

Substance M

An isolate from meditator urine

"SUBSTANCE M" A RESEARCH REPORT

Purpose of the Work

To develop a new drug with which to treat depression.

Introduction

A substance has been isolated from the urine of people who practice Transcendental Meditation (TM) that inhibits imipramine binding on blood platelets. It also correlates directly with feelings of vigor and is inversely correlated with fatigue and age.

History

As far back as 1976¹ it has been shown that TM changes the excretion of 5-hydroxyindole acetic acid (5HIAA). This is a serotonin metabolite and the role of serotonin in depression is well documented in the work of van Praag.^{2,3} An understanding of the role of neuronal serotonin in endocrine processes such as adrenal, thyroid, gonadal and prolactin functions⁴ was thought to be necessary since practitioners of TM exhibit long-term changes in their endocrine systems.⁵ Over a time of three years, a 10-fold decrease in thyroid-stimulating hormone was reported along with a 3-fold reduction in prolactin.

Given serotonin's role in endocrine processes and its role in stress,⁶ it was chosen by Walton, et al.⁷ as a fit subject for study at Maharishi International University (MIU) since TM studies show reductions in inappropriate responses to stress.^{8,9} The initial studies by Walton showed what Bujatti's did (increased 5HIAA with TM) but used a colorimetric technique involving nitrosonaphthol that was not specific for 5HIAA.¹⁰ On using a more specific reagent¹¹ a difference was seen between these two colorimetric techniques that had its highest value around meditation times (morning and evening). See Figure 1.

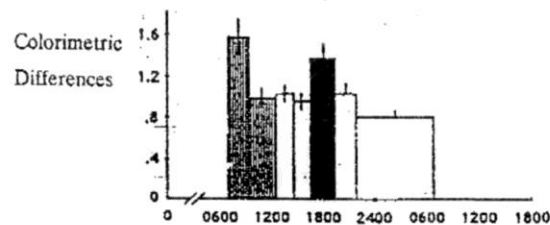


Figure 1. Urine Collection (Time of Day)

The next development came with a visit to MIU of an investigator from American Cyanamid who took back extracted samples of meditator and non-meditator urine. His results are summarized in Table 1.¹²

Table 1. The Effect of Partially Purified M Samples on Imipramine Binding and Serotonin Uptake^a

Subjects ^b	Substance M (mg/L)	% Inhibition	
		IMI Binding	5-HT Uptake
Group 1	11.9 ± 6	60.3 ± 12	38.7 ± 12
	1/4 dilution	38.8 ± 14	6.5 ± 1
Group 2	0.4 ± 0.2	7.9 ± 4	0.5 ± 0.3
	1/4 dilution	3.7 ± 2	0.0

^aSubstance M was separated from 24-h urine samples by Method II, which involved adsorption onto C18 reverse-phase extraction columns (Baker Chemical Co.) followed by washes with water then ethyl acetate and finally elution with acetonitrile. Concentrations of tritiated imipramine (IMI) and 5-HT were 2.5 nM and 1 μM, respectively. For 5-HT uptake the numbers of platelets per tube ranged from 3.4×10^6 to 6.3×10^6 . For IMI binding 100-200 μg of platelet membrane protein were used in each tube. Data are expressed as means ± SEM.

^bThe 5 Group 1 subjects were long-term (over 5 years) practitioners of the TM and TM-Sidhi program. The 5 Group 2 subjects had never practiced meditation or any other self-improvement techniques. There was one female in each group.

We considered these results remarkable but the serotonin (SHT) uptake numbers were consistent with Bujatti's work.

A collaboration with American Cyanamid was begun in which much chromatography was done, many HPLC peaks were collected and several repetitions of the imipramine-binding analysis were tried. Of course, as one can see from Table 1, there is a very large uncertainty (up to ±50%) in all these assays. Five repetitions of the HPLC peak-collecting and testing process were done and one peak was targeted for further study. This peak turned out to be a sesame-seed oil metabolite (sesame seed oil is in salad dressing). This metabolite does not act as an anti-depressant and does not inhibit serotonin uptake.

A second attempt is now underway and the next section describes the results-to-date.

Current Status

Given the uncertainty inherent in the imipramine-binding assay a dual strategy was taken. Psychological tests were administered along with the imipramine-binding assay in order to find

that substance (or group of substances) that correlate with both imipramine binding inhibition and some psychological parameter.

Chromatography - Simple

A new TLC method was developed that showed spots in the following pattern when sprayed with the non-specific 5HIAA nitrosaphthol reagent.

Figure 2.
TLC of Urine

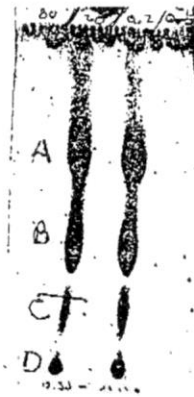
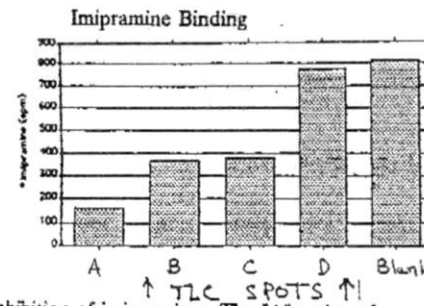
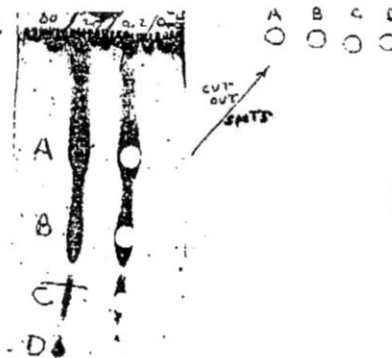


Figure 3.
TLC
of
Urine

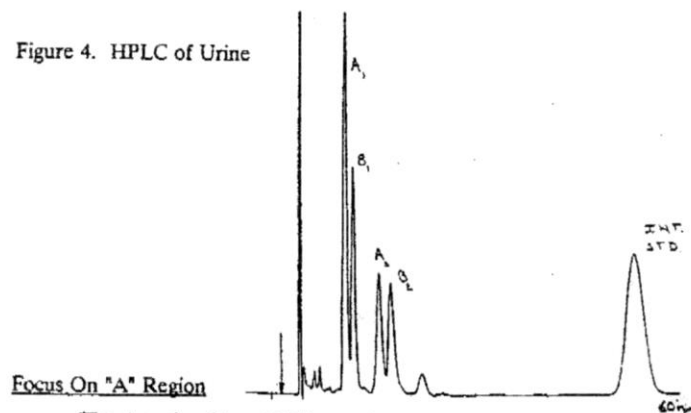


The TLC spots were cut out and tested for inhibition of imipramine. The "A" region of the TLC plate shows the maximum inhibition activity (smallest bar in bar-chart implies least imipramine left bound to the platelets after treatment of the platelets with spot A material).

Chromatography-High Performance Liquid (HPLC)

The HPLC chromatogram corresponding to the TLC plate is shown in Figure 4. It is noted that TLC spots A and B contained two HPLC peaks each and that spot C could not be seen under the HPLC conditions used.

Figure 4. HPLC of Urine



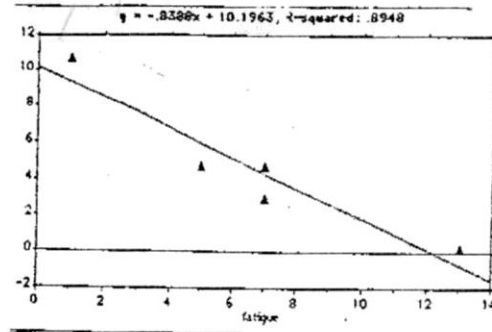
The A region (from TLC) contains two peaks (two distinct compounds) by HPLC: A₁ and A₂. In order to choose between them we subjected 6 subjects to a battery of pencil and paper psychological tests measuring 26 parameters. Overnight urine samples were collected the next morning and run through the HPLC. Correlations ($p \leq .01$) were seen with field independence (inversely proportional) age (inversely proportional) POMS (inversely proportional) vigor (directly proportional) fatigue (inversely proportional) health (directly proportional) good experiences during meditation (directly proportional) and the amount of the A₁ peak in the HPLC chromatograms.

A week-long study was also conducted on a meditating subject and his reports of his "efficiency" correlated with the A₁ peak on the chromatograms of his urine. A few of the graphs are shown in Figure 5.

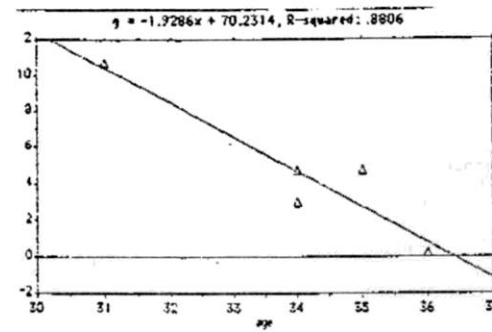
PEAK HT

5

A₁



A₁



A₁

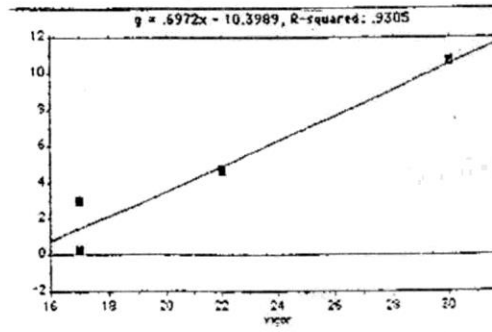
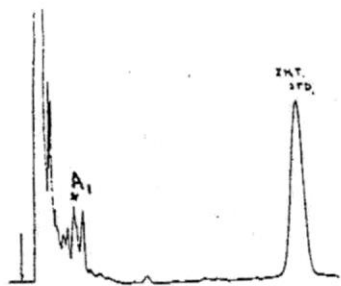


Figure 5. A₁ Peak ht Response Within a Meditating Group

HPLC URINE POOLS

6

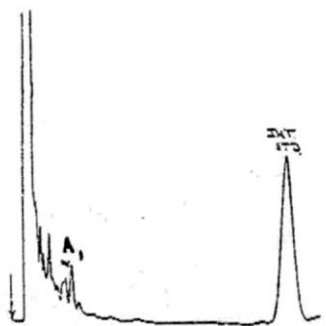


TM

n = 5

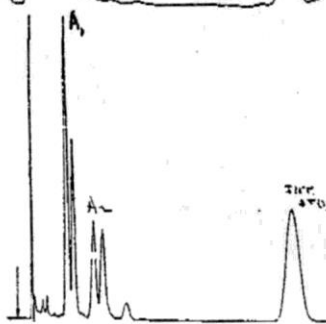
Average Age = 34

Overnight Collection

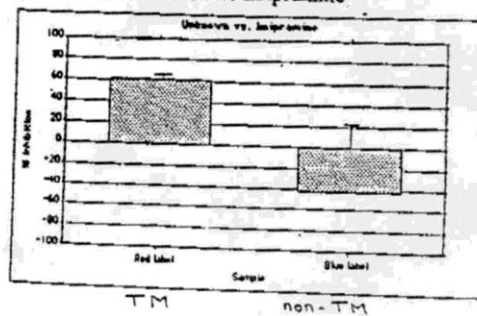


non-TM

n = 5



% Inhibition of Imipramine



URINE POOLS

n = 5

Figure 6. Urine Pools of Meditators (TM) vs. Non-Meditators (non-TM)

Since no other peak of the four (A_1 , A_2 , B_1 , B_2) showed correlation in the psychological or the urine pool test, A_1 is now the target compound.

Mass Spectroscopy

Due to the heat and air lability of A_1 it has been difficult to get mass data for A_1 .

Recently (April 9, 1990) Oneida Research Services (Whitesboro, NY) has run LC/MS on the four peaks (A_1 , A_2 , B_1 , B_2) and has given me good MW data on all four HPLC peaks. They have also indicated that there is a structural similarity among all four peaks. This is not surprising in light of the imipramine-binding inhibition displayed by spots A and B (See Figure 3) and the wet chemistry implications of the non-specific 5HIAA nitrosonaphthol reagent which both TLC spots (and presumably all four HPLC peaks?) react to.^{13,14} The LC/MS is also equipped with MS-MS capability so that the structure proposed can be rebutted or not.

Future Work

Since there is evidence for a substance in meditator urine that competes for the imipramine binding sites in blood platelets, I believe that it is worth while synthesizing that substance. Discovering its other properties is also of interest.

Urine-drinking is still practiced in parts of India. A former Indian prime minister is said to have drunk his own (M. Desai). What is the basis of this practice? A probable answer is documented in ancient Indian medicine (i.e. Ayurveda).¹⁵

Private conversations with an MIU collaborator (who is very well acquainted with Ayurveda) have indicated that the next possible application of this substance should be in a challenged immune system.

There was a 30 million dollar wholesale marketplace for antidepressants in 1983.¹⁶ Even though the obvious application for this substance ("Substance M") is as an anti-depressant. I believe that it may have other uses as well. Therefore I intend to synthesize it and submit it for further testing.

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¹⁶ Baum, Cerry. Personal conversation. National Institute of Health, Bethesda, MD.

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