

**Validity of Urinary Neurotransmitter Testing
with Clinical Applications of the
CSM™ (Communication System Management) Model**

R.W. Watkins, MD, MPH, FAAFP
Chief Medical Officer • Sanesco, International

Contents

1. Overview.....	1
2. Validity of CSM testing.....	1
3. Using NT testing as a clinical tool.....	2
4. Neurotransmitter Precursor Data and the CSM model.....	3
5. Neurotransmitters, the BBB, and the CSM model.....	5
6. Neurotransmitters, the Kidney, and the CSM model.....	7
7. Clinical Data and the CSM model.....	8
a. Depression and the CSM model.....	8
b. Parkinson's Disease and the CSM model.....	10
c. Diabetes, Metabolic Syndrome and the CSM model.....	11
d. ADD and the CSM model.....	12
e. PTSD and the CSM model.....	13
f. Autism and the CSM model.....	13
g. Hormonal Changes and the CSM.....	14
h. Migraines and the CSM model.....	16
8. Discussion and Conclusions.....	16
9. References.....	18

Validity of Urinary Neurotransmitter Testing with Clinical Applications of the CSM™ (Communication System Management) Model

Overview

This review article seeks to promote the use of urinary neurotransmitter (NT) testing as a non-invasive, reliable, and beneficial clinical tool that can yield important biomarkers for a variety of conditions and illnesses. There are two main points addressed herein: first, the validity of the testing as a laboratory measurement in terms of reproducibility, accuracy, and precision. The second to be addressed is one of clinical utility and application. The testing of urine to determine neurotransmitter levels has a long history preceding the development of pharmaceuticals that are currently in use that affect neurotransmitter balance. The use of amino acid precursors of the major neurotransmitters also has a long history in the treatment of a variety of medical conditions. The medical literature, as well as a wealth of clinical observation, continues to support the use of urine in testing for neurotransmitter levels and guiding therapies designed to bring balance to the nervous system.

Validity of CSM Testing

The first facet that needs to be explored is the level of scientific study surrounding the development, use, and evolution of the technologies which Sanesco's Laboratory Division uses to determine proper neurotransmitter levels. Each test was and continues to be held to the gold standard in NT testing –High Performance Liquid Chromatography (HPLC). Most of the analytes determined for Sanesco are indeed done by HPLC. The tests that are not done by HPLC are done by Enzyme-Linked Immuno Sorbent Assay (ELISA) testing. As both methodologies have been developed and primarily run in Europe, they have been subject to the scrutiny of a number of European third party and governmental offices. The scrutiny they withstand far exceeds that which is required in the U.S. under FDA guidelines and standards. In addition, all in-vitro diagnostics fulfill the regulations of the new European IvD directive (CE) and are both FDA and CLIA registered.

The third party companies mentioned above include BioRad ¹, a world leader in quality control in the areas of Immunology and Endocrinology. Controls for the Sanesco's testing are obtained from BioRad and reports are regularly sent to them to ensure the lab is getting consistent and precise measurements.

Another 3rd party company that has been involved with Sanesco quality control is UKNEQAS (United Kingdom National External Quality Assessment Service) ². UKNEQAS facilitates comprehensive external quality assessment service in laboratory medicine. Through education and the promotion of best practices, it helps ensure that the results of investigations are accurate, reliable, and comparable wherever they are

produced. UKNEQAS has a strict code of management and membership procedures that must be adhered to in retaining their membership status or “mark.”

In addition, analytes on the Sanesco panels have been certified as to their quality assurance by INSTAND e.V.³. INSTAND is a reference laboratory used for analyzing panels according to reference measurement procedures as well as for routine methods. The reference measurement laboratory is used for calibration purposes, both internally (for EQA-samples) and externally (calibration of materials for commercial companies to be used as secondary calibrators). The reference measurement laboratory is accredited according to the international standards DIN-EN-ISO/IEC 17025:2005, DIN-EN-ISO 15195:2004 and operates according to DIN-EN-ISO 15193:2004 (DIN-12286:1997) by the Deutscher Kalibrierdienst (DKD; German Calibration Service) a part of the Physikalisch-Technische Bundesanstalt (PTB; National Metrology Institute) in Brunswick and Berlin. INSTAND’s main goal in the development and maintenance of reference measurement procedures as specified in ISO 15193. The laboratory has reference measurement procedures for 30 analytes and participates in international EQA-surveys for reference institutions (IFCC/JCTLM surveys).

Finally, both technologies that are used by Sanesco’s Laboratory Division are being used by research facilities and university research centers throughout the world. To date, there have been almost 300 publications in leading medical journals citing these technologies.

In summary, the test quality is excellent in terms of its validity, reproducibility, accuracy, and precision. No other commercial laboratory offering neurotransmitter testing to clinicians has either the pedigree or the ongoing commitment to quality assurance that Sanesco’s Laboratory Division has demonstrated.

Using NT testing as a clinical tool

Thus, it has been established that the testing is of high quality. The next question that needs to be addressed is: How valid are the clinical applications? This is perhaps a bit more difficult to answer. Although there is a long history of measuring NTs and their metabolites in a variety of medical conditions in blood, CSF, urine, saliva, and even vitreous humor, the CSM (Communication System Management) model is relatively new. To date, there have been no large scale studies to look at outcomes data. That being the case, there is a tremendous amount of observational data on over 100,000 patients from thousands of clinicians over the past several years.

What has been demonstrated from this data is that if a patient is symptomatic with depression or poor sleep and their serotonin level (for instance) is low, one we can make an intervention using natural substances in the form of amino acid precursors and co-factors, then re-measure the patient’s NT levels and review their symptoms. In about 75% of cases, patients symptoms improve with treatment and that improvement is reflected in their improved NT levels following repeat testing.

The first of data (unpublished) that supports this conclusion was an internal review of 50 patients looking at symptom scores and the levels of NTs at first test and then at retest (6-9 weeks) and again at about 3 months (Table 1).

This is the definition of a useful clinical tool. This tool helps patients and their

practitioners explain why patients feel the way they do. Many patients report after seeing their test results that, for the first time, the way they have been feeling makes logical sense to them. To be clear, this article is not asserting that NT testing be used for diagnosing specific conditions or illnesses. With time, more research will be carried out on the CSM model. That is not the case currently. But such is the situation with any number of laboratory tests – as is the case with elevated liver enzymes. Having an elevated liver enzyme is not a diagnostic marker any more than is finding a low ferritin in a patient. They are simply biomarkers that point the way to possible underlying pathologies and open up a realm of differential diagnoses but in and of themselves are not in the strictest sense diagnostic markers.

The first number represents the improvement from the first to second test. The second number represents the improvement from the first to third test.

<u>Fatigue</u>	<u>Insomnia</u>	<u>Depression</u>
59% > 80%	48% > 81%	59% > 85%

*Improvement is defined as fewer symptoms as reported by patients on questionnaire submitted with each test.

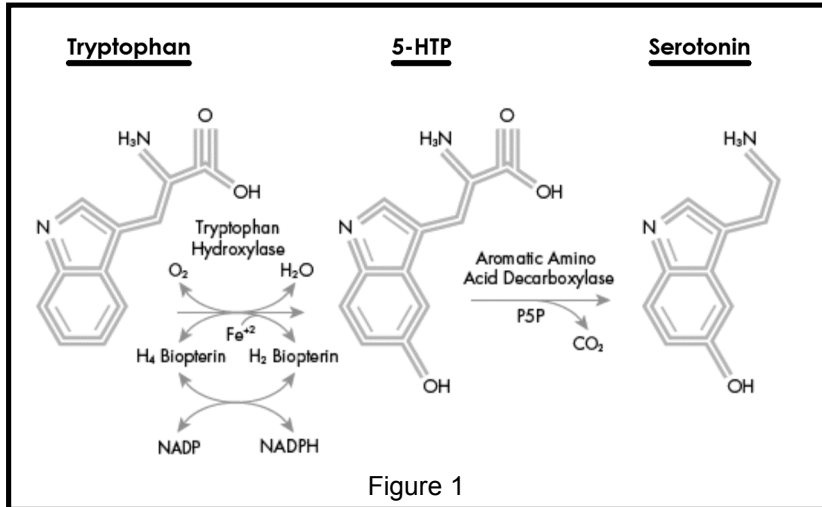
Table 1

Neurotransmitter Precursor Data and the CSM model

With that as a starting point, the focus will now shift to the early literature in neurotransmitter science. Insufficient neurotransmitter activity, particularly of serotonin and norepinephrine, is a central tenet of our current model of depression⁴. Beginning in the early 1970s, researchers came to understand the role of neurotransmitters in brain and body functions as well as their effect on mood. They also established a body of evidence indicating ingestion of certain amino acid precursors of neurotransmitters could increase the levels of those NTs in the brain⁵. Further, they found that these precursor molecules could be useful in treating patients with depression. In the late 1970s and 1980s, a tremendous amount of research examined the use of the serotonin precursors L-tryptophan and 5-hydroxytryptophan (5-HTP), and the norepinephrine precursors tyrosine and phenylalanine in depressed patients⁶. As neurotransmitter biochemistry and its role in mood disorders was further elucidated, pharmaceutical companies took notice. In December of 1987, the first selective serotonin re-uptake inhibitor (SSRI)—fluoxetine (Prozac)— was approved in the United States. Other SSRIs quickly followed and the era of development of a seemingly unending line of reuptake inhibitor anti-depressants was born.

5-Hydroxytryptophan (5-HTP) is an aromatic amino acid naturally produced in the body from L-tryptophan (LT) – an essential amino acid (see Figure 1). The clinical efficacy of 5-HTP is due to its ability to increase production of serotonin in the brain. Produced commercially by extraction from the seeds of the African plant *Griffonia simplicifolia*, 5-HTP has been used clinically for over 30 years. In addition to depression, the

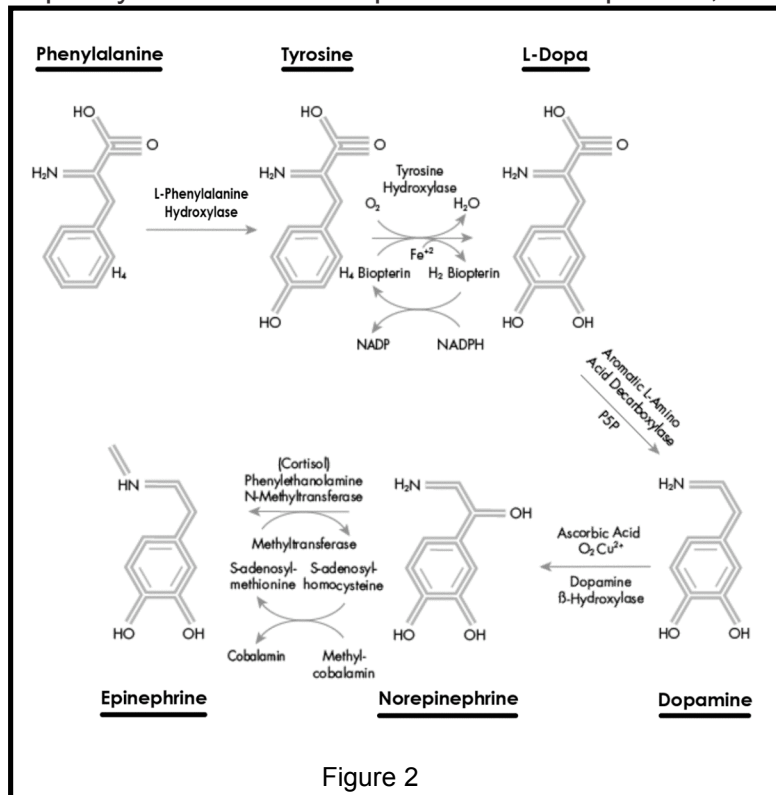
therapeutic administration of 5-HTP has been shown to be effective in treating a wide variety of conditions, including fibromyalgia, insomnia, binge eating associated with obesity, cerebellar ataxia, and chronic headaches. 5-HTP easily crosses the blood–brain barrier and effectively increases central nervous system (CNS) synthesis of serotonin⁷. Administration of



5-HTP has been associated with a significant increase in cerebrospinal fluid levels of 5-hydroxyindolacetic acid, the primary metabolite of serotonin, suggesting 5-HTP leads to an increased release of serotonin. Serotonin production in the human brain can be increased two-fold by oral intake of L-tryptophan as well⁸. In a recent review article, the researchers found 27 studies (11 were double-blind, placebo-controlled studies) where 5-HTP was evaluated for efficacy in the treatment of depression. Although results were mixed, the authors concluded that 5-HTP had at least limited efficacy in the treatment of depression and in some studies was found to be equally efficacious as standard antidepressants⁹.

In the same way, tyrosine is the precursor of norepinephrine, and L-phenylalanine is the precursor for tyrosine. Tyrosine and phenylalanine are also precursors of dopamine, which may also play a role in depression (see Figure 2).

Dopamine is typically thought of as playing more of a role in motivation and reward seeking behaviors than with serotonin¹⁰. Given the current number of animal studies available, there appears to be less scope for increasing norepinephrine synthesis with precursor loading than is the case for serotonin. In the pathway for norepinephrine synthesis, tyrosine hydroxylase is the rate-limiting enzyme in the pathway. It is normally about 75-percent saturated with tyrosine. This leaves little room to upregulate the enzyme¹¹.



Research on the use of norepinephrine precursors for treating depression is limited. To date, there have been only a couple of small studies using L-tyrosine that have shown efficacy in depression treatment^{12, 13}. There has been one negative study¹⁴. Relative to DL-phenylalanine (DLPA), a double-blind study was reported in 1979. In this study, DL-phenylalanine (150-200 mg/day) or imipramine was administered to 40 depressed patients (20 in each group) for one month. No statistical difference was found between the two groups based upon the Hamilton Depression Scale as well as a self-rating questionnaire. This led the researchers to conclude that DL-phenylalanine (DLPA) could have antidepressant properties¹⁵. In a 1980 study, 11 patients with major depressive disorder participated in an open study and were treated with DLPA (mean dose of 350 mg/day) for four weeks. This study produced negative results showing no statistical improvement in the test subjects¹⁶. However, the same researchers did another study later using higher dosages of DLPA. Here, 31 of 40 depressed patients responded to large doses of L-phenylalanine (up to 14 g/ day).¹⁷ Thus it seems a higher dose of DLPA might be warranted in cases of depression in which low norepinephrine activity is involved⁶.

The conception of the CSM model that continues to be developed at Sanesco is grounded in the desire to balance the nervous system on both the inhibitory and the excitatory side. While there has been little research to date supporting this model, there were studies in the early 1980's that looked at combining 5-HTP along with tyrosine in patients with depression. This approach provides precursors for both the serotonergic and catecholaminergic precursors. While both studies were small, the authors indicated that that the 5-HTP's effects in depression were potentiated by tyrosine ¹⁸.

The complexities of depression cannot be addressed using precursor molecules alone. As with many therapies in the integrative medical model, this does not have to be an "all or none" phenomena. We are seeing scores of practitioners combining precursor therapy with standard anti-depressant medications and seeing positive results. It seems that if there are adequate precursors, and thus substrate for the medications to work on, the patient often needs less medication, and the medication they use tends to be more effective. To date, there have been no reports of serotonin syndrome in the literature using 5-HTP either alone, in combination with catecholamine precursors or in combination with standard antidepressant medications⁹. That being the case, the practice should be carried out with caution and full knowledge of the potential problems such as serotonin syndrome in the instance of serotonin precursors or increases in blood pressure if tyrosine is used in the treatment.

Neurotransmitters, the BBB, and the CSM model

Thus far, it has been demonstrated that when giving precursor molecules, they are absorbed into the circulation and then move through the BBB (blood-brain barrier) and into the cerebrospinal fluid. Here they can effect changes in the neurotransmitter levels and improve clinical symptoms. The next question might be: How then do they show up in the urine so that we can make clinical decisions based upon reliable numbers? Some have erroneously postulated that NTs do not cross over the BBB back into the circulation. However, it is known that indeed they do cross back over the BBB and back into the circulation.

Although capillary beds in the periphery permit relatively free exchange of solutes between blood and the surrounding tissue, the microvasculature of the human brain presents a highly regulated, yet dynamic interface between the blood and central nervous system (CNS). This blood–brain barrier (BBB) protects the brain from potentially neurotoxic substances, facilitates exchange of nutrients (including NTs) and waste products between the brain and blood, and maintains an optimal extracellular environment for neuronal function. The selective permeability of the BBB is maintained by several constituents of the endothelium including (1) epithelial-like tight junctions (TJs) that limit paracellular diffusion of even small molecules; (2) carrier-mediated transport proteins that regulate the passage of nutrients from blood to brain, maintain inorganic ion balance in brain interstitial fluid, and efflux xenobiotics and metabolites from brain to blood, often coupled with the activity of enzymes catalyzing biotransformation at the endothelium; and (3) receptor-mediated and absorptive endocytotic mechanisms. Furthermore, the specialized functions of the endothelium are induced, maintained, and regulated via complex interactions with other cell types and the extracellular matrix of the brain, constituting a neurovascular unit¹⁹.

Transporters for the monoamine neurotransmitters, norepinephrine, and serotonin have been described in mouse cerebral microvessels, where it is speculated that they may play a role in clearance of these transmitters from synapses. Serotonin crosses both the luminal and abluminal (the outside side of the vessel) membranes of the BBB via the SERT (serotonin transporter)²⁰. In recent reports, the BBB has been shown to perform the brain-to-blood efflux transport of neurotransmitters, such as GABA^{21, 22}, L-Aspartate and L-Glutamate²³. Dopamine and Norepinephrine both cross the abluminal membrane of the BBB via the NET (Norepinephrine transporter)²⁴. Epinephrine uptake in human brain endothelial cells of the BBB has been demonstrated in vitro²⁵. Histamine crosses both abluminal and luminal cerebral endothelial membranes as well, with primary action being luminal release²⁶. This information suggests one of the general roles of the BBB in facilitating efficient transmission and transportation of neurotransmitters and neuromodulators²⁷.

Thus, the CNS uses a number of different methods to move and remove neurotransmitters in and around cells. Most of the NTs are repackaged through reuptake mechanisms and are recycled for use again in the future. Some, through enzymatic conversion, are metabolized into molecules that have no NT function and are considered waste which must also be cleared from the CNS through various transport mechanisms. It is clear however, that NTs are transported back and forth across the BBB and in and out of the peripheral circulation on a regular basis.

The next question might be: What is the significance of this in relation to what is seen in patients, before and after receiving supplements to support NT balance, and how does this translate into the results seen in urine testing? Once again, there is a lack of good research data in humans using the CSM model as we know it. However, some animal studies are available that reflect that model. Lynn-Bullock, et al, administered 5-HTP to Sprague–Dawley rats either orally or via intraperitoneal injection. The intensity of the immunoreactivity to serotonin and 5-hydroxytryptophan in the substantia nigra was maximal within 2 h of 5-hydroxytryptophan administration and returned to control levels by 24 h. This time course mirrored changes in HPLC measurements of 5-

hydroxytryptophan, serotonin, and the metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the urine²⁸. These results suggest that giving an NT precursor which increases levels of serotonin in the brain, is then reflected in a concomitant rise in the urine NTs and/or metabolites following administration. This is indeed what is believed to happen in the CSM model.

Neurotransmitters, the Kidney, and the CSM model

A question oft arises concerning the contribution the kidneys make to NT analysis in the urine. One of the issues can be whether the NTs are intact, or for the most part metabolized, before they are excreted. A number of research articles have shown that the major NTs are excreted intact in the urine under the influence of several specific renal transporters that filter neurotransmitters from the blood to the urine. These are the renal polyspecific cation transporters, hOCT1, hOCT2, hOCT3, as well as the SLC6 family of transporters (members include the transporters for the inhibitory neurotransmitters GABA and glycine, the monoaminergic transmitters norepinephrine, serotonin, and dopamine). It is these transporters that are responsible for monoamine elimination^{29,30}.

What about questions regarding the potential influence of renal neurotransmitter metabolism on urinary values? It is true that human kidneys can synthesize and metabolize most of the principle neurotransmitters. In addition, the kidneys are innervated and thus receive neural input from the nervous system which might also influence urinary NT testing interpretation. When renal nerve stimulation (RNS) on renal venous outflow and urinary excretion of endogenous norepinephrine, epinephrine, and dopamine was examined in anesthetized dogs, results indicate that renal nerves release dopamine as well as norepinephrine and that urinary catecholamine excretion is a poor indicator of intrarenal catecholamine release³¹.

However, many things can influence urinary excretion of NTs. Most of the data we have are from animal studies, but a high salt diet can influence catecholamine excretion³². In addition, catecholamine excretion can be influenced by the changes that come with age, gender, body mass index, kidney function, and smoking^{33, 34}. Within the CSM model however, it is believed that many of these variables will balance out because a large aspect of the model is to retest the patient. This insures that BMI, for instance, would not be seen as much of an issue over repeated testing. Something that cuts down on test-to-test variability of the NTs is instructing the patient to test at the same time of day using their second morning void. This bypasses issues such as the early morning catecholamine surge.

Furthermore, mental stress can cause renal vasoconstriction due to increased renal sympathetic nerve activity. Under stressful conditions, levels of dopamine, norepinephrine, and epinephrine all increase in human and animal models³⁵. As seen above, RNS of the kidney, can cause an increase in urinary catecholamine release. In a rat study, this increased release of catecholamines was attenuated by infusion of GABA. It was found that GABA suppresses sympathetic NT release and attenuates adrenergically-induced vasoconstriction in the rat kidney³⁶.

This is what is seen clinically as well and it certainly fits with the CSM model. It is not uncommon in the least to see a very high norepinephrine value, or glutamate value (both powerfully excitatory NTs) with low levels of GABA and/or serotonin (inhibitory NTs). An intervention is then made to raise GABA and subsequently the patient is retested and the elevated norepinephrine levels are seen to come back down into a more normal range. This is concomitant usually with a patient whose anxiety is attenuated.

Clinical Data and the CSM model

Looking deeper into clinical applications of the CSM model, one must review the available medical literature on the history of NT testing. The first application of looking at NTs in the urine occurred as early as the 1950s with the research of von Euler in the diagnosis of an adrenal tumor called pheochromocytoma. He and his colleagues looked at urinary levels of epinephrine and norepinephrine principally as diagnostic markers for the tumor³⁷⁻³⁹.

Depression and the CSM model

Since that time, many clinical conditions have been delineated and monitored via the use of urinary NT testing. Much literature has been written in the area of the diagnosis and treatment of depression using urinary NTs. Grossman, et al, conducted a study comparing urinary norepinephrine (NE) and its metabolites in unipolar or bipolar depressed patients with healthy volunteers. Their data suggested that unmedicated unipolar and bipolar depressed patients have a 'hyperresponsive' noradrenergic system (with elevated NE levels and turnover). This data therefore provided a framework which ties together plasma and urinary findings⁴⁰.

Roy, et al, examined urinary catecholamine and metabolite outputs in 28 unipolar depressed patients and 25 normal controls. The total group of depressed patients had significantly higher urinary outputs of norepinephrine (NE) and its metabolite normetanephrine (NM), and significantly lower urinary outputs of the dopamine metabolite dihydroxyphenylacetic acid (DOPAC), than controls. Patients who met DSM-III criteria for a major depressive episode with melancholia (N = 8) had significantly higher urinary outputs of normetanephrine than controls, whereas patients with a major depressive episode without melancholia (N = 7) and dysthymic disorder patients (N = 8) had levels comparable with controls. They postulated that the higher urinary outputs of norepinephrine and its metabolite, normetanephrine, reflected again, this dysregulation of the sympathetic nervous system seen in depression⁴¹. This study also shows the benefit of urinary NT testing in helping clinicians stratify patients into different diagnostic categories which may require different therapeutic approaches.

More exciting research in the area of catecholamines is found with regard to depression and heart disease. Otte, et al, studied urinary output of dopamine, norepinephrine, and epinephrine in patients with heart disease who also had depressive symptoms. They found that depressive symptoms were associated with elevated levels of norepinephrine excretion but not with epinephrine or dopamine⁴². They went on to explain that depression is common in patients with heart disease^{43,44} and is associated with an

increased risk of future cardiac events and mortality⁴⁵⁻⁴⁸. Let us look into why this might be so.

Enhanced activity of the sympathetic nervous system with increased concentrations of catecholamines has been proposed as one possible mechanism by which depressive symptoms may increase morbidity and mortality⁴⁹. This hypothesis is based on evidence suggesting that depressed patients without heart disease have elevated catecholamine (plasma here) levels⁵⁰⁻⁵², as previously noted in the urine. Earlier studies have also found increased sympathetic output in depressed patients with coronary heart disease, including increased heart rate⁵³ and decreased heart rate variability⁵⁴. Thus, altered autonomic tone may contribute to adverse cardiac outcomes in patients with depression⁴².

Otte, et al, went on to establish a plausible mechanism linking depressive symptoms with increased sympathetic activity resulting from enhanced activity of hypothalamic and extrahypothalamic corticotropin-releasing factor (CRF) in depressed patients⁴². It is well known that CRF is increased in healthy patients with depression⁵⁵, which leads to increased cortisol levels⁵⁶⁻⁵⁸. In addition, much research suggests that both hypothalamic and extrahypothalamic CRF activate the locus ceruleus in the brain, leading to an increase in norepinephrine⁵⁹⁻⁶². Thus, high CRF activity might lead to both elevated cortisol and norepinephrine levels⁶³. Then, norepinephrine can enhance forebrain CRF activity, leading to higher activity of both norepinephrine and CRF in depressed patients, possibly closing a feed-forward loop⁶⁴.

This might suggest that depressive symptoms are more closely related to increased sympathetic nervous system activity. This is reflected in increased norepinephrine levels as compared with the adrenomedullary system and reflected levels of epinephrine excretion⁴².

Again, the model proposed by Otte, coincides very nicely with the CSM model and what is seen clinically. It is common to see high levels of norepinephrine (NE) in depressed patients and in patients with insomnia – often in combination with low levels of serotonin and GABA. This could be at least partially due to the fact that many people have adrenal fatigue – which can result from having high levels of cortisol for too long which can lead then to chronically lower output of cortisol. Thus, the adrenomedullary system becomes exhausted and the patients are left “running on NE”. This is also a common pattern in carbohydrate intolerance or Metabolic Syndrome – both incidentally, associated with a higher risk of heart disease. By reestablishing HPA axis health by supporting the adrenals and supporting the inhibitory NTs, we see clinical improvement that coincides with the lab numbers.

An excellent review article by KJ Ressler and CB Nemeroff in 2000 on the role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders cited many studies in which urinary NT testing was conducted and tied the role of the HPA axis to this system as well⁶⁶. Looking at serotonin's role in depression, Linnoila, et al, measured urinary 5-HT and 5-HIAA levels in patients with major depression following a series of ECT treatments. They found that lithium

carbonate as well as the ECT decreased output of 5-HT in these patients⁶⁷. Once again, knowing the clinical status of a patient and their urinary levels of NTs may help guide therapy and help evaluate therapeutic response.

In a study using 150mg 5-HTP daily for seven days, Takahashi and colleagues measured urinary excretion levels and plasma concentrations of three 5-hydroxyindole compounds, 5-HTP, 5-HT and 5-HIAA. Clinical response to 5-HTP treatment appeared to have correlation with the biochemical measures in the depressed patients, that is, non-responders exhibited significantly lower excretion levels of 5-HT and 5-HIAA in urine, and lower plasma levels of 5-HT than did responders⁶⁸. They postulated that this may be an indication that non-responders did not utilize the 5-HTP to the same extent as the responders. As more data is gathered about different groups of patients, there may again be found a utility for urinary NT testing to help delineate those groups and thus improve treatment outcomes in non-responders.

In an article by Turner and Blackwell, an integrative approach to the treatment of IFN-induced depression was proposed. It is well known that IFN can increase serotonin reuptake and decrease serotonin synthesis. Studies have shown that selective serotonin reuptake inhibitors (SSRIs) can effectively treat IFN-induced depression in only 63-75% of cases. For the remaining patients, depression often necessitates dose reduction of or discontinuation of IFN therapy. The authors hypothesized that SSRIs are not fully effective because they affect only serotonin reuptake, not serotonin synthesis, and that effective treatment must address both uptake and synthesis. Citing a number of studies looking at the 5-HTP literature relating to increase of serotonin in CSF, plasma, and urine, they hypothesized that patients who become depressed on IFN will respond better to the synergistic combination of SSRIs plus 5-HTP⁶⁹. In our current pharmaceutical model of treatment for depression, it must be remembered that precursor therapy has been used successfully for many years.

Applying this to the CSM model, improvements are often seen in patients on antidepressants who begin using precursors in addition to the drugs. Many patients who have been on the drugs for years typically have low levels, sometimes very low levels, of serotonin on their urine assays. This occurrence may be because over years of using these re-uptake inhibitor drugs, more NT (e.g. serotonin in this case) is lost simply by passive diffusion. Restoring the levels of substrate upon which the drug will act has indeed been observed to improve clinical symptoms in countless patients using the CSM model. Often then, these patients are able to either get off the drugs they have been taking or at least moderate the dose and decrease side-effects the drugs may be having on them.

Parkinson's Disease and the CSM model

Many who use the CSM model in practice on a daily basis see the inverse relationship of dopamine and serotonin. Someone has low serotonin on their initial test and normal dopamine levels, an intervention is made to raise serotonin, and on retest, the patient's dopamine is low. The converse is true as well when dopamine is low and dopamine support is given. Serotonin levels subsequently go down. This scenario necessitates work for balance of both the excitatory and the inhibitory NTs.

In experimental animals levodopa displaces 5-HT from its storage sites⁷⁰ and both levodopa and DA exert an inhibitory influence on tryptophan hydroxylase, which governs the rate-limiting step in the synthesis of 5-HT⁷¹. This is seen on a larger scale in studies of Parkinson's Disease patients who are taking L-dopa and a dopa decarboxylase inhibitor for their disease. In a study by Siirtola, et al, levodopa caused a sharp rise in urinary dopamine levels, DOPAC, and HVA. Metanephrines did not change, but 5-HIAA was significantly decreased. The authors proposed that significant correlation between 5-HIAA excretion and clinical improvement of tremor during levodopa treatment may suggest that participation of 5-HT in the mechanism of tremor. Correlation analyses showed, moreover, that those patients who excreted less 5-HIAA during treatment showed better overall improvement ($P < 0.05$)⁷².

In a study by Mayeux, et al, CSF levels of 5-HIAA were drawn prior to and after 5-HTP was given orally. 5-HIAA levels increased on repeat tap in those who received the 5-HTP. This correlated with clinical improvement in their depression. It should be noted those with the greatest degree of depression and/or dementia had the lowest levels of 5-HIAA⁷³. Those who use NT support therapies often see tremor improve as well as mood elevation when NTs are balance in these patients. Typically high levels of dopamine in the urine and low levels of serotonin (as above) are seen in the urine. Treating these patients to raise their serotonin is often helpful in both the depression and the tremor. Numbers usually improve with retesting and improved NT balance coinciding with clinical improvements.

Diabetes, Metabolic Syndrome and the CSM model

In Caucasians, obesity expressed as the body mass index (BMI) correlates positively with the plasma insulin concentration and 24-hour urinary norepinephrine output but negatively with epinephrine output^{74,75}. In another study, Chinese subjects with the different manifestations of the metabolic syndrome: hyperinsulinemia and insulin resistance, the presence of elevated norepinephrine, and reduced epinephrine excretion were also closely associated with general and central obesity⁷⁶. Since obesity, particularly the centripetal form seen in Metabolic Syndrome, has been shown in numerous other studies to be associated with increased urinary excretion of norepinephrine and decreased excretion of epinephrine, the possibility that the sympathoadrenal system is involved in the lipid abnormalities associated with the centripetal form of obesity was investigated by Ward, et al⁷⁷. Their data suggested that (1) epinephrine plays an important role in regulating lipid and lipoprotein metabolism in humans, and (2) decreased adrenal medullary activity may contribute to the dyslipidemia (increased triglycerides and decreased HDL-C) commonly observed among the obese. The sympathoadrenal system therefore, along with hyperinsulinemia, may contribute to the increased cardiovascular risk associated with the insulin resistance syndrome. De Pergola, et al⁷⁸, in a very recent study, showed that patients with metabolic syndrome had higher insulin ($P < 0.001$) and FT3 ($P < 0.001$) serum levels and higher 24-h urinary noradrenaline ($P < 0.001$) than subjects without this syndrome and concluded that insulin and NE cooperate independently to the development of the metabolic syndrome.

Again, those who use the CSM model in patients on a daily basis see the above patterns all the time. The typical patient with metabolic syndrome or hyperinsulinemia will show a high NE with usually a low epinephrine – indicating the decreased medullary activity noted above. Looking at the HPA axis, we also see usually low adrenal function in these patients as well. This pattern is thought to be due to years of excursions of the blood sugar with the body using epinephrine and cortisol to sustain euglycemia. The adrenals get worn out and the patient is then “running” on NE, often with concomitant anxiety and/or hypertension. It also sets the patient up for increased inflammatory status. In a study by Szelényi⁷⁹, it was shown that higher levels in norepinephrine (NE) level were associated with increased levels of tumor necrosis factor (TNF)- α and interleukin (IL)-10 response both in the plasma and in the hippocampus of mice. They demonstrated that the TNF- α response was in direct correlation with the biophase level of NE.

There was a recent study by Brunner⁸⁰ who again showed that the neuroendocrine stress axes (HPA axis, sympatho-adrenal medullary and cardiac autonomic activity) are all activated in Metabolic Syndrome. The investigators noted that there is relative cardiac sympathetic (NE and epinephrine) predominance. Further, they stated the neuroendocrine changes may be reversible. This case-control study provided the first evidence that chronic stress may be a cause of Metabolic Syndrome. Those who use the CSM model know that many of these changes can be reversible. As blood sugar is regulated, exercise is instituted, weight loss manifests, and the HPA axis is balanced, there is a return of adrenal function and NT markers to more normal levels.

ADD and the CSM model

There have been numerous studies looking at urinary levels of catecholamines in children with ADD^{81, 82}. A number of studies have gone so far as to look at various diagnostic categories of the disorder by looking at the ratio of monoamines, catecholamines, and their metabolites. One such study by Rogness, et al⁸³, suggested that classifying groups by the biochemical variables may make biochemical-behavioral relationships visible that are obscured in more heterogeneous groups of children with ADD. Also, studying relationships between monoamines and their metabolites among diagnostic groups may provide clues to biochemical-behavioral relationships that are not evident when individual amines or metabolites are examined in isolation.

Another study looking at children with ADD with and without anxiety using 2-hour excretion of NE, epinephrine, and their metabolites concluded that children with ADHD may have a higher tonic activity of the noradrenergic system than controls, while children with comorbid ADHD/Anxiety may be differentiated from those with ADHD alone by higher adrenergic (epinephrine) activity⁸⁴.

Studies have also been done to determine the effect of treatment on urinary catecholamines and indoleamines in hyperactive children⁸⁵. Combining diagnostic capabilities of urinary testing along with helping physicians make better therapeutic choices is clearly where we want to go in the future. This kind of testing has not been conducted using the CSM model, but certainly could be done as better ways are found to diagnose and to choose appropriate therapies.

A recent study by Dvorakova, et al⁸⁶, illustrates this point. The study was performed in a randomized, double-blind, placebo-controlled design. Concentrations of catecholamines were higher in urine of ADHD patients compared to those of healthy children. Moreover, NE concentrations positively correlated with degree of hyperactivity of ADHD children. In ADHD patients, epinephrine and NE concentrations positively correlated with plasma levels of oxidized glutathione. The treatment of ADHD children with Pycnogenol (pine bark extract) caused decrease of dopamine (D) and a trend of epinephrine and NE to decrease with an increased GSH/GSSG ratio. In conclusion, the data provided further evidence for the over activity of the noradrenergic system in ADHD and demonstrated that epinephrine release may be increased, as well. Treatment of ADHD children with Pycnogenol normalized catecholamine concentrations, leading to less hyperactivity, and, consequently, to reduced oxidative stress. The study has been repeated twice by the research group⁸⁷.

PTSD and the CSM model

There is a fair amount of research examining biological correlates of posttraumatic stress disorder (PTSD) in patients that has suggested those with chronic PTSD have substantially different levels of catecholamines and cortisol compared to similarly traumatized patients who do not meet diagnostic criteria for PTSD^{88, 89}. In one study by Yehuda, et al⁹⁰, urinary catecholamines (dopamine, NE, and epinephrine) were measured in 22 Vietnam veterans looking at correlations with severity of symptoms in PTSD. They found that dopamine and NE, but not epinephrine were found to correlate with severity of symptoms. Mason, et al⁹¹, found that using the urinary NE/cortisol ratio, they could distinguish PTSD from all other patient groups (major depression, bipolar disease, and paranoid schizophrenia). The NE/cortisol ratio had a diagnostic sensitivity of 78% and a specificity of 94% for correct classification of PTSD in their study sample.

A study by Delahanty, et al, extended these findings by examining whether urinary hormone levels collected soon after a trauma were related to subsequent acute PTSD symptoms in child trauma victims. The researchers found that after removing the variance associated with demographic variables and depressive symptoms, urinary cortisol and epinephrine levels continued to predict a significant percentage (7–10%) of the variance in 6-week PTSD symptoms. Examination of boys and girls separately suggested that significance was primarily driven by the strength of the relationships between hormone levels and acute PTSD symptoms in boys⁹². These data suggest that urinary NT/HPA testing can indeed allow physicians to predict diagnostic outcomes and add diagnostic precision. Certainly, these can be important biomarkers of disease and improve our clinical tools.

Autism and the CSM model

There has been a significant amount of research in the area of autism with the use of urinary NT testing^{93, 94}. Barthelemy, et al, in France, have conducted most of the work in this area. They have found that significant differences in the urinary excretion of both DA and NE and their respective metabolites in autistic children compared to normal children. Autistic children showed low DA, high HVA (homovanillic acid), high NE, low MHPG (a metabolite of NE) urinary levels. They noted that these results were

consistent with previous findings on altered catecholamine metabolism in autistic children. They also suggested that autistic behaviors might be related to an abnormal functional imbalance among monoamines either at a molecular level or at a system level. Furthermore, they emphasized the special interest of urinary assays in pediatric research⁹⁵.

The same research group went on to show that very significant group and age effects were found for DA, HVA, 3MT, NE + E and 5HT that were determined from the urine of 156 autistic children aged two to 12 years 6 months, and compared with those of age-matched mentally retarded non-autistic and normal controls. High HVA, 3MT, NE + E and 5HT levels were found in autistic and non-autistic children. The DA, HVA, 3MT, NE + E, 5HT and 5HIAA levels decreased significantly with age in the three groups⁹⁶.

Sanesco has conducted its own reference range studies and found the same to be true. As children age, typically monoamines will decrease. The researchers went on to show that significantly decreased levels of DA and HVA were observed in autistic children on haloperidol, compared with non-medicated autistic children. This shows that with treatment, in this case pharmaceutical treatment, decreased levels of NTs can be seen and clinical pictures can be improved.

Clearly there is more involved with the autistic spectrum than rebalancing of NTs. However, while practitioners are dealing with the toxic, mineral, gut, and other imbalances that are almost always present in these children, balancing NTs can bring about more calm to patients and their families. On the other end of the curve, those practitioners using the CSM model have certainly observed the same situation in Alzheimer's Disease and other organic brain syndromes where mood and sleep, in particular, are often disturbed.

Hormonal Changes and the CSM

Many who treat menopausal women and even women with PMS know that it is not just about estrogen levels or fluctuations in the estrogen levels. In an era of limited use of estrogens, many of us have become aware of the research done with SSRIs and SNRIs with regard to using them as alternatives to estrogen therapies in the treatment of hot flashes^{97, 98, 99}. Obviously, it is known that these substances are not acting on the sex hormones levels, but on the neurohormone levels.

A very recent study by Haliloglu¹⁰⁰ looked at premenopausal (PM), naturally menopausal (NM) and surgically induced menopausal (SM) women in order to investigate the differences in serum cortisol, dehydroepiandrosterone sulfate (DHEA-S), follicle stimulating hormone (FSH) and estradiol (E2) levels on serum serotonin levels. They found increased serotonin levels in naturally menopausal women and postulated that this may be a compensatory mechanism resulting from decreased E2 levels. It is thought that there is strong interaction between E2 and the serotonergic system.

Again, this relationship is often seen in NT testing and therapy. Estrogen is known to be a powerful, natural SSRI in its own right¹⁰¹. When balancing NTs in women with hot flashes, it is very common to have this be the only therapy necessary. In other words,

many women do well with NT therapies alone and do not have to be placed on any kind of hormone therapy at all. This not only cuts the number of medications a woman needs, but most importantly, it may decrease her risk of cardiovascular diseases and hormonal cancers.

PMS (premenstrual syndrome) and PMDD (premenstrual dysphoric disorder) have also been the subject of much research in relation to NT balance. Most researchers have specifically looked at the serotonin pathways using pharmaceutical therapies. Therefore, there are inferences that can be made and principles that can be applied regarding the CSM model in these conditions.

The principle features of premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD) are the predictable, cyclic nature of symptoms that often begin in the late luteal phase of the menstrual cycle and usually remit shortly after the onset of menstruation. PMDD can be distinguished from PMS by the severity of symptoms and the predominance of mood symptoms particularly regarding the areas of personal relationships and the marital/family dynamic. Among the various treatment modalities available for these conditions, selective serotonin reuptake inhibitors (SSRIs) have emerged as first-line therapy. Several strategies have evolved in the literature. SSRIs can be administered continuously throughout the entire month, intermittently from ovulation to the onset of menstruation, or semi-intermittently with dosage increases during the late luteal phase¹⁰².

The role of SSRIs in the treatment of PMS/PMDD is quite well-established. Leading one to presume that a major part of these syndromes is played by disordered metabolism and/or signaling of serotonin pathways. A 2002 Cochrane review of 31 randomized controlled trials showed a reduction in overall symptomatology and included data on 844 women with premenstrual syndrome. They concluded that SSRIs were highly effective in treating premenstrual symptoms. Secondary analysis showed that they were effective in treating physical as well as behavioral symptoms¹⁰³. A more recent 2008 meta-analysis in Obstetrics and Gynecology by Shah, et al, which included 2,964 women, demonstrated that SSRIs were again effective in treating PMS and premenstrual dysphoric disorder (OR 0.40, 95% confidence interval [CI] 0.31-0.51). Intermittent dosing regimens were found to be less effective (OR 0.55, 95% CI 0.45-0.68) than continuous dosing regimens (OR 0.28, 95% CI 0.18-0.42). In addition, no SSRI was demonstrably better than another¹⁰⁴.

Using the CSM model, many physicians have seen profound improvements in mood and behavior in patients with PMS/PMDD. This usually involves starting with a patient with low serotonin levels (many of whom have been on SSRIs for their prior treatment) and often elevated NE levels as well. As serotonin levels and other inhibitory NTs are improved, we typically see NE levels decrease as the system is brought into balance. Not infrequently, patients with severe symptoms must remain on some level of medication to help manage their symptoms, but almost universally, they are managed with less medication and the medication they use is much more effective. This is much the same scenario that has been alluded to with depression, anxiety, and sleep disorders.

Migraines and the CSM model

There is considerable overlap between migraine headache (particularly menstrual migraine) and PMS/PMDD. Menstruation is a potent, predictable trigger in many migraineurs¹⁰⁵. Migraine headaches are more likely to occur in association with menses than at other times in the cycle¹⁰⁶⁻¹⁰⁸; and 40%–70% of those who suffer from migraines report a correlation between their menses and their migraine attacks.¹⁰⁹⁻¹¹²

Clearly, the pathophysiology of dysmenorrhea and/or PMS syndromes is quite complex, as is that of migraine headaches. But they do have some commonalities. Namely, there is a heightened perception of pain. A number of studies, including one by MacGregor, et al¹¹³ showed that in a group of 38 migraine sufferers that the incidence of migraines was higher during late luteal phase and early follicular phase. So, migraine prevalence and intensity have been shown to be associated with the decline of estrogen during the menstrual cycle. Thus, migraines seem to be triggered by the withdrawal of estrogen after levels of estrogen were higher earlier in the period¹¹⁴. We have seen above that withdrawal of estrogen in menopausal states is associated with a concomitant drop in serotonin. Remember that the serotonin pathways are usually regarded as one of the major components of pain mediation. Serotonin determines “pain” behavior and influences the perception of pain. It also produces vasoconstriction in artery walls. Decreases in serotonin can be associated with relaxation of vessels and is associated with excessive pulsation of the vessels^{115,116}. In addition, research by Deanovic, et al¹¹⁷, has shown that in a group of 14 migraineurs where 5HT and 5-HIAA were followed in the urine, that over the course of headache, a significant increase in 5-HIAA excretion rate was found. At the same time, the excretion of 5-HT was not significantly altered. After the migraine attack, a very pronounced lowering in excretion rate of both 5-hydroxy-indoles occurred. These findings led the researchers to support to the theory of an abrupt fall in total plasma 5-HT as a trigger mechanism for the painful phase of migraine.

Taken together, it might make sense that it is the drop in estrogen with the concomitant drop in serotonin that could be an underlying trigger for menstrual migraines. Those using the CSM model routinely see improvement in migraine headaches as they endeavor to balance the HPA axis and improve NT balance.

Discussion and Conclusions

At the outset of this article, we explored the depth and breadth of the extensive science and technology that has been used to develop neurotransmitter testing at Sanesco. The question was answered: Do we have a good test? These same and related technologies and procedures have been and continue to be used at major universities and research facilities throughout the world. This is evidenced by nearly 300 articles in leading medical and research publications whose researchers use these technologies in their work. The testing is sound in its reliability, reproducibility, and accuracy.

The second question, which is perhaps more difficult to answer than the first, was one of clinical application and validity of our CSM (Communication System Management) model as it relates to that testing. Urinary NT testing has been used in many different

clinical models for both diagnosis and management of a variety of clinical conditions for many, many years. There is precious little data in the medical literature concerning the CSM model in its current stage of evolution and its use in day-to-day clinic practice.

The CSM model looks at the patient as an integrated whole – how one hormone system relates to another and how NTs affect and influence those hormone systems while helping patients achieve a healthier lifestyle through nutrition, exercise, and mind-body connections. As many practitioners know, it is an extremely effective model and helps patients achieve balance in their health and lives. Regardless of this fact, the supporting medical research simply is not there as of this writing.

This is a problem not with the CSM model, but with many of our research models – many of which are based on a double-blind, placebo-controlled studies (DBPC). While the DBPC study is an effective vehicle for determining if using drug A produces outcome B in patient C, it has limitations when one is trying to determine the effect of balancing hormones and neurotransmitters across several neuroendocrine systems while at the same time effecting lifestyle change in a complex patient who may be on numerous medications. One can not have a “cafeteria style” research model where one hormone or NT is manipulated to see an outcome. The DBPC study is not an efficacious model for integrative medicine. Despite tens of thousands of patient data points and thousands of practitioners using urinary NT testing daily, there is little clinical data in the literature available to defend our CSM model at present. The purpose of this article is to share with the reader the data that are available and make inferences as they relate to the CSM model. The studies cited provide a compelling backdrop for these discussions.

At the outset of this work, it was mentioned that the neurohormonal testing done, is not to be taken as a diagnostic tool per se. Rather the testing is a valuable clinical tool the clinician has to measure certain biomarkers that relate to a variety of clinical conditions. These include but are not limited to: depression, anxiety, sleep disorders and insomnia, fatigue, pain syndromes, those with cravings, chronic neurological conditions, ADD and ADHD, menopause, andropause, PMS, and metabolic syndrome, diabetes, and possibly heart disease. These measurements of hormones and NTs are biomarkers and are used as indicators of what is going on in the neuroendocrine systems of the patient. They give us information to share with the patient, which is essential to their understanding of why they feel the way they do and gives the clinician a way to be more targeted in their treatment choices. Thus, the testing is valid, clinically useful, and easy to incorporate into a clinician’s busy practice. Also, the test kits are easy to use at home by the patient and are a cost-effective and safe means to obtain the required data.

As time moves on, more practitioners will be incorporating this technology into their practices for their patients. As this occurs, more research will be done to continue to confirm the validity of the CSM model. New biomarkers will be developed and new ways of interpreting the data will evolve as the number of clinical applications increase.

References

1. http://www.bio-rad.com/B2B/BioRad/br_community_home.jsp?BV_SessionID=@@@@1494402128.1238591281@@@@&BV_EngineID=cccfadeglikjhgcfnegcfkmdhkkdfm.0&loggedIn=false&country=HQ&lang=English&divName=Life+Science+Research
2. <http://www.ukneqas.org.uk/content/Pageserver.asp>
3. <http://www.instandev.de/en/about-instand-ev/>
4. Garlow S, Musselman D, Nemeroff C. The neurochemistry of mood disorders: clinical studies. In: Charney D, Nestler E, Bunney B, eds. *The Neurobiological Foundation of Mental Illness*. New York: Oxford University Press; 1999.
5. Growdon JH. Neurotransmitter precursors in the diet: their use in the treatment of brain diseases. In: Wurtman RJ, Wurtman JJ, eds. *Nutrition and the Brain Series*. Vol. 3. Berkeley, CA; Raven Press: 1979.
6. Meyers, S. Use of Neurotransmitter Precursors for Treatment of Depression. *Altern Med Rev* 2000;5(1):64-71.
7. Birdsall, T. C. 5-Hydroxytryptophan: a clinically-effective serotonin precursor. *Altern Med Rev* 1998;3, 271-280.
8. Young SN, Gauthier AM. Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 1981;44:323-327.
9. Turner EH, Loftis JM, Blackwell AD. Serotonin a la carte: Supplementation with the serotonin precursor 5-hydroxytryptophan. *Pharmacology & Therapeutics* 109 (2006) 325 – 338.
10. Arias-Carrión O, Poppel E. Dopamine, learning, and reward-seeking behavior. *Acta Neurobiol Exp (Wars)*. 2007;67(4):481-8.
11. Young SN. Behavioral effects of dietary neurotransmitter precursors: basic and clinical aspects. *Neurosci Biobehav Rev* 1996;20:313-323.
12. Gelenberg AJ, Wojcik JD, Growdon JH, et al. Tyrosine for the treatment of depression. *Am J Psychiatry* 1980;137:622-623.
13. Goldberg IK. L-tyrosine in depression. *Lancet* 1980;2:364-365.
14. Gelenberg AJ, Wojcik JD, Falk WE, et al. Tyrosine for depression: a double-blind trial. *J Affect Disord* 1990;19:125-132.
15. Beckmann H, Athen D, Olteanu M, Zimmer R. DL-phenylalanine versus imipramine: a double-blind controlled study. *Arch Psychiatr Nervenkr* 1979;227:49-58.
16. Mann J, Peselow ED, Snyderman S, Gershon S. D-phenylalanine in endogenous depression. *Am J Psychiatry* 1980;137:1611-1612.
17. Sabelli HC, Fawcett J, Gusovsky F, et al. Clinical studies on the phenylethylamine hypothesis of affective disorder. *J Clin Psychiatry* 1986;47:66-70.
18. van Praag HM. In search of the mode of action of antidepressants: 5-HTP/tyrosine mixtures in depression. *Adv Biochem Psychopharmacol*;1984;39:301-314.
19. Hawkins BT, Egleton RD. Pathophysiology of the blood-brain barrier: animal models and methods. *Curr Top Dev Biol*. 2008;80:277-309.
20. Wakayama, K, Ohtsuki, S, Takanaga H, Hosoya, K, and Terasaki, T. Localization of norepinephrine and serotonin transporter in mouse brain capillary endothelial cells, *Neurosci. Res.* 2002;44:173-180.
21. Kakee, A., Takanaga, H., Terasaki T., Naito, M., Tsuruo, T., Sugiyama, Y. Efflux of a suppressive neurotransmitter, GABA, across the blood-brain barrier. *J. Neurochem.* 2001;79: 110-118.
22. Takanaga, H., Ohtsuki, S., Hosoya, K., Terasaki, T. GAT2/BGT-1 as a system responsible for the transport of g-aminobutyric acid at the mouse blood-brain barrier. *J. Cereb. Blood Flow. Metab.* 2001;21:1232-1239.
23. Hosoya, K., Sugawara, M., Asaba, H., Terasaki, T. Blood-brain barrier produces significant efflux of L-aspartic acid but not D-aspartic acid: in vivo evidence using the brain efflux index method. *J. Neurochem.* 1999; 73:1206-1211.
24. Ohtsuki, S. New aspects of the blood-brain barrier transporters; its physiological roles in the central nervous system. *Biol. Pharm.* 2004;27(10):1489-1496.
25. Hardebo, J. E. and Owman, C. Characterization of the in vitro uptake of monoamines into brain microvessels. *Acta Physiol Scand.* 1980;108(3): 223-229.

26. Huszti, Z., Deli, M. A., and Joo, F. Carrier-mediated uptake and release of histamine by cultured rat cerebral endothelial cells. *Neurosci.Lett.* 1995;184(3):185-188.
27. R.D. Egleton and T.P. Davis, Development of neuropeptide drugs that cross the blood-brain barrier, *NeuroRx* 2 (2005), pp. 44–53.
28. Lynn-Bullock CP, Welshhans K, Pallas SL, Katz PS. The effect of oral 5-HTP administration on 5-HTP and 5-HT immunoreactivity in monoaminergic brain regions of rats. *J Chem Neuroanat* 2004 May;27(2):129-38.
29. Hayer-Zillgen M.,Bruss, M., and Bonisch, H. Expression and pharmacological profile of the human organic cation transporters hOCT1, hOCT2 and hOCT3. *Br.J.Pharmacol.* 2002;136(6): 829-836.
30. Chen, N. H., Reith, M. E., and Quick, M. W. Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. *Pflugers Arch.*2004;447(5): 519-531.
31. Kopp, U., Bradley, T., and Hjemdahl, P. Renal venous outflow and urinary excretion of norepinephrine, epinephrine, and dopamine during graded renal nerve stimulation. *Am.J.Physiol.* 1983;244(1):E52-E60.
32. Morgunov N, Baines AD. Renal nerves and catecholamine excretion. *Am J Physiol.* 1981;240(1):F75-81.
33. Olsson T, Viitanen M, et al. Catecholamine excretion in old age. *Aging (Milano).* 1991;(3):263-8.
34. Cucho JL, Kuchel O, et al. Sex differences in urinary catecholamines. *Endocr Res Commun.* 1975;2(8):549-59.
35. Tidgren B, Hjemdahl P. Renal response to mental stress and epinephrine in humans. *Am J Physiol.* 1989 Oct;257(4 Pt 2):F682-9.
36. Fujimura S, Shimakage H, et al. Effects of GABA on noradrenaline release and vasoconstriction induced by renal nerve stimulation in isolated perfused rat kidney. *Br J Pharmacol.* 1999;127(1):109-14.
37. von Euler, US, and Luft, R. Noradrenaline output in urine after infusion in man. *Br.J.Pharmacol.Chemother.* 1951;6(2): 286-288.
38. von Euler, US, and Hellner, S. Excretion of noradrenaline, adrenaline, and hydroxytyramine in urine. *Acta Physiol Scand.* 1951;22(2-3): 160-167.
39. von Euler, US. [Adrenalin and noradrenalin in the urine of normal subjects and patients with pheochromocytoma.] *Med. Welt.* 1951;20(13): 406-407.
40. Grossman F, Potter WZ. Catecholamines in depression: a cumulative study of urinary norepinephrine and its major metabolites in unipolar and bipolar depressed patients versus healthy volunteers at the NIMH. *Psychiatry Res.* 1999 Jul 30;87(1):21-7.
41. Roy A, Pickar D, Douillet P, Karoum F, Linnoila M. Urinary monoamines and monoamine metabolites in subtypes of unipolar depressive disorder and normal controls. *Psychol Med.* 1986;Aug;16(3):541-6.
42. Otte C, Neylan TC, Pipkin S, Browner WS, Whooley MA. Depressive symptoms and 24-hour urinary norepinephrine excretion levels in patients with coronary disease: findings from the Heart and Soul Study. *Am J Psychiatry.* 2005 Nov;162(11):2139-45.
43. Rudisch B, Nemeroff CB: Epidemiology of co-morbid coronary artery disease and depression. *Biol Psychiatry* 2003; 54:227–240.
44. Blumenthal JA, Lett HS, Babyak MA, White W, Smith PK, Mark DB, Jones R, Mathew JP, and Newman MF: Depression as a risk factor for mortality after coronary artery bypass surgery. *Lancet* 2003; 362:604–609.
45. Lesperance F, Frasere-Smith N, Talajic M, Bourassa MG: Five-year risk of cardiac mortality in relation to initial severity and one-year changes in depression symptoms after myocardial infarction. *Circulation* 2002; 105:1049–1053 <http://ajp.psychiatryonline.org.libproxy.lib.unc.edu/cgi/jlink?linkType=ABST&journalCode=circulation&resid=105/9/1049>.
46. Frasere-Smith N, Lesperance F, Talajic M: Depression following myocardial infarction: impact on 6-month survival. *JAMA* 1993; 270:1819–1825 <http://ajp.psychiatryonline.org.libproxy.lib.unc.edu/cgi/jlink?linkType=ABST&journalCode=jama&resid=270/15/1819>.
47. Carney RM, Freedland KE: Depression, mortality, and medical morbidity in patients with coronary heart disease. *Biol Psychiatry* 2003; 54:241–247.
48. Musselman DL, Evans DL, Nemeroff CB: The relationship of depression to cardiovascular disease: epidemiology, biology, and treatment. *Arch Gen Psychiatry* 1998; 55:580–592.

49. Chrousos GP, Gold PW: A healthy body in a healthy mind—and vice versa—the damaging power of "uncontrollable" stress. *J Clin Endocrinol Metab* 1998; 83:1842–1845.
50. Gold PW, Chrousos GP: Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs. low CRH/NE states. *Mol Psychiatry* 2002; 7:254–275.
51. Wong ML, Kling MA, Munson PJ, et al. Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotrophin-releasing hormone. *Proc Natl Acad Sci USA* 2000; 97:325–330.
52. Veith RC, Lewis N, Linares OA, et al: Sympathetic nervous system activity in major depression: basal and desipramine-induced alterations in plasma norepinephrine kinetics. *Arch Gen Psychiatry* 1994; 51:411–422.
53. Carney RM, Freedland KE, Veith RC, Cryer PE, Skala JA, Lynch T, Jaffe AS: Major depression, heart rate, and plasma norepinephrine in patients with coronary heart disease. *Biol Psychiatry* 1999; 45:458–463.
54. Carney RM, Blumenthal JA, Stein PK, Watkins L, Catellier D, Berkman LF, Czajkowski SM, O'Connor C, Stone PH, Freedland KE: Depression, heart rate variability, and acute myocardial infarction. *Circulation* 2001; 104:2024–2028.
55. Holsboer F: The rationale for corticotrophin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *J Psychiatr Res* 1999; 33:181–214.
56. Deuschle M, Schweiger U, Weber B, Gotthardt U, Korner A, Schmider J, Standhardt H, Lammers CH, Heuser I: Diurnal activity and pulsatility of the hypothalamus-pituitary-adrenal system in male depressed patients and healthy controls. *J Clin Endocrinol Metab* 1997; 82:234–238.
57. Rothschild A: The hypothalamic-pituitary-adrenal axis and psychiatric illness, in Psychoneuroendocrinology. Edited by Wolkowitz O, Rothschild A. Washington, DC, *American Psychiatric Publishing*, 2003, pp 139–163
58. Wolkowitz OM, Epel ES, Reus VI: Stress hormone-related psychopathology: pathophysiological and treatment implications. *World J Biol Psychiatry* 2001; 2:115–143.
59. Arlt J, Jahn H, Kellner M, Strohle A, Yassouridis A, Wiedemann K: Modulation of sympathetic activity by corticotropin-releasing hormone and atrial natriuretic peptide. *Neuropeptides* 2003; 37:362–368.
60. Koob GF: Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry* 1999; 46:1167–1180.
61. Valentino RJ, Page M, Van Bockstaele E, Aston-Jones G: Corticotropin-releasing factor innervations of the locus coeruleus region: distribution of fibers and sources of input. *Neuroscience* 1992; 48:689–705.
62. Melia KR, Duman RS: Involvement of corticotropin-releasing factor in chronic stress regulation of the brain noradrenergic system. *Proc Natl Acad Sci USA* 1991; 88:8382–8386.
63. Roy A, Pickar D, De Jong J, Karoum F, Linnoila M: Norepinephrine and its metabolites in cerebrospinal fluid, plasma, and urine: relationship to hypothalamic-pituitary-adrenal axis function in depression. *Arch Gen Psychiatry* 1988; 45:849–857.
64. Koob GF: Corticotropin-releasing factor, norepinephrine, and stress. *Biol Psychiatry* 1999; 46:1167–1180.
65. Ruo B, Rumsfeld JS, Hlatky MA, Liu H, Browner WS, Whooley MA: Depressive symptoms and health-related quality of life: the Heart and Soul Study. *JAMA* 2003; 290:215–221. <http://ajp.psychiatryonline.org.libproxy.lib.unc.edu/cgi/ijlink?linkType=ABST&journalCode=jama&sid=290/2/215>
66. Ressler KJ, Nemeroff CB: Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depress Anxiety*. 2000;12 Suppl 1:2-19.
67. Linnoila M, Miller TL, Bartko J, Potter WZ. Five antidepressant treatments in depressed patients. Effects on urinary serotonin and 5-hydroxyindoleacetic acid output. *Arch Gen Psychiatry*. 1984 Jul;41(7):688-92.
68. Takahashi S, Takahashi R, Masumura I, Miike A. Measurement of 5-hydroxyindole compounds during L-5-HTP treatment in depressed patients. *Folia Psychiatr Neurol Jpn*. 1976;30(4):463-73. [Abstract]
69. Turner EH, Blackwell AD. 5-Hydroxytryptophan plus SSRIs for interferon-induced depression: synergistic mechanisms for normalizing synaptic serotonin. *Med Hypotheses*. 2005;65(1):138-44.
70. Ng KY, Chase TN, Colburn RW, Kopin IJ, Dopamine: Stimulation-induced release from central neurons, *Science*. 1971;172:487.
71. van Woert MH, Bowers MB, The effect of L-Dopa on monoamine metabolites in Parkinson's disease, *Experienti*. 1970;26:161.

72. T. Siirtola, V. Sonninen and U.K. Rinne. Urinary excretion of monoamines and their metabolites in patients with Parkinson's disease: Response to long-term treatment with levodopa alone or in combination with a dopa decarboxylase inhibitor and clinical correlations. *Clin Neurol Neurosurg.* 1975;78(2):77-88.
73. Mayeux R, Stern Y, Sano M, Williams JB, Cote LJ. The relationship of serotonin to depression in Parkinson's disease. *Mov Disord.* 1988;3(3):237-44.
74. Troisi RJ, Weiss ST, Parker DR, et al: Relation of obesity and diet to sympathetic nervous system activity. *Hypertension* 17:669-677,1991
75. Ward KD, Sparrow D, Landsberg L, et al: The relationship of epinephrine excretion to serum lipid levels: The Normative Aging Study. *Metabolism* 43:509-513, 1994
76. Lee, Z.S.K. , Critchley, J.A.J.H., Tomlinson, B., et al, Urinary Epinephrine and Norepinephrine Interrelations With Obesity, Insulin, and the Metabolic Syndrome in Hong Kong Chinese. *Metabolism*, 2001;Vol 50(2) (February):135-143.
77. Ward KD, Sparrow D, Landsberg L, The Relationship of Epinephrine Excretion to Serum Lipid Levels: The Normative Aging Study. *Metabolism*, 1994;43(4):509-513.
78. De Pergola G, Giorgino F, Benigno R. Independent Influence of Insulin, Catecholamines, and Thyroid Hormones on Metabolic Syndrome. *Obesity* (2008) 16, 2405–2411.
79. Szelényi J, Vizi ES. The catecholamine cytokine balance: interaction between the brain and the immune system. *Ann N Y Acad Sci.* 2007 Oct;1113:311-24
80. Brunner EJ, Hemingway H, Walker BR, Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation.* 2002 Nov 19;106(21):2659-65.
81. Rapoport JL, Mikkelsen EJ, Ebert MH, Urinary catecholamines and amphetamine excretion in hyperactive and normal boys. *J Nerv Ment Dis.* 1978 Oct;166(10):731-7.
82. Hanna GL, Ornitz EM, Hariharan M. Urinary catecholamine excretion and behavioral differences in ADHD and normal boys. *J Child Adolesc Psychopharmacol.* 1996 Spring;6(1):63-73.
83. Rogeness GA, Maas JW, et al. Attention deficit disorder symptoms and urine catecholamines. *Psychiatry Res.* 1989 Mar;27(3):241-51.
84. Pliszka SR, Maas JW, et al. Urinary catecholamines in attention-deficit hyperactivity disorder with and without co-morbid anxiety. *J Am Acad Child Adolesc Psychiatry.* 1994 Oct;33(8):1165-73.
85. Zametkin AJ, Karoum F, Linnoila M, et al. Stimulants, urinary catecholamines, and indoleamines in hyperactivity. A comparison of methylphenidate and dextroamphetamine. *Arch Gen Psychiatry.* 1985 Mar;42(3):251-5.
86. Dvoráková M, Jezová D, Blazíček P, et al, Urinary catecholamines in children with attention deficit hyperactivity disorder (ADHD): modulation by a polyphenolic extract from pine bark (pycnogenol). *Nutr Neurosci.* 2007 Jun-Aug;10(3-4):151-7.
87. Dvoráková M, Sivonová M, Trebatická, et al. The effect of polyphenolic extract from pine bark, Pycnogenol on the level of glutathione in children suffering from attention deficit hyperactivity disorder (ADHD). *Redox Rep.* 2006;11(4):163-72.
88. Yehuda, R. Post-traumatic stress disorder. *The New England Journal of Medicine.* 2002;346, 108–114.
89. Lemieux AM, Coe CL, Abuse related posttraumatic stress disorder: evidence for chronic neuroendocrine activation in women. *Psychosomatic Medicine* 1995;5,105–115.
90. Yehuda, R, Southwick S, Giller EL, Ma X, Mason JW, Urinary catecholamine excretion and severity of PTSD symptoms in Vietnam combat veterans. *Journal of Nervous Mental Disorders* .1992:180,321–325.
91. Mason JW, Giller EL, Kosten TR, Harkness L. Elevation of urinary norepinephrine/cortisol ratio in posttraumatic stress disorder. *J Nerv Ment Dis.* 1988:176(8):498-502.
92. Delahanty DL, Nugent NR, Initial urinary epinephrine and cortisol levels predict acute PTSD symptoms in child trauma victims. *Psychoneuroendocrinology.* 2005;30, 121–128.
93. Ferrari P, Bursztejn C, Dreux C, Braconnier A, Zarifian E, Lancrenon S, Fermanian J. Disorders of catecholamine metabolism in infantile autism. Comparative study of 22 autistic children. *Encephale.* 1989:Mar-Apr;15(2):255-62.
94. Launay JM, Bursztejn C, et al. Catecholamines metabolism in infantile autism: a controlled study of 22 autistic children. *J Autism Dev Disord.* 1987 Sep;17(3):333-47.
95. Barthelemy C, Bruneau N, Cottet-Eymard JM, et al. Urinary free and conjugated catecholamines and metabolites in autistic children. *J Autism Dev Disord.* 1988 Dec;18(4):583-91.

96. Martineau J, Barthélémy C, Jouve , Muh JP, Lelord G. Monoamines (serotonin and catecholamines) and their derivatives in infantile autism: age-related changes and drug effects. *Dev Med Child Neurol*. 1992;Jul;34(7):593-603.
97. Soares CN, Joffe H, Viguera AC. Paroxetine versus placebo for women in midlife after hormone therapy discontinuation. *Am J Med*. 2008 Feb;121(2):159-162.e1.
98. Stearns V, Beebe KL, Iyengar M, Dube E. Paroxetine controlled release in the treatment of menopausal hot flashes: a randomized controlled trial. *JAMA*. 2003 Jun 4;289(21):2827-34.
99. Speroff L, Gass M, Constantine G, Olivier S. Efficacy and tolerability of desvenlafaxine succinate treatment for menopausal vasomotor symptoms: a randomized controlled trial. *Obstet Gynecol*. 2008 Jan;111(1):77-87.
100. Haliloglu B, Benli Aksungar F, Ilter E, Temelli Akin F. Serotonin dilemma in postmenopausal women: is it low or high? *Maturitas*. 2008 Jun 20;60(2):148-52.
101. Koldzic-Zivanovic N, Seitz PK, Watson CS, Cunningham KA, Thomas ML. Intracellular signaling involved in estrogen regulation of serotonin reuptake. *Mol Cell Endocrinol* 2004 Oct 29;226(1-2):33-42.
102. Steiner M, Pearlstein T, Cohen LS, Endicott J, et al. Expert guidelines for the treatment of severe PMS, PMDD, and comorbidities: the role of SSRIs. *J Womens Health (Larchmt)*. 2006 Jan-Feb;15(1):57-69.
103. Wyatt KM, Dimmock PW, O'Brien PM. Selective serotonin reuptake inhibitors for premenstrual syndrome. *Cochrane Database Syst Rev*. 2002;(4):CD001396.
104. Shah NR, Jones JB, Aperi J, Shemtov R, Karne A, Borenstein J. Selective serotonin reuptake inhibitors for premenstrual syndrome and premenstrual dysphoric disorder: a meta-analysis. *Obstet Gynecol*. 2008 May;111(5):1175-82.
105. Mannix LK. *Journal of Women's Health*. June 1, 2008, 17(5): 879-891.
106. MacGregor EA. Oestrogen and attacks of migraine with and without aura. *Lancet Neurol* 2004;3:354.
107. MacGregor EA, Frith A, Ellis J, Aspinall L, Hackshaw A. Incidence of migraine relative to menstrual cycle phases of rising and falling estrogen. *Neurology* 2006;67:2154.
108. Stewart WF, Lipton RB, Chee E, Sawyer J, Silberstein SD. Menstrual cycle and headache in a population sample of migraineurs. *Neurology*. 2000;55:1517.
109. Dzoljic E, Sipetic S, Vlajinac H, et al. Prevalence of menstrually related migraine and nonmigraine primary headache in female students of Belgrade University. *Headache*. 2002;42:185.
110. Cupini LM, Matteis M, Troisi E, Calabresi P, Bernardi G, Silvestrini M. Sex-hormone-related events in migrainous females. A clinical comparative study between migraine with aura and migraine without aura. *Cephalalgia*.1995;15:140.
111. Granella F, Sances G, Zanferrari C, Costa A, Martignoni E, Manzoni GC. Migraine without aura and reproductive life events: A clinical epidemiological study in 1300 women. *Headache* 1993;33:385.
112. MacGregor EA, Chia H, Vohrah RC, Wilkinson M. Migraine and menstruation: A pilot study. *Cephalalgia*. 1990;10:305.
113. MacGregor EA, Frith A, Ellis J, Aspinall L, Hackshaw A. Incidence of migraine relative to menstrual cycle phases of rising and falling estrogen. *Neurology*. 2006;67:2154.
114. Somerville BW. Estrogen-withdrawal migraine. I. Duration of exposure required and attempted prophylaxis by premenstrual estrogen administration. *Neurology* 1975;25:239.
115. G. R. Martin, "Vascular receptors for 5-hydroxytryptamine: distribution, function, and classification," *Pharmacol. Ther.*, 1994;**62**, 283.
116. K. F. Izzati-Zade. The Role of Serotonin in the Pathogenesis and Clinical Presentations of Migraine Attacks. *Neuroscience and Behavioral Physiology*. 2008;38(5):501-505.
117. Deanovi Z, Iskri S, Dupelj M. Fluctuation of 5-hydroxy-indole compounds in the urine of migrainous patients. *Biomedicine*. 1975;23(9):346-9.



Sanesco International
1010 Merrimon Avenue
Asheville, NC 28804

For more information: 866-670-5705
info@Sanesco.net
www.Sanesco.net