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Detection of Psychotomimetic N,N-Dimethylated Indoleamines in the Urine of Four Schizophrenic Patients

By H. TANIMUKAI, R. GINTHER, J. SPAIDE, J. R. BUENO and H. E. HIMWICH

Pollin, Cardon and Kety (18) investigated the effects of large doses of various amino acids in combination with a monoamine oxidase (MAO) inhibitor on the behaviour of schizophrenics. They found that methionine in the presence of such an inhibitor was capable of producing behavioural changes which may 'represent a biochemically induced acute flare-up of a chronic schizophrenic process on the one hand, or a toxic delirium superimposed upon chronic schizophrenia on the other'. Brune and Himwich (8) confirmed the clinical results of Pollin *et al.* On the basis of their previous work indicating that tryptamine appeared in increased concentrations in the urine before and during the activation of psychotic symptoms, they suggested that under loading conditions the formation of various N,N-dimethylated indoleamines might be facilitated in the body. The tertiary indoleamines so formed might mediate the psychotic effect of methionine with a MAO inhibitor on schizophrenic patients.

According to this suggestion tryptophan provides primary indoleamines such as tryptamine and serotonin as precursors of N,N-dimethylated indoleamines, while methionine acts as a methyl donor. It is a well known fact to pharmacologists that N,N-dimethylated indoleamines such as N,N-dimethyltryptamine (DMT), bufotenin, psilocin, psilocybin, and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) exhibit psychotomimetic effects in man (2, 13, 19, 22), and also cause behavioural disturbances in experimental animals (15). The production of such N,N-dimethylated indoleamines from the naturally occurring indoleamines has been demonstrated with mammalian tissues by the enzymatic studies of Axelrod (3, 4). Moreover, experimental data supporting the above suggestion of Brune and Himwich (8) have been reported from various laboratories (1, 5, 6, 7, 16, 21). In order to check this hypothetical suggestion we undertook to determine whether or not such psychotomimetic amines are present in the body fluids of schizophrenic patients, at least at the time when they are in active psychotic states.

Methods

Subjects

Four male patients with chronic schizophrenia, but with active symptoms, and ranging in age from 40–58 years were studied on a controlled diet which excluded all preformed catechol and indoleamines. Three of these patients were of the paranoid type and the fourth belonged to the catatonic group. Psychoactive drugs were withheld from four to six weeks prior to the initiation of the study and throughout the experimental period.

After a seven-day control period, the patients were given dietary supplements of DL-methionine or L-cysteine as well as tranylcypromine (a MAO inhibitor), or tranylcypromine plus L-cysteine according to the protocol (see Figure 1).

Urine

Urine was collected on a 24-hour basis and was refrigerated in bottles at 4·5 °C. during the daily collection. After the volume and specific gravity were measured and the pH adjusted to 2 with 6N HCl, the entire volume of 24-hour collection was stored in a deep freeze at −20 °C. until analysed.

Paper and thin-layer chromatography

A procedure for the concentration and purification of urinary indoleamines and their

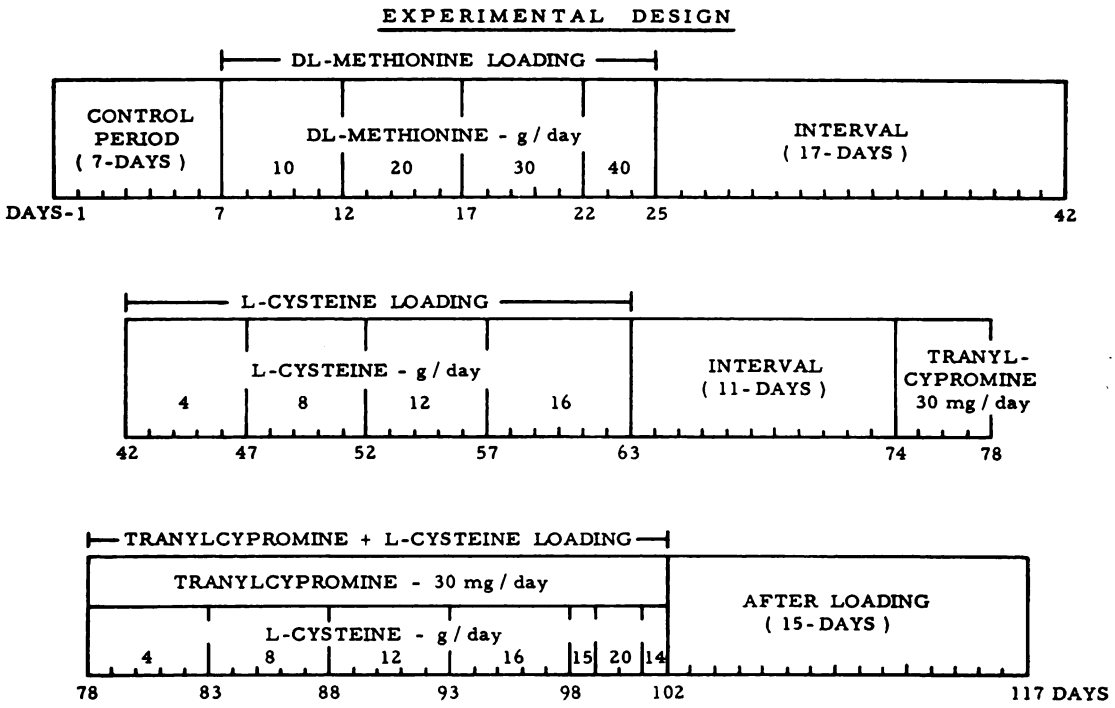

FIG. 1.—Experimental schedule for the loading of the dietary supplements and medication with tranylcypromine, a monoamine oxidase inhibitor.

identification by two-dimensional paper and thin-layer chromatography was recently developed in this laboratory (23). The same procedure was adopted in this study (see Fig. 2). For paper chromatography Whatman No. 1 paper was used and the two solvent systems were *n*-butanol-acetic acid-water (12 : 3 : 5) and 20 per cent aqueous KCl. For thin-layer chromatography, a 250 μ thickness of silica gel G layer was used, with isopropanol-aqueous ammonia (10 per cent) and water (8 : 1 : 1) and *n*-butanol-acetic acid-water (12 : 3 : 5) as the developing solvents. p-Dimethylaminocinnamaldehyde (DMCA) (1 per cent in 6N HCl, mixed with ethanol 1 : 9) was used as a spray reagent for visualisation.

Gas-liquid chromatography

The purified indoleamine concentrates from the urine samples were also analysed by gas-liquid chromatography (11) by preparing the trimethylsilyl (TMS) derivatives before and after treatment with acetone. The sample, one half of the purified residue, was dissolved in 100 μl. of dimethyl formamide, and treated with 100 μl. of hexamethyl disilazane (HMDS) and 200 μl. of acetone, whereby the primary amines were converted to eneamines and the free hydroxyl groups to TMS derivatives. The quantity of injection was suitably adjusted (10 μl.) to get a sizable peak for identification. Methylene unit values (10) were measured by interpolation between retention times for pairs of even numbered straight chain saturated hydrocarbons varying from C_{16} to C_{22}.

RESULTS

Paper and thin-layer chromatography

The diagrammatic representations of paper and thin-layer chromatograms of indoleamines prepared from the urine of schizophrenic patients, as visualized with the DMCA reagent, are shown in Figures 3 and 4 respectively. The R_f values and colours of the main spots on these

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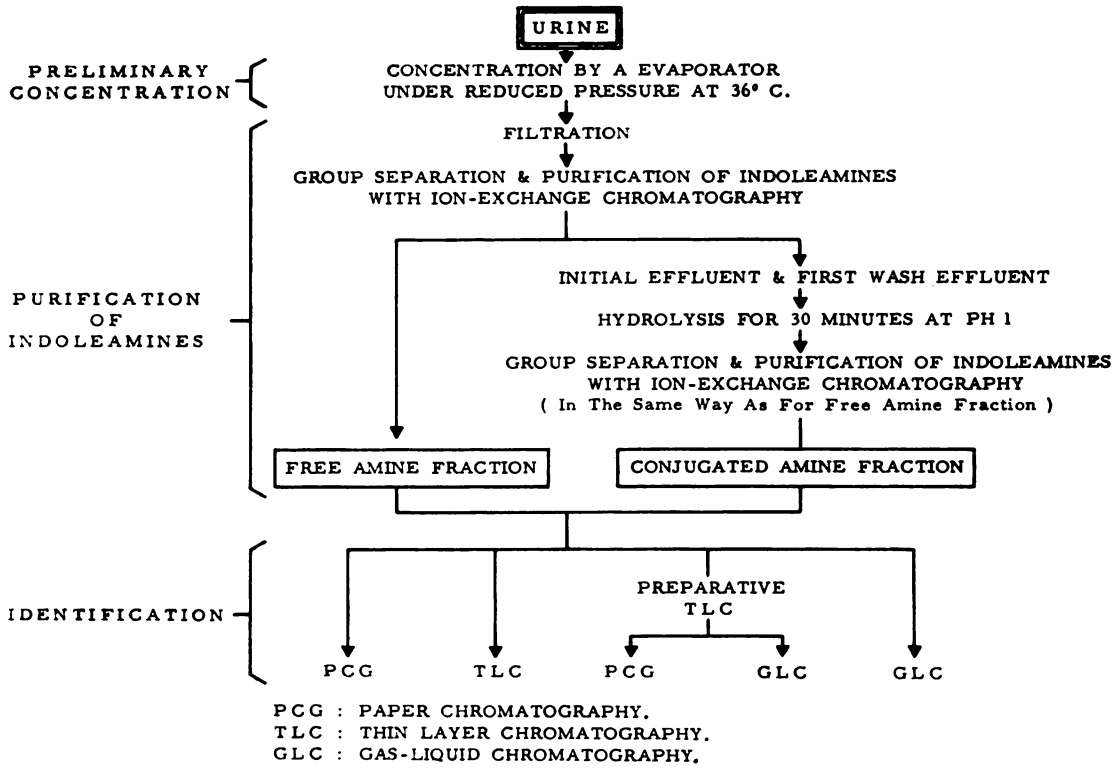

FIG. 2.—Schematic representation of the methods used for detecting urinary indoleamines.

chromatograms are listed in Tables I and II. Although the R_f values of these compounds can vary to some extent from case to case due to differences in the salt concentration in the sample, one can identify every spot in question since the colour and relative position of the individual spots on the chomatograms remain consistent.

The urine samples collected from the patients, on the different dietary supplements, were examined. Since the thin-layer chomatographic method is much more sensitive than the paper method, the following descriptions are mainly concerned with the results obtained by the thin-layer chromatographic study if not otherwise stated.

The results concerning the occurrence of N-methylated indoleamines are summarized in Table III. In the free amine fractions, tryptamine, serotonin, tryptophan and two unidentified DMCA positive spots (nos. 4 and 5 in Figure 4) were always noted regardless of the dietary supplements or the presence of a MAO inhibitor. During methionine or cysteine loading, in two out of four patients faint spots corresponding to bufotenin, DMT and/or N-methylserotonin (NMS) were also found. After tranylcypromine was given either with or without cysteine the tryptamine and serotonin spots enlarged, and a spot corresponding to bufotenin was detected in each of the four patients studied.

Free NMS was also observed in three out of four patients during tranylcypromine administration both with and without cysteine. In three patients thin-layer chromatography of the free amine concentrates from urine samples revealed a purple spot corresponding to DMT. However, the R_f values in both the dimensions were very close to 5-MeODMT. In two of the patients a faint blue spot corresponding to 5-MeODMT was also obtained.

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TABLE I

Nos. as in Fig. 3	Compounds	R_f BuOH-HAc-H_2O[a]	R_f KCl[b]	Colour[c]
1	Tryptamine	·70	·54	Purple
2	Serotonin	·49	·37	Blue
3	Tryptophan	·50	·62	Purple
4	Unidentified	·68	·78	Purple
5	Unidentified	·60	·71	Purple
6	Bufotenin	·60	·51	Blue

R_f Values and coloration of indoleamines and related compounds on paper chromatograms.
a BuOH-HAc-H_2O: n-butanol-acetic-water(12 : 3 : 5)
b KCl: 20 per cent aqueous potassium chloride
c The colours were examined within 10 minutes after spraying with 1 per cent solution of p-dimethylaminocinnamaldehyde in ethanol-6NHCl (9 : 1) (DMCA reagent).

TABLE II

Nos. as in Fig. 4	Compounds	R_f IpOH-NH_3-H_2O[a]	R_f BuOH-HAc-H_2O[b]	Colour[c]
1	Tryptamine	·80	·75	Purple
2	Serotonin	·68	·70	Blue
3	Tryptophan	·56	·64	Purple
4	Unidentified	·34	·46	Purple
5	Unidentified	·65	·94	Purple
6	Bufotenin	·88	·50	Blue
7	N, N-Dimethyltryptamine	·94	·56	Purple
8	N-Methylserotonin	·65	·60	Blue
9	5-Hydroxytryptophan	·55	·57	Blue
10	Kynuramine	·82	·68	Red-purple
11	5-Methoxy-N,N-dimethyl-tryptamine	·92	·54	Blue

R_f values and coloration of indoleamines and related compounds on thin-layer chromatograms.
a IpOH-NH_3-H_2O: isopropanol-aqueous ammonia-water (8 : 1 : 1).
b BuOH-HAc-H_2O: n-butanol-acetic acid-water (12 : 3 : 5)
c The colours were examined within 10 minutes after spraying with 1 per cent DMCA reagent.

TABLE III

Number of patients excreting N-methylated indoleamines as determined by paper and thin-layer chromatography (total number of patients was 4)

Loading	Bufotenin F*	Bufotenin C†	N-Methylserotonin F	N-Methylserotonin C	N-Dimethyltryptamine F	N-Dimethyltryptamine C	5-Methoxy-N-Dimethyltryptamine F	5-Methoxy-N-Dimethyltryptamine C
Methionine	2	1	1	0	2	1	0	0
Cysteine	1	3	1	0	2	0	0	0
Tranylcypromine ..	4	3	3	0	3	1	2	1
Tranylcypromine + Cysteine	4	3	4	0	3	0	2	0
After cessation of loading	1	3	0	0	1	0	0	0

* F = Free form.
† C = Conjugated form.

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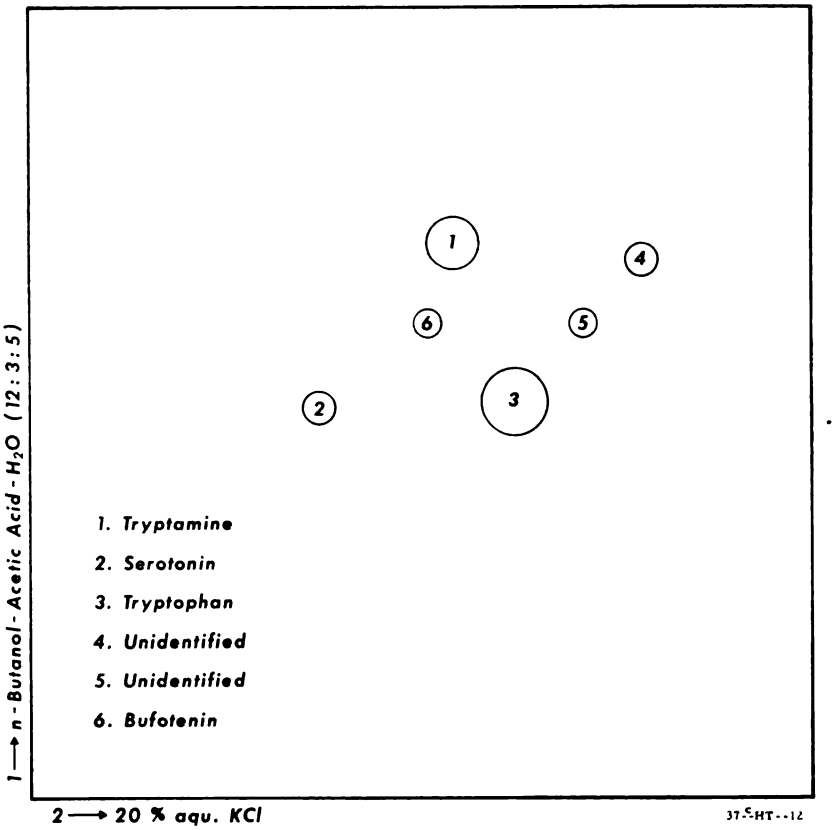

FIG. 3.—Diagrammatic representation of a paper chromatogram of indoleamines prepared from urine of a schizophrenic patient.

In the conjugated amine fraction tryptamine was consistently and serotonin was inconsistently found in the absence of a MAO inhibitor. Bufotenin was revealed in one patient under methionine medication, and in three patients under cysteine loading, although the spot was very faint. When tranylcypromine was given conjugated tryptamine as well as serotonin were seen in every urine sample, and in three out of four patients conjugated bufotenin was also clearly disclosed. Urinary excretion of conjugated tryptamine, serotonin and bufotenin continued with rather increasing concentrations while the patients received the combined medication of tranylcypromine and cysteine. Neither DMT nor NMS was found in conjugated forms except for one patient who excreted conjugated DMT.

Paper chromatographic study revealed a spot suspected of being bufotenin in both free and conjugated amine fractions in two patients under MAO blockade either with or without cysteine administration, but the incidence of the detection was less frequent than in the thin-layer study. No N-methylated indoleamines other than bufotenin were found in any urine sample by the paper chromatographic study.

Small amounts of bufotenin as well as DMT seem to be excreted in the urine of some schizophrenic patients in the absence of MAO blockade. In the presence of MAO blockade the urinary excretion of these psychotomimetic

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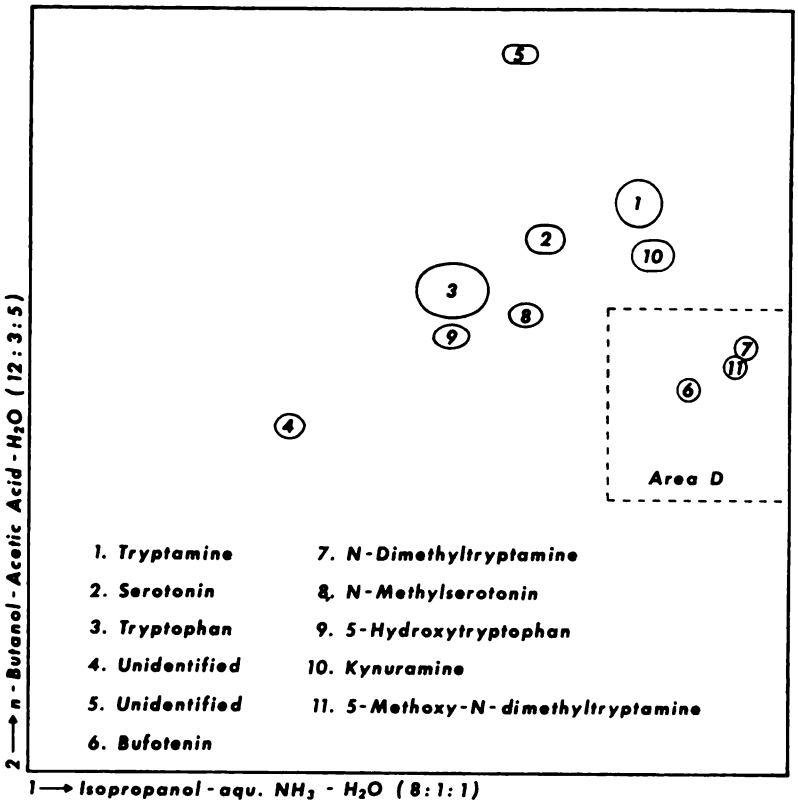

FIG. 4.—Diagrammatic representation of a thin-layer chromatogram of indoleamines prepared from urine of a schizophrenic patient.

indoleamines increased. Bufotenin appeared, under the same conditions, in the urine of all the schizophrenic patients examined, and identification was confirmed by the gas-liquid chromatographic studies described below.

In addition to the above mentioned indoleamines we noted kynuramine on the thin-layer chromatograms both in free and conjugated forms under tranylcypromine administration. This is a confirmative finding for that of Perry *et al.* (17) who first reported this amine as a normal constituent in human urine.

Gas-liquid chromatography

Representative gas-liquid chromatograms obtained from the urine samples are shown in Figure 5. The chromatogram showed a peak corresponding to bufotenin with a methylene unit value identical with that of authentic compound. This peak was unaffected when the fraction was also treated with acetone. Two different columns 10 per cent F-60 and 7 per cent F-60 + 1 per cent EGSP-Z gave similar results. Spiking was also noticed in the peak size of bufotenin, when authentic bufotenin was added to the urine sample. The amount of bufotenin excreted in the urine was calculated from the size of the peak in the gas-liquid chromatogram and was approximately estimated to be as little as 2–5 μg./day for each of the free and conjugated forms during MAO blockade. These values are similar to those estimated semi-quantitatively by thin-layer chromatographic

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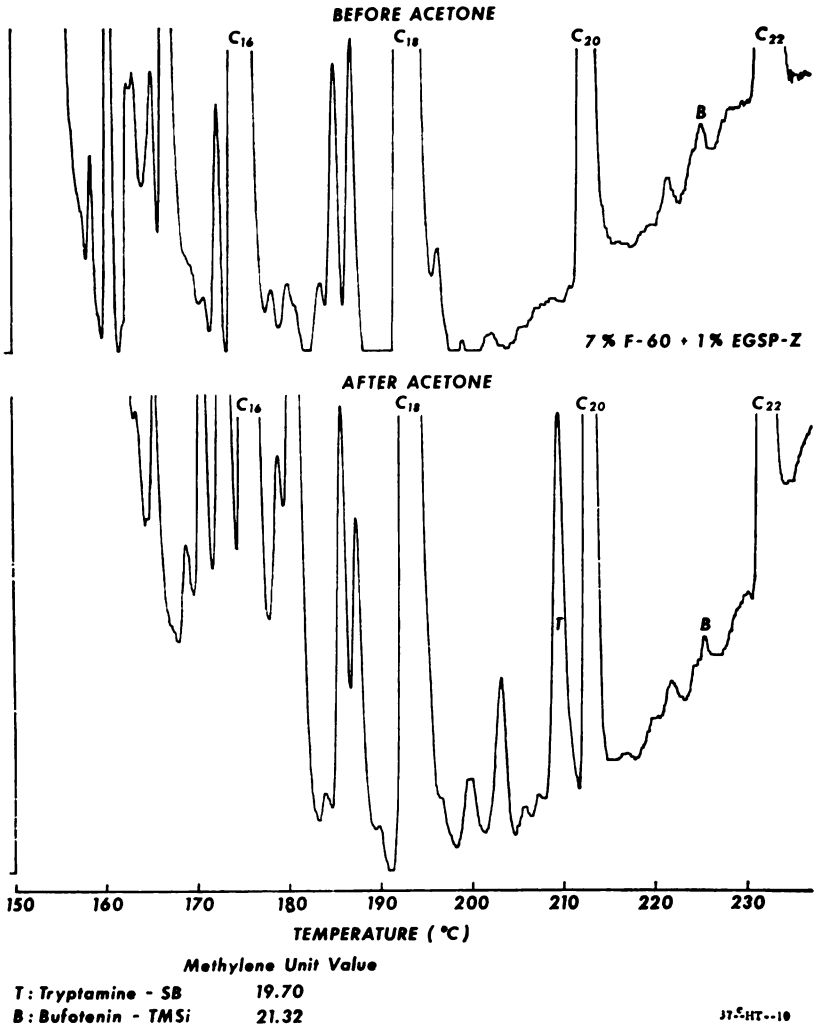

(By the Method of Capella & Horning)

FIG. 5.—Gas-liquid chromatograms of urinary amines from a schizophrenic patient (S.P.). Both chromatograms were obtained from the same sample injected with standard even-numbered straight chain saturated hydrocarbons (C_{16}–C_{22}). All hydroxyl groups were converted to trimethylsilyl ether groups. The sample in the lower portion of the figure was injected after treating with acetone where primary amines were converted to eneamines.

Condition:
Instrument—F & M Model 400 Hydrogen flame ionization detector.
Column—6′ × ¼″ glass U tube with 7 per cent F-60 + 1 per cent EGSP-Z on Gas Chrom Q (Applied Science Laboratories).
Temperature—150 to 230°C 2°/min.

T: Acetone condensation product of tryptamine.

B: Trimethylsilyl ether of bufotenin.

study. DMT and NMS were not identifiable on the gas-liquid chromatograms due to overlapping peaks in this region, and 5-MeODMT could not be detected in the volume of urine used in these experiments.

Discussion

With large volumes of urine samples (containing 100–150–200 mg. creatinine) we could clearly demonstrate the presence of bufotenin in the urine of each of four schizophrenic patients examined when they were receiving tranylcypromine, a MAO inhibitor, both with and without cysteine. The identification was made by three different chromatographic methods, i.e. paper, thin-layer, and gas-liquid.

Bufotenin was excreted both in the free and conjugated forms. In the absence of MAO blockade bufotenin seems to be excreted in amounts of less than 1 μg. per day and only a very faint spot was observed on the thin-layer chromatogram in some cases. However, when tranylcypromine was given bufotenin was found in the urine of all four schizophrenic patients. The total amount excreted was estimated to be 4–10 μg. per day, one-half in the free and one-half in the conjugated form, and we have evidence from another investigation (24) that the free form of bufotenin may be psychotomimetic.

The thin-layer chromatograms of free amine fractions showed occasional spots corresponding to NMS and DMT, and also to 5-MeODMT, which occurred only in urine samples of patients treated with a MAO inhibitor. It appears that NMS, DMT and 5-MeODMT are excreted in the urine when bufotenin is excreted, since the enzyme, non-specific N-methyltransferase, which N-methylates serotonin to form bufotenin through NMS, has also been shown to catalyze the N-methylation of tryptamine and many other amines (3, 4).

The wide distribution of bufotenin and DMT in higher plants has been reported by several investigators (12). We have carefully excluded, however, all known sources of preformed catechol and indoleamines from the diet of our patients. Therefore, it seems more likely that bufotenin and other N-methylated indoleamines in the present study were formed endogenously rather than from exogenous sources.

We also made clinical observations of the behavioural symptoms of these patients during the entire period of the experiment (20), and correlated the biochemical findings with the behavioural changes. The worsening of the mental and behavioural symptoms started in all four patients almost simultaneously about two weeks after free bufotenin and other N, N-dimethylated indoleamines increased in the urine. Such elevated levels of free N,N-dimethylated indoleamines were maintained during the exacerbation of the mental symptoms and decreased gradually to the premedication levels with concomitant improvement in behavioural symptoms, which started 5 days after the combined medication was discontinued. These correlations between biochemical and clinical observations, coupled with the reports that these N,N-dimethylated indoleamines exhibit psychotomimetic effects on human beings (2, 13, 19, 22) are compatible with the suggestion of Brune and Himwich (8) on the role of N,N-dimethylated indoleamines in explaining the psychotomimetic effects of certain amino acids on schizophrenic patients in the presence of a MAO inhibitor, although alternative hypotheses are equally possible and have not been ruled out. A catecholamine hypothesis in which a psychotogenic O-methylated catecholamine is regarded as a causal agent of schizophrenia is also possible. We, however, are inclined to attach greater importance to indoleamines since in our previous experiments urinary catecholamine level was related more to motor activity and/or emotional changes than to changes in the schizophrenic symptomatology (9, 10, 14).

The results presented here were obtained with four schizophrenics. For our data to be better understood a comparison of these observations on four schizophrenics with observations on normal patients under similar conditions will be necessary. Work on these lines is now in progress.

Summary

In addition to tryptamine and serotonin, we found bufotenin (5-hydroxy-N,N-dimethyltryptamine) both in free and conjugated forms in the urine of four schizophrenic patients

under dietary control when they were receiving tranylcypromine, with or without cysteine loading. The amount of bufotenin was estimated to be as little as 4–10 μg. per 24 hr. urine; one-half in the free, one-half in the conjugated form. In the absence of monoamine oxidase blockade bufotenin was also excreted in some patients, but less than 1 μg. per day.

N-methylserotonin, N, N-dimethyltryptamine and 5-methoxy-N, N-dimethyltryptamine were also disclosed, but only by thin-layer chromatography and especially when the patients were receiving tranylcypromine with or without cysteine. Increases of urinary bufotenin and other N-methylated indoleamines were observed about two weeks before the mental and behavioural symptoms of the schizophrenic patients worsened, and these elevated levels continued during the period of behavioural exacerbations.

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