References and Notes

1. N. A. Giorgio and M. Tabachnick, *J. Biol. Chem.* **243**, 2247 (1968); J. S. Marshall and J. Pensky, *J. Clin. Invest.* **48**, 508 (1969); U. S. Seal and R. P. Doe, in *Proc. Int. Congr. Endocrinol. 2nd* (Series 83, Excerpta Medica, London, 1964), p. 325.
2. J. Pensky and J. S. Marshall, *Arch. Biochem. Biophys.* **135**, 304 (1969).
3. See, for instance, W. O. McClure and G. M. Edelman, *Biochemistry* **5**, 1908 (1966); G. M. Edelman and W. O. McClure, *Accounts Chem. Res.* **1**, 65 (1968).
4. L. Stryer, *J. Mol. Biol.* **13**, 482 (1965).
5. D. J. R. Laurence, *Biochem. J.* **51**, 168 (1952).
6. J. S. Marshall and J. Pensky, *Arch. Biochem. Biophys.* **146**, 76 (1971).
7. R. F. Steiner, J. Roth, J. Robbins, *J. Biol. Chem.* **241**, 560 (1966).
8. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
9. Tetramet, Abbott Laboratories.
10. J. H. Oppenheimer, M. I. Surks, J. C. Smith, R. Squef, *J. Biol. Chem.* **240**, 173 (1965); A. Raz and D. S. Goodman, *ibid.* **244**, 3230 (1969).
11. A. Gordon and T. Goutsoftides, *Acta Endocrinol.* **62**, 217 (1969).
12. S. Ainsworth and M. T. Flanagan, *Biochim. Biophys. Acta.* **194**, 213 (1969).
13. J. H. Oppenheimer and R. R. Tavernetti, *J. Clin. Invest.* **41**, 2213 (1962).
14. J. S. Marshall and L. S. Tompkins, *J. Clin. Endocrinol. Metab.* **28**, 386 (1968).
15. G. L. Tritsch, C. E. Rathke, N. E. Tritsch, C. M. Weiss, *J. Biol. Chem.* **236**, 3163 (1961).

16. Supported in part by NIH grants AM 15308 and AI 06349 and PHS grant AM 05618. We thank D. Waugh for advice and criticism, and J. Matarazzo and J. Page for helpful discussions.

27 September 1971; revised 3 December 1971 ■

Biogenic Amine Metabolites in Cerebrospinal Fluid of Depressed and Manic Patients

Abstract. *A reduction in 5-hydroxyindoleacetic acid in cerebrospinal fluid was found in depressed and manic patients both while they were symptomatic and also after treatment. The concentration of homovanillic acid was initially reduced and then tended to increase after treatment.*

Considerable attention has been directed to possible abnormalities of biogenic amine metabolism in both depression and mania (*1*). The concentrations of amines and their metabolites in patients' urine have been studied but interpretation is difficult because only a small percentage of urinary amine metabolites originate in the brain. For example, it has been calculated that less than 1 percent of urinary 3-methoxy-4-hydroxymandelic acid in the dog originates in its brain. Urinary 3-methoxy-4-hydroxyphenyl glycol may be an exception to this (*2*).

Consequently, several investigators have examined amine metabolites in cerebrospinal fluid (CSF). A significant decrease in the concentration of CSF 5-hydroxyindoleacetic acid (5HIAA) has been reported in depressed patients (*3*); in addition, the increase in 5HIAA in CSF, usually observed after large doses of probenecid are given, was lower than that in control subjects. This suggests a decrease in the turnover of brain serotonin in depressed patients (*4*). Other studies suggest that 5HIAA concentrations in CSF return to normal after patients recover from depression and that values are normal in hypomanic patients (*3*). Unfortunately, the interpretation of much of this data is made difficult by the uncertain description of the patients studied and the large range of concentrations found.

We studied severely ill patients with affective disorders who were hospitalized on a special research ward. A period of 7 to 21 days lapsed before drug therapy was started. During this period no drugs were given, and a detailed clinical, psychological, and biological evaluation was made. The patients received a diet designed for the study of the metabolism of biogenic amines. Patients were diagnosed as manic-depressive if there was a history of previous hospitalization for both manic and depressive episodes and if two psychiatrists agreed on the current clinical state of the patient. Unipolar, or primary depressive, illness was diagnosed if there was no history of previous manic episodes, and if there was no other significant psychiatric abnormality or organic brain syndrome. A daily clinical evaluation of each patient's behavior was made by a trained research nursing staff (*5*) and recorded on a specially designed form containing symptoms of both mania and depression. These forms were developed for the systematic rating of manic patients (*6*). The ratings can be considered reliable, in view of the fact that there was little variation between the ratings of individuals making the determinations.

Lumbar punctures were performed between 9:00 a.m. and 11:00 a.m. while the patient was in a recumbant position, before he was allowed to rise, and before he had eaten. The first puncture was performed before treatment, and, in most instances, the second was done after clinical recovery and before the patient was discharged from the hospital. Several of the patients had been receiving drugs the second time (indicated in Table 1). The CSF was acidified with HCl and immediately frozen. Homovanillic acid (HVA) was estimated by the method of Gerbode

Table 1. Concentration of 5HIAA and HVA in CSF; before and after treatment.

Patient	5HIAA (ng/ml)		HVA (ng/ml)	
	Before	After	Before	After
		Manic-depressed, depressed		
1	16.9*		84.9*	
2	21.8		6.8	
3	19.8	17.5	7.6	48.5
4	20.9	15.7	23.6	
5	8.2	29.6†	10.6	58.6†
6	< 7.0		25.6	
Mean	15.8‡	20.9‡	26.5	53.6
		Manic-depressed, manic		
7	< 7.0	9.6		
1	< 7.0*	19.4*§		16.7*§
8	12.4||	< 7.0||¶	35.7||	58.1||¶
8	42.1||	20.6||¶	52.7||	98.5||¶
Mean	17.1‡	14.2‡	44.2	57.8
		Unipolar depressives		
9	< 7.0		19.9	
10	< 7.0		57.2	
11	< 7.0		26.7	
12	14.7	21.6#	20.7	68.2#
13	17.8		33.8	
14	< 7.0		8.1	
Mean	10.1‡		27.7	

* These concentrations are found in the same patient (No. 1) at different clinical stages of his illness. † Recovered from depression—moderate anxiety. ‡ These means were calculated with the value of < 7 ng being regarded as 7. The actual values were probably lower and the true means would therefore be lower. § Receiving lithium carbonate. || These concentrations are found in the same patient (No. 8) at different clinical stages of his illness. ¶ Mania remitted; mild depression. # Little change in clinical condition; had received large doses of L-dopamine for 5 weeks.

and Bowers (*7*). Four milliliters of CSF were used, and a recovery tube was assayed with each determination. Recovery of the substances averaged 74 percent for 20 determinations. The method of Korf and Valkenburgh-Sikkema (*8*) was used to estimate concentrations of 5HIAA. Tubes were incubated with *o*-phthalaldehyde for 3 minutes rather than for 10 minutes. Recovery of the 5HIAA that had been present in 2 ml of CSF averaged 96 percent for 24 analyses. The results presented are corrected for recoveries. The minimum amount of 5HIAA, in cuvettes, which gave reproducible fluorescent readings was 2.5 ng. Therefore, because of dilution during the extraction, those CSF samples which, after extraction, gave less than 2.5 ng of 5HIAA in the cuvette are recorded as < 7 ng/ml (*9*).

Twenty-four separate CSF specimens were obtained from 14 patients (Table 1). Other results obtained by the same assays have been reported as follows: HVA in CSF in neurological patients, 42 ($\pm$ 19) ng/ml; and 5HIAA in CSF in unspecified "non-depressed" controls, 40 (4 to 81) ng/ml (*4*). A small number of control specimens (from various neurological patients) studied by us showed the following concentrations: HVA in CSF, 14.5 to 77.1 ng/ml (4 patients); and 5HIAA in CSF, 4.1 to 49.1 ng/ml (5 patients). These values do not represent normal control subjects. The individuals are neurological patients (as are most subjects on whom CSF studies are done and reported by others) who often have a central nervous system disease and, in many instances, are receiving medication. Thus, considerable caution must be exercised in extrapolating from this data. Certainly, the range noted by us as well as others (*4, 19*) is so wide that it limits the value of using a mean concentration.

It can be seen (Table 1) that the 5HIAA values were somewhat low in all three groups of patients, and HVA was low in the two groups of depressed patients, but apparently normal for the patients in the manic state. There was a small increase in 5HIAA in the specimens taken after treatment from the manic-depressed, depressed patients and a slight drop in 5HIAA in the manic patients. The number of HVA assays is smaller, but they indicate a considerable increase in concentration after treatment.

Analysis of the data showed no difference in CSF concentrations in depressed patients based on severity of the depression and no difference between those who were retarded and those who were agitated. The latter distinction was somewhat arbitrary in that the same patients may show features of agitation and retardation at different times. Also, motor retardation does not exclude the possibility of psychic agitation.

Table 2. 5HIAA and HVA in CSF. Psychotic and neurotic patients; before treatment.

	5HIAA (ng/ml)	HVA (ng/ml
	Psychotic	
	< 7.0	19.9
	< 7.0	26.7
	12.4	35.7
	42.1	52.7
	< 7.0	8.1
	< 7.0	
	< 7.0	
Mean	12.8	28.6
	Neurotic	
	16.9	84.9
	14.7	20.7
	8.2	10.6
	20.9	23.6
	19.8	7.6
	< 7.0	25.6
	17.8	38.8
	21.8	6.8
Mean	15.9	27.3

Table 2 shows the results of the patients being divided into psychotic and neurotic groups based on the presence or absence of delusions, respectively (*10*). The 5HIAA concentrations were lower in the psychotic than they were in the neurotic group. The mean CSF concentration of 5HIAA for the psychotic patients was 12.7 ng/ml while the mean for the neurotic group was 15.9 ng/ml. There was, however, a value of 42.1 ng/ml for one psychotic patient. The other six psychotic patients had amounts with a mean of less than 8 ng/ml, about half the mean for the neurotic group. (This division of patients, as with the other classifications made concerning patients, was independent of any knowledge of the CSF concentrations.)

While it has been reported that 5HIAA in CSF reflects changes in brain serotonin metabolism (*11*), more recent observations suggest that this compound in lumbar fluid is derived primarily from the spinal cord (*12*). This raises doubt as to whether the amount of 5HIAA in the lumbar fluid can be used as an index of brain serotonin metabolism. However, if the abnormal findings noted here do arise as a result of an alteration in serotonin metabolism in the spinal cord, then it would prompt consideration of whether there may be an important disturbance in amine metabolism in depression and mania in areas other than the brain.

The observation of a relative reduction in 5HIAA in CSF in both depressed and manic patients contributes to suggestions that depression and mania are not necessarily bipolar in nature (*13*). Other supporting observations include reports of similar changes in sleep patterns (*14*), in electrolyte changes in both manics and depressives (*15*), and in the response to lithium in both manic and selected depressed patients (*16*).

The failure of 5HIAA in CSF to change after clinical recovery in all of the patients may indicate that the concentrations are normal for these people.

The initial reduction of HVA in CSF in three depressed patients was followed by a relative increase after treatment. However, the number of subjects is small and the concentration of HVA decreased in one patient. It is generally presumed that HVA is primarily derived from dopamine, and there is little evidence of a significant disturbance of dopamine in depression. It has been suggested that HVA in CSF is derived primarily from brain capillaries (*17*). Our findings cannot be accounted for by differences in the physical activity of the patients in that there was no difference in HVA in the groups of retarded and the agitated patients. Changes in urinary HVA have been noted in patients receiving lithium (*18*).

J. MENDELS, A. FRAZER
R. G. FITZGERALD, T. A. RAMSEY
J. W. STOKES

Department of Psychiatry and Pharmacology, University of Pennsylvania, and *Veterans Administration Hospital, Philadelphia*

References and Notes

1. J. J. Schildkraut, *Amer. J. Psychiat.* **122**, 509 (1965); A. Coppen, *Brit. J. Psychiat.* **133**, 1237 (1967); I. P. Lapin and G. F. Oxenkrug, *Lancet* **1969-1**, 132 (1969); J. Mendes and J. Stinnet, in *Textbook of Biological Psychiatry* [Interscience (Wiley), New York, 1972].
2. J. W. Maas and D. H. Landis, *Psychosom. Med.* **28**, 247 (1966).
3. S. J. Dencker, U. Malm, B. E. Roos, B. Werdinuss, *J. Neurochem.* **13**, 1545 (1966); M. B. Bowers, G. R. Heninger, F. Gerbode, *Int. J. Neuropharmacol.* **8**, 255 (1969).
4. H. M. Van Pragg, J. Korf, J. Puite, *Nature* **255**, 1259 (1970); N. R. Tamarkin, F. K. Goodwin, J. Axelrod, *Life Sci. (I)* **9**, 1397 (1970).
5. W. Bunney and D. A. Hamburg, *Arch. Gen. Psychiat.* **9**, 280 (1963)
6. S. K. Secunda and J. Mendels, in preparation.
7. F. A. Gerbode and M. B. Bowers, *J. Neurochem.* **15**, 1053 (1968).

8. J. Korf and T. Valkenburg-Sikkema, *Clin. Chim. Acta* **26**, 301 (1969).
9. With the dilution during extraction, 2.5 ng HIAA in the cuvette would yield 7 ng/ml in CSF, assuming 85 percent recovery. Therefore, if, after extraction, there were < 2.5 ng of HIAA in the cuvette, the results are expressed as < 7 ng/ml in CSF.
10. It should be noted that a patient diagnosed as a manic-depressive, depressed, might be neurotic. Our diagnosis was based on a history of at least one prior manic and one prior depressive episode requiring hospitalization. For the study, the illness at the time needed not to have been of psychotic proportions (manic-depressive *illness*).
11. H. Anderson and B. E. Roos, *Acta Pharmacol. Toxicol.* **26**, 293 (1968); G. W. Ashcroft, *Proc. Roy. Soc. Med.* **62**, 1099 (1969).
12. M. Balat and B. Zivkovic, *Science* **173**, 738 (1971).
13. P. C. Whybrow and J. Mendels, *Amer. J. Psychiat.* **125**, 141 (1969); J. Court, *Brit. J. Psychiat,* **115**, 372 (1969); J. Mendels, *Concepts of Depression* (Wiley, New York, 1970).
14. J. Mendels and D. R. Hawkins, in *The Psychobiology of Depression*, T. A. Williams, M. M. Katz, J. Shields, Eds., in press; *Arch. Gen. Psychiat.,* in press.
15. A. J. Coppen, D. M. Shaw, A. Mangoni, *Brit. J. Med.* **2**, 295 (1962); J. Mendels, A. Frazer, S. K. Secunda, Society of Biological Psychiatry Annual Meeting, Washington, D.C. 1 to 2 May 1971.
16. J. Mendels, S. K. Secunda, W. L. Dyson, *Arch. Gen. Psychiat.*, in press.
17. G. Bartholini, R. Tissot, A. Pletscher, *Brain Res.* **27**, 163 (1971).
18. F. S. Messiha, D. Agallianos, C. Clower, *Nature* **225**, 868 (1970).
19. After this report was submitted for publication, R. Papeschi and D. J. McClure [*Arch. Gen. Psychiat.* **25**, 354 (1971)] reported a reduction in HVA concentration in CSF in depression.
20. Supported in part by research funds from the Veterans Administration, by research training grant TR-29 from the Veterans Administration and by NIMH training grant 5-TO1-MH-11178. We thank Michael Kane and Steven Seltzer for technical assistance.
21 June 1971; revised 13 December 1971

Proprioceptive Illusions Induced by Muscle Vibration: Contribution by Muscle Spindles to Perception?

Abstract. *When vibration of 100 hertz was applied to the tendon of the biceps or the triceps muscle, the subject made a systematic misjudgment of the angle at the elbow. During contraction the error could be as much as 40 degrees. The subject thought that the elbow was in the position that it would have assumed if the vibrated muscle had been stretched.*

The mechanisms underlying kinesthesia or "position sense" have long been debated. Sherrington (*1*) attributed position sense solely to the central perception of the discharges from the appropriate proprioceptive end organs. Others (*2*), following Helmholtz, have argued that the sensory centers are informed by recurrent pathways of the dispatch of commands from the higher motor centers, and that this of itself can lead to changes in the perception of position. Both mechanisms are now usually accepted as being capable of paying a part. Sherrington (*1*) produced the first evidence that muscle spindles are sensory end organs and suggested that they should be included among the mechanoreceptors that contribute to kinesthesia. Since the arrival of the electronic era it has been shown that the muscle spindle afferents do indeed signal the mechanical state of the muscle, but there has been a parallel accumulation of evidence against the spindle discharges having any access to conscious sensation. Instead, the spindle has been seen as reserved for the subconscious control of movement, notably by the cerebellum. The various receptors in joints have accordingly been allocated the sole responsibility for providing the peripheral contribution to kinesthesia.

The evidence for this view has been firmly based on human studies. First, the regional anesthetization of a finger joint produces a gross loss of awareness of the position of the finger when it is moved passively, even though the muscle spindles in the relevant muscles may be presumed to be behaving normally (*2*, *3*). Second, the passive stretching of a muscle in the conscious subject by pulling upon the exposed tendon fails to produce any clear proprioceptive sensation (*4*). Thus the muscle spindle has appeared to be excluded from contributing to sensation. Until recently, this view was supported by the inability of electrophysiologists to discover a cortical representation of spindle afferents by the recording of an evoked potential in response to stimulation of a muscle nerve at an appropriate strength. However, with refinements in technique such projections have now been amply demonstrated in both cat and monkey (*5*). The following proprioceptive illusion, which may be produced in the normal human subject, suggests that under suitable circumstances spindle afferent discharges can contribute to perception.

The illusion has been produced as follows. The blindfolded subject sat with his upper arms lying parallel and horizontal on a support. His forearms were free to move in the vertical plane. A light wooden splint was tied to each forearm and connected via a string to a potentiometer to allow recording of the angle at the elbow. The recording arrangement was nonlinear, but gave a reproducible reading that was accurate to within 2°; this was adequate for the present experiments. One arm was designated as the experimental arm. The spindles in either its biceps or its triceps muscle were then excited by manually applying a physiotherapy vibrator to the appropriate tendon (Pifco vibrator; frequency of vibration, 100 hz; amplitude of movement, of the order of 0.5 mm when loaded). The other arm was designated as the tracking arm, and the subject was asked to keep it aligned with the vibrated arm. The tracking arm thus was an objective indication of the subject's estimate of the position of the vibrated arm. The subject was told to maintain the position of the vibrated arm against gravity, but was asked not to oppose any movement which tended to take place when the arm was vibrated or moved by the experimenter. Essentially similar results were found when the arm commenced moving reflexly from a position of complete rest against a stop.

Figure 1 shows the typical effect of vibrating the biceps muscle. Shortly after the vibration began, the vibrated arm started to move into flexion under the influence of the tonic vibration reflex. This phenomena is now well known and is attributed to the excitation of the spindle primary endings by the vibration; this excitation is believed to lead to a stretch reflex type of response, although there may well also be contributions from higher centers (*6*). The initial part of the reflex movement was not perceived by the subject, but when movement of some 10° had occurred the subject became aware of the motion and began to move his tracking arm also. But the tracking arm moved more slowly than the vibrated arm so that the misalignment between them increased progressively.

After the vibrated arm had moved through about 40°, its movement was gently arrested without the subject's knowledge. As a result of the reflex movement itself, a long string was gently pulled tight; one end of the string was attached to the splint and the other was fixed. The subject then had a strong sensation that his arm was being moved in the opposite direction to that in which it had just been