*INDOLURIA IN SCHIZOPHRENIA:

II. CHROMATOGRAPHIC STUDY ON 40 SCHIZOPHRENIC AND 10 NORMAL SUBJECTS

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Introduction

In Part I of this communication the theoretical basis for the investigation was outlined. Details of a statistical study were given and it was suggested that these confirmed a greater incidence amongst schizophrenics than normals of subjects excreting an excess of indole substances. In addition the question of hepatic dysfunction was investigated in 48 schizophrenic patients using the Cephalin-Cholesterol Flocculation Test. The results of this investigation were essentially negative.

The present paper describes a chromatographic study of urine samples from 40 schizophrenic and 10 normal subjects. The object was to investigate the possibility of differences in the type of indoles excreted.

Experimental Observations

In this part of the project it was planned to study a smaller series of schizophrenic patients, both from the clinical aspect and as regards the pattern of urinary excretion of indolic substances. It was anticipated that identification of the various indolic substances should be possible and that it might be possible to correlate any differences so found with clinical status or diagnostic grouping.

Material

This consisted of 40 schizophrenic patients, 15 women and 25 men. The mean age was $43 \cdot 5 \pm 7 \cdot 5$ years. Amongst the women the diagnostic categories were represented thus: Catatonic 2, Hebephrenic 7, Paranoid 6. Amongst the men there were 8 Catatonic, 6 Paranoid, and 11 Hebephrenic patients. It will be appreciated that these categories are primarily descriptive. Many patients over a period of years show a variety of symptoms which would strictly entitle them to placement in other diagnostic categories. For the purposes of this study the paranoid subgroup may be defined as an illness in which ideas of persecution with or without ideas of grandeur are prominent and persistent. Catatonic schizophrenia similarly means an illness in which alternations between stupor and impulsive excitement have been prominent and in which abnormalities in the motor sphere (i.e. gait and posture) have been noted. Hebephrenia means an illness in which blunting and incongruity of affect, hallucinosis and a rather rapid deterioration of the personality are prominent features.

Most of these patients had been in hospital a long time and there was no reasonable doubt about the diagnosis. However, they were all interviewed by

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the author personally, the ward sister kindly supplied information about them, and the case notes were inspected. The mean length of illness for the whole group was $17 \cdot 2 \pm 7 \cdot 6$ years. In addition ten normal subjects were studied (5 male, 5 female), the mean age being $26 \cdot 3 \pm 8 \cdot 4$ years.

Methods

Once again "night urine" samples were examined. A concentration procedure based on that described by Dalgleish (1955) was employed. (1) Deactivated charcoal was prepared by adding slowly, with stirring, 100 g. of activated charcoal to a solution of 8 g. of stearic acid in 500 ml. of ethanol. The mixture was stirred for 60 minutes, was diluted with 5 litres of water and the charcoal was then collected on a Buchner funnel. The charcoal was then washed with more water and air-dried. (2) Fifty ml. of urine was adjusted to approximately pH 4 with acetic acid and was then shaken with 1 g. of deactivated charcoal. The mixture was then poured into a sintered glass funnel and the adsorbent collected on the sintered glass disc. It was then washed with further water to remove salts and aliphatic substances. Then 25 ml. of 7 per cent. aqueous phenol was added to the funnel and the eluate collected in a beaker. A further 10 ml. of distilled water was then passed over the charcoal and this eluate was also collected, giving a total volume of approximately 35 ml. (3) The eluate was evaporated under a water-pump vacuum and on a water-bath (the temperature was kept below 80° F.). Two further portions of 5 ml. of distilled water were added to the retort during the distillation to assist in the elimination of the phenol. The final volume lay between 5 and 15 ml. (4) One hundred microlites of the concentrate so prepared was then applied to the right-hand lower corner of sheets of Whatman No. 1 chromatography paper ($33 \cdot 5 \times 31 \cdot 5$ cm.) and two-way ascending chromatograms were run (based on the method described by Jepson, 1955). The first solvent was Isopropanol-Ammonia and the second was Butanol-Acetic acid. The runs were performed in all-glass tanks (one for each solvent), the papers being held in position by steel clips attached to steel cross-wires (the tanks and fittings were supplied by Messrs. Shandon). The first run was allowed to proceed for about 16 hours, and the second run for about 10 hours. The papers were then dried in a fume-cupboard and subjected to location agents. (5) Two "papers" were prepared from each concentrate. The first paper was dipped in a ninhydrin-acetic solution (Jepson and Steven, 1953), dried and heated in an electric oven set at 110° F., for two minutes. The paper was examined under ultra-violet light provided by a Hanovia Chromatolite. Fluorescent spots were outlined in lead pencil, then this same paper was dipped in an Ehrlich solution, dried, and the coloured spots again outlined in pencil (broken line).

The second paper was examined initially under U.V.L., without any pretreatment, and fluorescent spots were outlined in pencil (solid line), then this second paper was dipped in a sulphanilic acid reagent, dried and then the spots were outlined in pencil (broken line).

Reagents Used

(a) *Ninhydrin-Acetic Acid*

Ninhydrin 0·2 g. made up with acetone to 90 ml. To this was added 10 ml. of glacial acetic acid. This reagent had to be made up freshly for each set of papers.

(b) *Ehrlich Reagent*

Ten g. of p-dimethylamino-benzaldehyde was dissolved in concentrated hydrochloric acid and made up to 100 ml. Ten ml. of this stock solution was diluted for use with 40 ml. of acetone.

(c) *Sulphanilic Acid Reagent*

Sulphanilic acid 4·5 g. was dissolved in 45 ml. of concentrated hydrochloric acid and made up with distilled water to 450 ml. (Stock solution A.). Sodium nitrite 25 mg. was dissolved in 500 ml. of distilled water (Stock solution B.). When required 10 ml. of A. was added to 10 ml. of B., and the mixture was allowed to stand for five minutes. To this was then added sodium carbonate 20 ml. of a 10 per cent. W/V aqueous solution. This latter was added cautiously as the mixture effervesces rather vigorously.

Identification

Each "batch" of papers run consisted of two "urine samples" (two papers each) and one reference paper. On to the right-hand lower corner of this paper was "spotted" 10 microlites of a solution containing tryptophan, 5-hydroxy tryptamine, tryptamine, indolyl-acetic acid and 5-hydroxyindoleacetic acid (1 mg. per ml. of each substance). The reference paper was subjected to the ninhydrin-acetic acid and Ehrlich procedure.

In any sample in which a spot for indole-acetic acid was found the identity was confirmed by later running a third paper and treating it with an acid oxidizing reagent; a fresh saturated solution of potassium persulphate was prepared and to 1 drop of this was added 10 ml. of concentrated hydrochloric acid and 40 ml. of acetone.

It should be made clear that all these location reagents were used in shallow polythene trays as "dips".

Identification was effected by:

1. Comparison of Rf. values.
2. Comparison with reference paper.
3. Colour reactions with Ehrlich "dip".
4. Fluorescence under U.V.L., both in untreated papers and after treatment with ninhydrin-acetic acid.
5. Colour reactions with sulphanilic acid "dip".
6. Colour reactions in selected cases with acid oxidizing reagent.

In Table I below are shown the Rf. values obtained, using this technique, for the reference substances already listed. In Table II are shown the colour reactions for these reference substances.

TABLE I

Rf. Values for Reference Substances

Reference Substances	Rf. Values × 100	
	Isopropanol-Ammonia	Butanol-Acetic Acid
5-hydroxy-indolyl-acetic acid	15–20	72–80
5-hydroxy-tryptamine	54–60	44–50
3-indolyl-acetic acid	30–36	85–95
Tryptamine	74–84	70–76
Tryptophan	20–26	44–52

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TABLE II

Colour Reactions of Reference Substances

Reference Substances	Fluorescence with U.V.L.	Fluorescence after Ninhydrin-acetic Acid	Ehrlich Reagent	Sulphanilic Acid Reagent	Acid Oxidizing Reagent
5 H.I.A.A.	—	—	purple-blue —blue	chocolate	pink-purple
5 H.T.	—	green-blue	blue-purple —grey-blue	chocolate	—
I.A.A.	—	—	pink-purple —grey	—	bright pink
tryptamine	—	green-blue	purple	—	—
tryptophan	—	—	purple	—	—

RESULTS

These are shown in Tables III, IV and V.

TABLE III

Indole Substances Detected in Urine Concentrates from 40 Schizophrenic Patients

Patient	5 H.I.A.A.	I.A.A.	Indoxyl Sulphate	Trypto-phan	Q.F.B.	"X"	Other Spots
J.D. ..	X	X	X	X	X	X	B.G.E. Rf. 10–30
H.R. ..	X	X	X	X	X	X	
J.J. ..	X	X	X	X	X		
H.H. ..	X		X				
M.S. ..		X	X	X			OE Spot at Rf. 15–45 and PRE Spot 50–90
J.R. ..			X	X			PRE Spot at 52–90
M.L. ..				X			PRE Spot at 52–90
I.G. ..		X	X	X			
O.R. ..	X		X	X	X		
H.L. ..	X	X	X	X		X	
J.L. ..	X	X	X	X	X		
A.L. ..			X	X	X		
I.B. ..		X	X				
M.G. ..			X	X			
M.D. ..	X	X	X	X	X	X	PRE Spot at Rf. 50–85
M.C. ..	X	X	X	X	X	X	
W.R. ..			X	X			
W.W. ..	X	X	X	X	X		PRE Spot at Rf. 52–85
J.D. ..	X	X	X	X	X		
T.McC. ..	X	X	X	X	X	X	
H.McI. ..		X	X	X	X	X	
R.M. ..	X	X	X	X	X	X	
T.R. ..	X	X	X	X	X		
N.R. ..	X		X	X	X		Two OE Spots at Rf. 15–45 and 50–85 (both blue with U.V.L.)
W.B. ..	X	X	X	X	X	X	
A.G. ..	X		X	X			
W.A. ..		X	X	X	X		
N.K. ..	X	X	X	X	X		
J.B. ..	X	X	X	X	X		
J.R. ..		X	X	X	X	X	
H.S.T. ..	X	X	X	X			
J.S. ..	X		X	X		X	
W.R. ..			X	X			
J.R.W. ..	X	X	X	X			
R.C. ..	X	X	X	X	X		

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TABLE III (*continued*)

Patient	5 H.I.A.A.	I.A.A.	Indoxyl Sulphate	Trypto-phan	Q.F.B.	"X"	Other Spots
D.McW. ..	X	X	X	X			OE spot at Rf. 15–45—blue U.V.L.
R.O. ..	X		X	X	X	X	OE spot at Rf. 15–45—blue U.V.L.
R.McC.M.		X	X	X		X	
D.J. ..	X	X	X	X	X		
J.G. ..			X	X			

TABLE IV

Indole Substances Detected in Urine Concentrates from 10 Normal Subjects

Subjects	5 H.I.A.A.	I.A.A.	Indoxyl Sulphate	Trypto-phan	Q.F.B.	"X"	Other Spots
a	X	X	X	X	X		
b	X		X	X			
c	X	X	X	X		X	
d	X	X	X	X			
e	X		X	X	X	X	
f	X	X	X	X			
g			X	X			
h	X	X	X	X			
i	X		X	X		X	
j	X		X	X	X		
No. of subjects excreting various indole substances	9	5	10	10	3	3	
Per cent. incidence	90	50	100	100	30	30	

Spot Q.F.B. (quickly fading blue) occupied a position approximately that expected of serotonin (higher Rf. in IprA). However, the colour was different, it appeared earlier and the response to ninhydrin-acetic acid and U.V.L., was equivocal. The possible identity of this spot will be discussed later. The spot was termed Q.F.B., as it appeared to be identical with that recently reported by Riegelhaupt in schizophrenic urine (1958). Spot "X" (Rf. 22–70) gave pale purple, fading to blue-grey with Ehrlich. There was no response to the other location reagents. It was considered possibly to be indolylacetyl-glutamine. The spot found in a few urines at Rf. 15–45, giving a blue fluorescence (untreated) with U.V.L., and an orange reaction with Ehrlich's reagent was thought to be kynurenine.

The other spot at Rf. 50–90 (52–85) gave a purple-red reaction with Ehrlich's reagent but no fluorescence with U.V.L. This spot was thought to be γ-(3-indolyl)-butyric acid but this supposition was not confirmed by use of the acid oxidation reagent. This may be due to the fact that the colour with Ehrlich's reagent was pale and Ehrlich's reagent is very much more sensitive than the acid-oxidation reagent.

The numbers of schizophrenic subjects excreting these various indolic substances (excluding kynurenine and the other spot, supposed to be γ-(3-indolyl)-butyric acid) are shown in Table V.

TABLE V

The Number of Patients Excreting Various Indolic Substances (40 Subjects)

Substances	H.I.A.A.	I.A.A.	T.	I.	Q.F.B.	"X"
No. of patients excreting ..	26	27	39	40	23	13
Per cent. of total No. of patients	65·0	67·5	97·5	100	57·5	32·5
Total No. of patients ..	40	40	40	40	40	40

Discussion

The observations on the 40 schizophrenic subjects reveals that, apart from tryptophan and indoxyl sulphate which were more or less constant, the substances detected were 5 H.I.A.A. (65 per cent.) spots "Q.F.B." (57·5 per cent.) and "X" (32·5 per cent.). Haverback *et al.* (1956) using Udenfriend's quantitative method, found low normal values for the excretion of 5 H.I.A.A. in the schizophrenics they studied. Buscaino and Stefanachi (1958) found no significant difference as regards 5 H.I.A.A. between their schizophrenic and normal samples. These authors used a qualitative colour reaction described by Sjoerdsma *et al.* (1955), a chromatographic technique and also Udenfriend's quantitative method. They comment that in only 85 per cent. of cases in which the colour reaction had been positive was 5 H.I.A.A. detected in the chromatogram.

Leyton (1958) found that 20 per cent. of his schizophrenic subjects excreted less 5 H.I.A.A. than did the controls. Perhaps relevant to this is the fact that Lauer *et al.* (1958) found that their schizophrenic subjects did not respond to a "tryptophan load test" with increased excretion of 5 H.I.A.A. while all their control subjects showed a 100 per cent. increase in the excretion of this compound.

Spot "X" is believed by the author to be indole acetyl glutamine. This spot was detected in 32·5 per cent. of schizophrenic and 30 per cent. of normal samples. Leyton (1958) similarly found no significant difference in the excretion of this compound as between normals and schizophrenics. Buscaino and Stefanachi found indole acetyl glutamine less commonly in the samples from schizophrenic subjects.

Indole acetic acid (I.A.A.) was found in 27 schizophrenic urine samples (67·5 per cent.) as compared with 5 normal samples (50 per cent.) Buscaino and Stefanachi found I.A.A. slightly less frequently in their schizophrenic samples while Leyton found no significant difference. Ross (1913) originally reported that schizophrenics excreted an excess of I.A.A.

V. M. Buscaino (1952) quotes another Italian, Gullotta, as demonstrating in 1929 an excess of I.A.A., in schizophrenic urines. Similarly Sherwood (1958) is reported as having found an excess of I.A.A. in schizophrenia, phenylketonuria and terminal cancer cases. Out of interest the present author examined urine from five cases of carcinomatosis and one case of phenylketonuria. As expected the latter urine gave intense spots for I.A.A., and indolelactic acid (I.L.A.). However, I.A.A., was not detected in any of the cancer cases though one sample gave a very intense "Q.F.B." spot.

Spot "Q.F.B." appears to be identical with that reported by Riegelhaupt (1958) who so named it because it faded quickly. Rodnight and Aves (1958) report a spot with similar Rf. values and staining reactions. They named it "U 2", found that it was seen more commonly in schizophrenic urine and suggested that it was a skatole derivative. Buscaino and Stefanachi found a spot with similar Rf. values in isopropanol-ammonia and butanol acetic acid. The colour with Ehrlichs seems to have been similar to that found by Rodnight and Aves and by the present author.

Buscaino (1958b) suggests that, though he thinks "Q.F.B." may be 5–6 dimethoxy tryptamine, other workers doubt whether it is an indole at all. However, there seems to be no doubt that, whatever the identity of Q.F.B., may in fact be, it is excreted more commonly in schizophrenic urine.

The author inclines to the view that "Q.F.B." may represent more than one

substance as was found by Riegelhaupt (1958) who attempted to isolate it. If Q.F.B. does represent 5–6 dimethoxy tryptamine this finding may have some relevance. The general view of biochemists is that methoxy derivatives are the result of the activity of intestinal bacteria on dietary protein. Apparently there are exceptions to the rule; thus methoxy derivatives of oestrogen have been detected in human urine (Fotherby, 1958); Axelrod has detected methoxy-adrenaline in rat urine while Hoagland (1958) quotes Gaudette and Scott (1958) as detecting 3-methoxyadrenaline and 3-methoxy-4-hydroxy mandelic acid in rabbit urine after the administration of adrenaline labelled with ^{14}C.

Whether the process of methoxylation occurs through bacterial action within the gut lumen or as a metabolic process within the body proper is to some extent an academic question at the present time. What is important is that four different laboratories have reported the more frequent detection, in schizophrenic as opposed to non-schizophrenic urine, of a substance variously called "Q.F.B." and "U 2". The author believes that the Rf. values and staining properties reported suggest that these different workers are in fact talking about the same substance. This finding may be due to dietary differences or other environmental artifacts of the sort noted by Horwitt (1956). This is a problem which clearly merits further research.

The only clinical correlation observed in this study was between the excretion of spot "Q.F.B." and the catatonic subgroup. There were ten catatonic subjects in all and seven of these were found to excrete Q.F.B. (70 per cent.). This incidence is higher than that for the whole group of 40 subjects (57·5 per cent.) but as the numbers are small the author does not feel justified in laying too much emphasis on this finding. No correlations, positive or negative, were found between indole excretion, age, length of illness or other diagnostic features.

Summary

1. Concentrates were made from urine samples collected from 25 male and 15 female schizophrenic subjects, 10 normal subjects were also studied.
2. These concentrates were used in two-way ascending chromatograms and an attempt was made to identify the indoles detected.
3. One "abnormal" spot ("Q.F.B.") was found in 23 schizophrenic subjects and in 7 out of the 10 catatonic patients (70 per cent. incidence).
4. These results are discussed in relation to other reports on the excretion of indoles in schizophrenia.

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