Reports and Letters

Aromatic Excretory Pattern of Schizophrenics

It is now well recognized that certain aromatic derivatives, such as mescaline and lysergic acid diethylamide (LSD-25), can induce in normal persons transient mental disturbances that bear certain resemblances to the symptoms of schizophrenia. It has been suggested that schizophrenia may involve a metabolic error that results in the biosynthesis of a similar hallucinogen. Such a hypothesis is strengthened by the fact that the hallucinogen LSD-25 has an antagonistic action on certain biological effects of the tranquilizing agents reserpine and Frenquel, α-(4-piperidyl)benzhydrol hydrochloride (*1*).

The known hallucinogens and tranquilizing agents differ greatly one from another in structural complexity, but all possess one common and perhaps significant structural characteristic—an activated aromatic ring. It is tempting to hypothesize, therefore, that, if any natural hallucinogen existed, it would also belong to this broad class of activated aromatic compounds. On the basis of these assumptions, one might expect to find differences between the aromatic excretory patterns of schizophrenics and normal individuals.

Reports of such differences have already been made. Young *et al.* (*2*) have reported the presence of abnormal diazo-coupling compounds in the urine of schizophrenics. Sano (*3*) has reported that tests for certain indole derivatives are positive in a higher percentage of the urines of schizophrenics than they are in the urines of normal individuals. As a first step toward establishing whether or not aromatic pathways are altered in the schizophrenic state, we have set out to confirm and extend these results (*4*).

Descending chromatograms on urine were run using a 4/1/1 butanol-acetic acid-water solvent with diazotized sulfanilic acid as the color developer (*2*). One group consisted of the morning urines of 104 females from Essondale, the British Columbia Mental Hospital. Of these, 52 were schizophrenics, 36 were patients with other types of mental disorders, and 16 were members of the staff. All 104 persons were receiving the normal hospital diet, and all the samples were taken on the same day. Each of the chromatograms was assigned a quantitative rating based on the number and intensity of spots. Ratings of the chromatograms, done independently by two observers, agreed exactly in 78 cases and differed by only one point in the other 26 cases; 0.05 ml of urine was used for each chromatogram.

There are two problems that arise in comparing semiquantitatively the chromatograms of different urines. One concerns the relative dilution of the urine, and the other the amount of a given material represented by the developed spot. Customarily, the urine volume spotted on the chromatogram is normalized to a constant creatinine concentration, while the amount of material developed on the paper is estimated by the area of the spot or by its photoreflectance. The creatinine excretion by schizophrenics is abnormal and variable (*2, 5*), and this, coupled with our observation that diluting or concentrating the urine within physiological limits had little effect on either the size or intensity of spots developed, convinced us that spotting a constant volume of overnight urine was a satisfactory technique. We discovered also that assigning a quantitative rating based on the number and intensity of spots was as satisfactory a technique for differentiating chromatograms as the more laborious measurement methods and, in addition, was just as reproducible and much faster. We have therefore reported only these quantitative ratings.

The results, which are summarized in Table 1, indicate clearly that, taken over-all, there are more diazo-coupling compounds in the urine of schizophrenics than there are in the urine of nonschizophrenics. The greatest differences between the chromatograms of schizophrenic and normal urine appeared to be in the R_f regions 0.15 to 0.3, 0.45 to 0.6, and 0.8 to 0.9. A more exact study of the location and color of the "abnormal" spots will be reported later (*6*). The day-to-day variation in the aromatic excretory patterns of given individuals, whether schizophrenic or nonschizophrenic, makes it hazardous to say that any given chromatographic spot is exclusively schizophrenic. The differences between schizophrenics and nonschizophrenics are quantitative rather than qualitative, and they become apparent when the over-all aromatic excretory pattern is considered.

Based on a scale of 11, the average value for schizophrenics was 6.0 ± 2.5 as compared with 2.8 ± 1.3 for normal individuals. Of the 13 schizophrenics with a chromatographic rating of 3 or less, 10 were responding well to therapy (insulin shock, electroshock, psychiatric treatment); of the 19 schizophrenics with a rating of 8 or more, 18 had shown no response to treatment. Longitudinal studies on selected schizophrenics are now in progress to determine whether the aromatic excretion pattern shifts toward normal in patients who are responding to treatment and whether patients who show a more normal pattern on first admission are more responsive to treatment. Further studies on more than 700 individuals, including many normal persons and about 400 new admissions to the mental hospital, are in excellent agreement with the results detailed here. In

Table 1. Results of chromatograms of urines of normal individuals and schizophrenics. Each chromatogram was rated by assigning one point for each faint spot, two points for each spot of average intensity, and three points for each peculiarly intense spot; the highest rating attained by this method was 11. No prior selection of patients was made. After the chromatographic work had been completed, the diagnoses were obtained from the hospital files. Since some of the patients had been recently admitted, the diagnoses are not necessarily unequivocal.

Subject		No. (and percentage) of subjects with a rating of					Avg. rating	Avg. deviation
Type	No.	11–8	7–5	4	3	< 3		
Schizophrenic	52	19 (37%)	10 (19%)	10 (19%)	10 (19%)	3 (6%)	6.0	2.5
Patients with other mental disorders	36	1 (3%)	6 (17%)	3 (9%)	10 (28%)	16 (43%)	2.9	1.4
Normal	16	0 (0%)	1 (6%)	5 (31%)	3 (19%)	7 (44%)	2.8	1.3

more than 80 percent of the new admissions of schizophrenics, the diagnosis was predictable from the chromatographic results.

Sano (*3*) has reported that the cold Millon's test, the Davis reaction, and the Mitsuba reaction are positive in some 40 to 50 percent of the urines of schizophrenics as opposed to 1 to 5 percent of the urines of normal individuals. Our evidence to date indicates that the results from these tests generally correlate well with the chromatographic ratings, but that the chromatographic ratings give a slightly more sensitive differentiation between schizophrenics and nonschizophrenics.

Reported R_f values for histidine derivatives in the solvent system used suggest that histidine derivatives are not responsible for any of the spots at $R_f > 0.1$. Confirming evidence has been obtained from studies of the changes in excretion pattern of persons on restricted diets (*6*). Four of the chromogens have been tentatively identified as indican, 3-hydroxyanthranilic acid, dihydroxyphenylalanine, and 5-hydroxyindoleacetic acid. Further studies are in progress .

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References and Notes

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Studies of Chlorotetracycline Biosynthesis and the Preparation of Chlorotetracycline-C^{14}

As part of an investigation of the metabolism of *Streptomyces aureofaciens* Duggar, a number of studies have been made of the sources and intermediate states of the carbons in the chlorotetracycline (CTC) (*1*) that is accumulated by this organism. The organism studied was a mutant designated as *S. aureofaciens* BC-41, which is a descendant, through a series of mutation treatments, of the original A-377 soil isolate of Duggar.

Table 1. Carbon-14 incorporation into chlorotetracycline.

Labeled substrate	Incorporation (48-hour addition) (%)
Starch-C^{14}, uniformly labeled (*8*)	17.3
D-Glucose-1-C^{14} (*4*)	8.6
D-Glucose-2-C^{14} (*4*)	6.3
D-Glucose-6-C^{14} (*4*)	14.5
D-Glucose-C^{14}, uniformly labeled	12.2
D-Fructose-1,6-C^{14} (*4*)	3.4
Glycine-2-C^{14} (*8*)	52
L-Methionine-CH_3-C^{14}	48
D,L-Serine-3-C^{14}	18
Glycerol-1-C^{14}	6.5
Sodium acetate-1-C^{14}	13
Sodium acetate-2-C^{14}	15
Ethanol-2-C^{14}	7.4
Sodium formate-C^{14}	3.8
Formaldehyde-C^{14}	3.2

Measurements were made of the extent of carbon-14 transfer from a number of C^{14}-labeled metabolites to the carbon skeleton of CTC. For these experiments, the nutrient medium contained, as carbon sources, starch, corn-steep liquor, lard oil, and calcium carbonate (*2*). The weight ratio of labeled substrate added to CTC that was subsequently formed was less than 0.04. The CTC from each fermentation was isolated chromatographically. The product's radioactive purity was demonstrated by catalytic hydrogenation (*3*) of the radioactive CTC to a subsequently chromatographically isolated tetracycline (TC) of the same molar radioactivity.

Those metabolites significantly incorporated are presented in Table 1. Metabolites not significantly incorporated (< 3 percent) when added at zero hours were D,L-alanine-2-C^{14}, D,L-histidine-2-C^{14}, D,L-leucine-2-C^{14}, D,L-glutamic acid-2-C^{14}, D,L-methionine-2-C^{14}, adenine-8-C^{14}, guanine-8-C^{14}, urea-C^{14}, glycerol-1-C^{14}, sodium acetate-2-C^{14}, sodium carbonate-C^{14}, and phenol-1-C^{14}. Substrates not significantly incorporated when added at 48 hours, a time of rapid CTC production, were D-glucitol-1-C^{14} (*4*), L-arabinose-1-C^{14} (*4*), D-arabinose-1-C^{14} (*4*), D-arabinose-5-C^{14} (*4*), D-ribose-1-C^{14} (*4*), D-xylose-1-C^{14} (*4*), lactic acid-1-C^{14}, lactic acid-2-C^{14}, succinic acid-2-C^{14}, glycine-1-C^{14}, sodium carbonate-C^{14}, and shikimic acid-C^{14} (*5, 6*).

The CTC prepared from a fermentation to which starch-C^{14} had been added at the beginning of the fermentation cycle had a specific radioactivity 0.8 to 0.9 that of the starch, indicating that, under these fermentation conditions, 80 to 90 percent of the chlorotetracycline carbon originated from starch. The incorporation data can be considered evidence against a pathway from starch to CTC involving either fructose, pentoses, the Krebs cycle, carbon dioxide fixation, or shikimic acid (*6*).

The good incorporations observed from a number of metabolites known to be sources of one-carbon groups and the marked differences in extent of incorporation observed between the two differently labeled glycines and the two methionines indicate a role in the biosynthesis for one-carbon groups. The possibility of preferential appearance of carbon-14 from such donors in the 4-dimethylamino group of CTC was tested by degrading CTC, which was prepared from a fermentation containing glycine-2-C^{14}, with alkali (*7*) and isolating the resulting dimethylamine. Forty percent of the CTC radioactivity was found in the 4-dimethylamino group, which was approximately 4 times that expected from random labeling.

Pure, crystalline chlorotetracycline-C^{14} has been isolated in good yields from fermentations carried out in the presence of starch-C^{14} and in the presence of glycine-2-C^{14}. The specific radioactivity of the CTC that results is limited only by the radioactivity of the starch or glycine available.

The carbon-14 incorporations observed from the afore-mentioned simple substrates suggest that *S. aureofaciens* has the capacity to build the complete CTC molecule from simple materials. Related experiments have shown that *S. aureofaciens* spores can germinate, form mycelium, and biosynthesize CTC in a glycerol-mineral medium containing glycerol as the sole source of carbon and containing ammonium ion as the sole source of nitrogen. The concentrations of CTC accumulated during the glycerol-mineral-supported fermentation cycles were less than those accumulated during the corn-steep-supported cycles, but they show clearly that the organism is able to construct the entire CTC structure from simple beginnings.

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References and Notes

1. The trademark of the American Cyanamid Company for CTC is Aureomycin.
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