the proportion of endogenous osmotic solutes accumulated by *Chlorella emersonii* in the light and dark. Plant Cell Environ. 6:227–234.

Stifer, A. 1959. Salt uptake in plants. Biol. Revs. Cambridge Phil. Soc. 34:159–220.

Sze, P. 1993. A biology of algae, 2nd ed. William C. Brown Publisher, Dubuque, IA. 259 pp.

Waaland, J.R. 1981. Commercial utilization. Pages 741–762 *in* C.S. Lobban and M.J. Wynne, eds. The biology of seaweeds. University of California Press, Berkeley, CA.

President's Column

"Those who adapt survive,
Those who adapt well, thrive,
Those who do not adapt, perish"

In this, my final President's Report, I am pleased to tell you that the status of our Academy and the strength of its journal are both excellent. The strength and vision of the incoming leadership, including particularly Dr. Susan Nodurft, our new President, are such that plans for the Year 2000 Annual Meeting (back in Biloxi) are well underway. Indeed, if all goes as planned, that meeting will boast a number of significant new features and could prove to be one of our most diverse and interesting yet. I hope that all members will make plans to submit abstracts, bring students, and encourage colleagues to join us at that meeting.

My inaugural message (summarized above) stressed the relevance of social Darwinism to the life history of non-profit organizations such as ours. The activities of the Academy during this year, and its current state of vigor, provide evidence that we have indeed "*adapted well*." As one example, the gamble of a new meeting site in Tupelo was well rewarded. Not only do we now have another tested home for the annual meeting, but in doing so, we have also reached out to our members who live and work in North Mississippi. This provides a balance that the Academy has long sought in the geographical location for the annual meeting.

I encourage the members of the Academy to actively embrace the spirit of innovation that will be necessary to continue the process of adaptation. Symposia planned for the Year 2000 Annual Meeting spring from newly developed interactions with personnel from the Mississippi Institutions for Higher Learning and NASA, as well as with research faculty in the nursing profession. Productive change is also evident in the elimination of an under-served Microbiology Division and incorporation of Divisions of Sociology and (at least tentatively) History and Philosophy of Science. As I have said previously, we need to maintain our expansion and make efforts to serve the needs of the next "new" group of scientists and educators, i.e., the Information Technologists. This group is comprised of individuals who take decidedly non-traditional approaches to issues of science and technology, at least from the perspective of the tenured and tenure-track academic faculty, who comprise the majority of our current members. To attract this young, vital and dramatically growing group will require yet greater change in the goals and approaches adopted by the Mississippi Academy of Sciences. The extent to which we can reach out to groups such as this will determine our rate of growth and, ultimately, our success or failure.

Education Members

Organizations that assist the Mississippi Academy of Sciences in its efforts to promote science in Mississippi

Alcorn State University
Belhaven College
Delta State University
Mississippi-Alabama Sea Grant Consortium
Mississippi Delta Community College
Mississippi Gulf Coast Community College
Mississippi State University
MSU Agricultural & Forestry Expt. Station
Mississippi University for Women
Pearl River Community College
University of Mississippi
University of Mississippi Medical Center
University of Southern Mississippi

Executive Officer's Column

When we first considered going to Tupelo, it was with great trepidation. In recent memory we had never had an Annual Meeting in the northern part of the state. (Tupelo is not exactly centrally located.) The convention people there told us that they routinely hosted successful conventions, but we assumed that was just hype! They were correct. Tupelo gave us a great meeting! We attracted a record number of abstracts and had some excellent quality science. We successfully attracted large numbers of attendees from Ole Miss, a school that has contributed few members recently. We had a large and interesting equipment exhibit. This positive experience has led us to include Tupelo in our cycle of meeting sites.

Summer seems like a quiet time for the MAS. We are not yet actively engaged in preparing for our next Annual Meeting. But summer is also the time when we welcome our new President-Elect and a new Director. New Division Heads are also getting acclimated to their jobs. Our summer Board meeting is actually a key time to begin setting an agenda for the Meeting a year and a half into the future. Our Y2K meeting is already planned and now needs to be set into motion. Our Y2K+1 meeting needs to start getting our attention.

The Academy is currently in excellent shape. Membership has been steady over the last few years; financially, we have a slight positive balance; the Journal is thriving with Ken Curry as Editor. The Junior Academy is moving towards more participation in their national meeting. However, we cannot be complacent. Member participation in Academy activities is always critical. Be a volunteer. If you want to become more involved in any aspect of the MAS, contact me or one of the other Board members. We always need fresh ideas and help from our members. If you have suggestions for our WEB site, please pass them along. My expertise as a WEB master is exceedingly limited, but I have a teenaged son whose WEB dabblings far exceed my concerted efforts. He's always willing to tell me what to do.—John Boyle

Mississippi Junior Academy of Sciences

MJAS is looking forward to an exciting year. The annual board meeting will be held at the Museum of Natural History in Jackson on Saturday, September 18. Dr. Margot Hall will be working with MAS and MJAS as the Corporate Coordinator. She will be mailing out a letter requesting corporate donations to the Academy. Many MAS and MJAS members are not aware of the background and the financial needs of our organization. Below is an excerpt from her letter to potential sponsors.

"The Mississippi Academy of Sciences (MAS) is an organization composed of scientists, mathematicians, engineers, technologists, and science educators, from research institutes, business, industry, government, military installations, medical centers, reference labs, schools, colleges, and universities in the state. The mission of the Academy is to support the scientific needs of the state through enhanced scientific education, basic and applied research, and professional service. To accomplish this the Academy currently:

a) sponsors the Mississippi Junior Academy of Sciences (MJAS) for pre-college students

b) sponsors scientists to work with individual students at the undergraduate and graduate university level

c) publishes the Journal of the Mississippi Academy of Sciences which is distributed to over 1000 scientists and educators

d) hosts an annual meeting for approximately 1000 participating scientists, educators, and students, providing them with an opportunity to

present their research

e) hosts the annual Junior Academy (MJAS) research paper competition

Our organization exists to encourage the pursuit of basic and applied science and science education at all levels in the state of Mississippi. Dedicated professionals donate their time and energy without charge to the Academy. However, there are areas under our purview which cost money. For example, each year Mississippi students (grades 9–12) are encouraged to conduct scientific research projects guided by their teachers and volunteer local scientists. The students present their papers at a statewide competition (MJAS) held each January. Outstanding papers from each of five scientific subdivisions are selected and the authors receive awards. Additionally a class winner is selected from the ninth and tenth grade competitors. The students with the two best papers receive the Best Research Paper and the Clyde Sheely Awards. All of the winners receive plaques. The students with the two best papers receive an all-expense-paid trip to the MAS annual meeting and the National Junior Academy of Sciences meeting ($1000/student), and their papers are published in the Journal of the Mississippi Academy of Sciences ($250/paper).

In addition to the Junior Academy expenses, there are several other costly areas for the Mississippi Academy of Sciences. We encourage undergraduate and graduate students to join the MAS and we subsidize their memberships. Students receive benefits of membership (excluding the journal) and pay only 20% of the normal membership dues. Likewise, their participation in the annual meetings is at a reduced rate (40%). Since one of the purposes of the MAS annual meetings is to create a forum in which Mississippi students can present and publish their research findings (abstracts), we wish to be as inclusive (and inexpensive) as possible. Also, we wish to continue the tradition of offering certificates and small monetary awards to the students with the winning abstracts in many of the 13 divisions of the MAS. Unfortunately, this has put strain on the budget. In addition to the usual costs for publishing a journal and offering an annual meeting, we invite a nationally renowned speaker to give the Dodgen Lecture at the annual meeting. We are currently limited in the support we can offer the presenter.

We are inviting you to join us in our effort to motivate, recognize and enhance the scientific interests of our young investigators. Donations may be made to the general fund or in a restricted category (please see the enclosed circular), and checks should be made payable to the Mississippi Academy of Sciences and mailed to me at the address given below. There are currently three categories:

Corporate Patron	$1000
Corporate Director	$ 500
Corporate Sponsor	$ 250

In recognition of your support of the Mississippi Academy of Sciences (MAS) and/or the Mississippi Junior Academy of Sciences (MJAS), you/your corporation will be listed as one of the sponsors at the National Junior Academy Competition, in the Journal of the Mississippi Academy of Sciences, and in the MAS annual meeting program. Additionally, you will receive a certificate of recognition, and you will be invited to attend the annual meeting with its social activities, such as the Exhibitors Wine and Cheese Party. We sincerely hope that you will find the Academy deserving of your philanthropy, and will join us in providing our potential young scientists with the key experience which may help decide their future careers and contributions to the state. Please find enclosed some information about the Academy and the Junior Academy and if you have any questions, please don't hesitate to contact me at: (601) 266-4942 (office) or 266-4908 (secretary) or e-mail Margot.Hall@usm.edu."

If you are interested in becoming a MAS-MJAS sponsor or know of someone that is, please contact Dr. Hall. Information contain in this column is graciously excerpted from materials compiled by Drs John Boyle, Rosalina Hairston, and Margot Hall.—Joan Messer

Science Education Divisional Report

The Science Education Division of the Mississippi Academy of Sciences had 18 platform and five poster presentations this year at the Tupelo Meeting. These numbers reflect a decrease in the average numbers of presentations over the last several years. However, there was a diversity of topics ranging from distance learning, to self-directed learning centers for elementary students, to the production of seafood inspection safety program training videos, to preparing preservice teachers through the use of field trips, to identifying native plants in Walter Anderson's murals, to physics curricular enhancements, to biomedical research internships, to simulated habitats for a high school botanical garden, to the use of webpage and multimedia in science laboratory management, to connecting teaching practices to facilitate attitudinal changes in field courses, to learn to work case studies, to providing pedagogical knowledge to graduate students within a teaching assistant orientation session, and to wetlands environmental awareness programs. Platform presentations involved 357 attendees or an average of 20 per session (the smallest attendance in a session was 14 and the greatest attendance was represented by 44). General comments by attendees concerning the range of interdisciplinary sciences, both formal and informal at the graduate and undergraduate levels were extremely positive.

During the Divisional Meeting, the Y2K Chair-Elect was selected, Mr. Willie Heard, J.L. Scott Marine Education Center and Aquarium. Dr. John Ammons, Mississippi Delta Community College is the Y2K Chair for the Science Education Division. The Y2001 Chair-Elect was also selected, Dr. Joyce Mullins, Pearl River Community College. Two action items resulted from this Division Meeting relative to awards, specifically, that the MAS review providing graduate and undergraduate awards in each Division using a consistent format for the submission of competitive applications. The second issue related to the type award which should be presented by each Division. These action items were given to the MAS President-Elect, Dr. Susan Nodurft, Pearl River Community College to present at the MAS Summer Board Meeting; Dr. Ammons, Y2K Science Education Chair, will also ensure these action items are discussed at the MAS Summer Board Meeting.

It has been a pleasure to work with the MAS as the Chair-Elect and Chair of the Science Education Division, Sharon H. Walker, J.L. Scott Marine Education Center and Aquarium, Institute of Marine Sciences, The University of Southern Mississippi.—Sharon Walker

Visit the website of the Mississippi Academy of Sciences
http://www.msstate.edu/Org/MAS/MAS.HTML

First call for abstracts for the 2000 Annual Meeting of the Mississippi Academy of Sciences

The Mississippi Academy of Sciences

will hold its **2000** annual meeting
Thursday and Friday, **February 24 and 25**
at the Broadwater Resort East in
Biloxi, Mississippi

Abstracts are due no later than 1 November 1999. A form for abstract submission is included in this issue. You may submit the abstract electronically through the Academy website: http://www.msstate.edu/Org/MAS/MAS.HTML

MISSISSIPPI ACADEMY OF SCIENCES ABSTRACT FORM/MEMBERSHIP FORM

ABSTRACT INFORMATION

Abstract title _____

Name of presenting author(s) _____

Telephone _____ Email _____

Name of Academy member _____

(One author must be a current member of the MAS; 2000 membership dues must be paid.)

Check the division in which you are presenting

___ Agriculture and Plant Science	___ History and Philosophy of Science	___ Science Education
___ Cellular, Molecular and Dev. Biology	___ Math., Computer Sci. and Statistics	___ Social Sciences
___ Chemistry and Chemical Engineering	___ Marine and Atmospheric Sciences	___ Zoology and Entomology
___ Geology and Geography	___ Physics and Engineering	
___ Health Sciences	___ Psychology and Behav. Neuroscience	

Type of presentation

___ Poster presentation ___ Workshop

___ Lecture presentation ___ Invited symposium

If the presenting author for this paper is also presenting in another division, please list the other division: _____

Audio-visual equipment needs

___ 2" x 2" slide projector

___ Overhead projector

Other audio-visual equipment including computers and computer projection equipment must be provided by the speaker.

MEMBERSHIP INFORMATION

New ___ Renewal ___

Mr. Ms Dr. _____

Address _____

City, State, Zip _____

School or Firm _____

Telephone _____ Email address _____

PLEASE INDICATE DIVISION WITH WHICH YOU WISH TO BE AFFILIATED _____

Regular member $25	Student member $5	Life member $ 250
Educational $150	Corporate Patron $1000	Corporate Donor $500

CHECKLIST

The following MUST be DONE:

___ 1. Enclose copy of abstract (even if abstract has been submitted electronically)

___ 2. Complete and enclose abstract form /membership form(this form)

___ 3. Enclose the following payments (make check payable to Mississippi Academy of Sciences):

- ___ $25 per abstract
- ___ $25 regular membership fee OR $5 student membership fee (2000 membership must be paid for abstract to be accepted)

___ 4. You must supply a check # _____ or P.O. # _____ (credit cards are not accepted)

In addition you MAY preregister at this time:

___ Enclose the following payments:

- ___ $12 regular member (Preregistration before Feb. 1, 2000)
- ___ $5 student member (Preregistration before Feb. 1, 2000)

NOTE: Late abstracts will be accepted with a $10 late fee and only if there is room in the appropriate division. They will be published in the April issue of the MAS JOURNAL.

MISSISSIPPI ACADEMY OF SCIENCES—ABSTRACT INSTRUCTIONS
PLEASE READ ALL INSTRUCTIONS BEFORE YOU SUBMIT YOUR ABSTRACT

- Your paper may be presented orally or as a poster. Oral presentations are generally 15 minutes. The speaker should limit the presentation to 10–12 minutes to allow time for discussion. Instructions for poster presentations are given on the reverse side of this sheet.
- Enclose a personal check, money order, institutional check, or purchase order for $25 publication charge for each abstract to be published, payable to the Mississippi Academy of Sciences. The publication charge will be refunded if the abstract is not accepted.
- At least one author must be a member of the Academy at the time the paper/poster is presented. Payment for membership of one author must accompany the abstract.
- Attendance and participation at all sessions requires payment of registration. Complete pre-registration information will be sent in January.
- Note that two separate fees are associated with submitting a paper for presentation at the annual meeting of the Mississippi Academy of Sciences. An abstract fee is assessed to defray the cost of publishing abstracts and a membership fee is assessed to defray the costs of running the Academy. Preregistration payment ($12 regular; $5 student) may accompany the abstract, or you may elect to pay this fee in January or pay full registration costs at the meeting.
- Abstracts may be submitted typed or printed on clean white paper. Abstracts received in this form will be scanned into a computer. Leave ample margins and use a sanserif type font to help minimize errors in scanning.
- Abstracts may be submitted as a WordPerfect (Windows or DOS), ASCII, ANSI, or .RTF file on a PC readable diskette. Formatting should be minimal. This abstract submission form and the appropriate fees should be sent by US mail even if a diskette is used for the abstract.
- Abstracts may be submitted by e-mail or entered directly through the MAS website. The URL is http://www.msstate.edu/Org/MAS/ MAS.HTML. This abstract submission form and the appropriate fees should be sent by US mail even if the abstract has been submitted electronically.
- Submit your abstract and appropriate fees to the Abstracts' Editor, John Boyle, TO BE RECEIVED NO LATER THAN NOVEMBER 1, 1999.

Dr. John Boyle
Mississippi State University
Dept. of Biochemistry
P.O. Drawer 9650
Mississippi State, MS 39762

FORMAT FOR ABSTRACT

- Your abstract should be informative, containing: (a) a sentence statement of the study's specific objectives, unless this is given in the title; (b) brief statement of methods, if pertinent; (c) summary of the results obtained; (d) statement of the conclusions. It is not satisfactory to state, "The results will be discussed."
- Your abstract, including a concise, descriptive title, author(s), location where work was done, text and acknowledgment, may not exceed 250 words.
- The title should be all capital letters. Use significant words descriptive of subject content.
- Authors' names start a new line.
- The institution where your research was done should include city, state, and zip code. Do not include institutional subdivisions such as department.
- The abstract should be one paragraph, single spaced, starting with a 3-space indentation.
- Use standard abbreviations for common units of measure. Other words to be abbreviated, such as chemical names, should be spelled out in full for the first use, followed by the abbreviation in parenthesis. Do not abbreviate in the abstract title.

- Special symbols not on your printer or typewriter must be in black ink.
- Use italics for scientific names of organisms.
- Begin authors' names on a new line. Place an asterisk (*) after the presenter(s), if there are multiple authors.
- Use superscripts for institutional affiliations where necessary to avoid ambiguity.
- Refer to these examples as guides.

EXAMPLES OF TITLES AND AUTHORS:

[single author, no ambiguity about designated speaker or affiliation]

AN EXPERIMENTAL MODEL FOR CHEMOTHERAPY ON DORMANT TUBERCULOUS INFECTION WITH PARTICULAR REFERENCE TO RIFAMPICIN

Joe E. Jones, Mississippi State University, Mississippi State, MS 39762

Abstract body starts here . . .

[two authors, one designated speaker, different affiliations, but no ambiguity]

AN EXPERIMENTAL MODEL FOR CHEMOTHERAPY ON DORMANT TUBERCULOUS INFECTION WITH PARTICULAR REFERENCE TO RIFAMPICIN

Joe E. Jones and Ralph A. Smith*, Mississippi State University, Mississippi State, MS 39762 and University of Mississippi Medical Center, Jackson, MS 39216

Abstract body starts here . . .

[two authors, both designated as speakers, different affiliations, but no ambiguity]

AN EXPERIMENTAL MODEL FOR CHEMOTHERAPY ON DORMANT TUBERCULOUS INFECTION WITH PARTICULAR REFERENCE TO RIFAMPICIN

Joe E. Jones* and Ralph A. Smith*, Mississippi State University, Mississippi State, MS 39762 and University of Mississippi Medical Center, Jackson, MS 39216

Abstract body starts here . . .

[three authors, one designated speaker, different affiliations]

AN EXPERIMENTAL MODEL FOR CHEMOTHERAPY ON DORMANT TUBERCULOUS INFECTION WITH PARTICULAR REFERENCE TO RIFAMPICIN

Joe E. Jones[1], Ralph A. Smith[1]*, and Alice D. Doe[2], [1]Mississippi State University, Mississippi State, MS 39762 and [2]University of Mississippi Medical Center, Jackson, MS 39216

Abstract body starts here . . .

GUIDELINES FOR POSTER PRESENTATIONS

- The Academy provides poster backboards. Each backboard is 34" high by 5' wide. Mount the poster on the board assigned to you by your Division Chairperson. Please do not draw, write, or use adhesive material on the boards. You must provide your own thumb tacks.
- Lettering for your poster title should be at least 1" high and follow the format for your abstract. Lettering for your poster text should be at least 3/8" high.
- Posters should be on display during the entire day during which their divisional poster session is scheduled. They must be removed at the end of that day.
- Authors must be present with their poster to discuss their work at the time indicated in the program.