

STUDIES IN SCHIZOPHRENIA—VI
PAPER CHROMATOGRAPHY OF URINARY INDOLES AND THE
“GLYOXYLIC ACID” REACTION

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THE following is a short report on the results obtained from 204 two-way paper chromatograms for urinary indoles. The material examined came from 58 schizophrenics, 34 organic psychoses, 58 affective psychoses (33 complicated by physical disease, and 25 not), 48 neurotics etc. (18 complicated by physical disease and 30 not) and 6 mentally normals.

The urines were with few exceptions, first morning specimens from new admissions, sent to the laboratory for routine examinations. They came from 98 men and 100 women from three and two admission wards respectively, and 6 female staff. The specific gravity was on the average 1.020, with average variations of ± 0.008 .

The tests carried out for the purpose of this examination were:

1. *Two-way paper chromatography*: 2 ml. of clear urine contained in a 10 ml. beaker were concentrated *in vacuo* over phosphoric oxide. In this way a four- or five-fold reduction in volume could be obtained within 24 hours. The volume was measured and the equivalent of about 100 μ l of the original specimen submitted to ascending two-dimensional chromatography using the solvent system of Jepson (7). If the concentrate could not be used at once it was stored overnight at 4° C. in a 5 ml. screw-capped bottle. The concentrate was spotted 3 cm. from the lower right-hand corner of 10 inches square Whatman No. 4 filter paper using a platinum loop constructed to hold 5 μ l. of water. Each spot was dried in a stream of warm air (about 45° C.) before a further drop of concentrate was added. Loaded papers were first run in isopropanol-water-ammonia (200–20–10) (IPRAM) overnight (16 hours), the lid of the tank being sealed with 50 per cent. glycerol solution. After thorough drying in a stream of unheated air, the second run was made at right-angles to the first in n-butanol-water-acetic acid (120–50–30) (BuA) for six hours. After drying in an unheated air-stream the papers were drawn through Ehrlich reagent (Jepson (7)) contained in a shallow trough (10 ml. of a 10 per cent. solution of p-dimethylaminobenzaldehyde in pure HCl diluted to 50 ml. with redistilled acetone). The papers were observed continuously for the first 5–10 minutes, periodically for the next hour and finally examined the following morning. Spots were ringed with soft pencil as they appeared. Notes were made of colours, their intensity and speed of development and of any colour changes as they became established.

2. The "glyoxylic acid" ("g.a.") reaction (as described by Fleischhacker and Lancaster (5)), followed by chloroform (or carbon tetrachloride) extraction. The glyoxylic acid reagent was used throughout and specific gravities were not adjusted.

3. The Obermeyer test for indican. Attention was paid to the question of treatment of patients. Diagnoses were obtained only after all laboratory tests had been completed, and as a rule several weeks after admission in order to give the clinician time to make his diagnosis. The schizophrenic group consisted of idiopathic and also a number of "symptomatic" schizophrenics; the "organic" group consisted mainly of patients suffering from arteriosclerotic and senile dementias and also a few with Huntington's Chorea and epileptic and other psychoses. The complications of the affective and neurotic group (the latter containing also some patients with psychopathic personality) were mainly vascular and heart disease, metabolic disorders, alcoholism and peptic ulcers.

RESULTS

1. Chromatography

The number of spots excluding urea, varied between two and fourteen per person in the following way:

Number of spots	..	2-4	5-7	8-10	More than 10
Number of persons	..	54	97	46	7

On the whole there was no essential difference in the number of spots per person in connection with sex or age, and, as far as could be ascertained, the administration of drugs (e.g. sodium amytal, and, in a few cases Largactil), nor is there any clear relation to the concentration of indican as demonstrated by the Obermeyer reaction. Other workers too (Rodnight and Aves (11), Curzon (3)) have not found the connection in this respect as claimed by Sano (12). It is true that when the Obermeyer reaction was weakly positive, only 2 to 4 spots were more frequently found; but often with a very outspoken Obermeyer reaction the number of spots was also small. The number of spots was often somewhat larger in urines of high specific gravity and somewhat smaller when the specific gravity was low; but again this was not invariable.

In the different groups the average number of spots per person were as follows:

Schizophrenic	6.3	Organic psychoses	6.2
Complicated affective	6.3	Uncomplicated affective	6.1
Complicated neuroses	6.1	Uncomplicated neuroses	5.5
Mentally normal	6.0				

Patients with more than 8 spots were somewhat more frequent in the schizophrenic, complicated affective and complicated neurotic than in the other groups.

Altogether over 30 different spots were seen, a number of them very faint and soon disappearing, others more intense and lasting for several hours or even longer. The colours seen were yellow, brown, pink, purple, blue, grey, green and intermediate shades. Excluding those of the yellow, yellow-brown, brown series, there were 26 different spots seen, some very rarely. Of these, 13 appeared in more than 10 per cent. and have positions which agree quite well with those on the chart published by Buscaino and Stefanachi (1); but only 7 spots appeared in 40 per cent. or more of the material (including indoxyl

sulphate, present in all). It is with these 7 spots that we are dealing in this communication.

It may be mentioned in brief that of the rarer spots none apparently were characteristic of any groups, except possibly spot 12 (see later).

Slight variations in technique (temperature, filter paper, and so on) can introduce variations in the position and intensity of spots, as can be seen from charts published by different authors, and this has to be borne in mind when considering the following map. The spots are numbered in order of frequency with which they were found in our material.

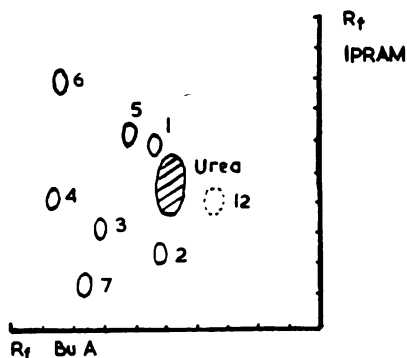
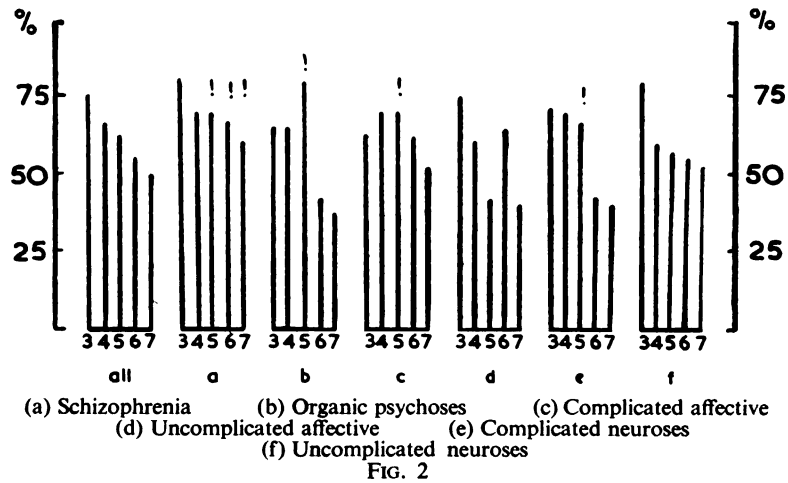


FIG. 1

1. Indoxyl sulphate.
2. Tryptophan.
3. Probably indolyacetyl glutamine.
4. Probably 3-indolyacetic acid.
5. A not definitely defined substance, possibly a skatol derivative (Buscaino and Stefanachi's 3, Leyton's S, Riegelhaupt's Q.F.B. (although it often did not fade very quickly in our material), Rodnight's U.2).
6. Position similar to that of tryptamine, but it is probably not this substance (Rodnight and Aves (11)).
7. Probably 5-hydroxy-indolyl acetic acid.
12. Slightly bluish-green, Rf 42/35, appearing late (after 20–45 minutes) and still visible after 18 hours (? Buscaino and Stefanachi's 8 (1) and Riegelhaupt's 54/33 (10)). Present in about 15 per cent. of schizophrenic, organic and complicated psychoses, mainly in urines yielding at least 6 spots, independent of high-intensity spots, sex and age.

The following histogram illustrates the distribution of spots 3 to 7 in the different groups. Spots (1) which appeared in all, and (2) which appeared in 88 per cent. to 96 per cent. of all groups are omitted. In each group all these spots appeared in at least 40 per cent. of urines examined.

We have marked by ! those spots where frequency of appearance may have some characteristic meaning particularly in combination with the others in this or that group, e.g. spot 5 is a little more frequent in schizophrenics and other "organic" conditions; spot 12 did not appear in any of the uncomplicated neurotics, but it was present only in 15 per cent. of the other groups. If spot 4 is omitted, the number of the more common spots is highest in schizophrenics, which agrees with Rodnight and Aves' findings. On the other hand, it was not possible to find any obvious difference between idiopathic and symptomatic schizophrenia or arteriosclerotic and senile dementia.



It is clear from this that none of these substances as such can be of any diagnostic significance and the purely qualitative aspect of indole-compound chromatography in urines will not lead us any further. Papers published by other authors (Sano (13), Curzon (3) and Leyton (8)) confirm this view. On the other hand there are some differences which need not necessarily be due to external circumstances. They may be an indication of metabolic variations in different individuals, either as a consequence of disturbed functions, or as predisposing them. It can also not be excluded that differences in pattern are not only of a qualitative but also of a quantitative nature.

We found great difficulty in evaluating the intensity of spots except where variation from "average" was extreme. Although quite a number of faint or very faint spots were seen, only 33 from 24 people were thought to be of striking intensity, amongst them, most often, spot 6. They were all found in chromatograms with at least 6 spots and in all 6 groups to the same extent. The results of authors who have attempted semi-quantitative estimations (Curzon, Rodnight and Aves, Leyton, Sano (13) and Buscaino and Stefanachi (2)) show several contradictions as can be seen from their papers. If one considers the mere frequency of spots in the different groups, there are marked variations between Rodnight and Aves and ourselves with regard to indolylacetyl glutamine and 5-hydroxyindolacetic acid which we found somewhat more often, and 3-indolylacetic acid which was more frequent in their material.

From all this it appears that much more rigid metabolic and chromatographic techniques have to be used, if conclusive information is desired.

2. The "Glyoxylic Acid" ("g.a.") Reaction

In order to demonstrate dysmetabolism of tryptophan in schizophrenics Riegelhaupt (9) applied the "g.a." reaction to urines. He found it positive in many cases and took this, like other authors later on (Curzon, Leyton and to a certain degree also the present authors (4, 5, 6)), as a confirmation of this hypothesis. Subsequently, we have shown that it was positive not only in idiopathic schizophrenic, but also in many other organic psychoses and even some mentally normal people when they were acutely physically ill (Fleischhacker and Lancaster). It was also observed in a few cases of acute mental disorders that the reaction was negative when it was expected to be

positive (Fleischhacker, Lancaster and Wheeler). In this investigation the results in the different groups were as described previously (Fleischhacker and Lancaster), but the number of positive and doubtful tests was smaller than expected in this more acute material, thus confirming our former experience (Fleischhacker, Lancaster and Wheeler).

Riegelhaupt (9) had tentatively concluded that there is no connection between the outcome of the "g.a." test and indican excretion, and we have shown that this is correct in principle (Fleischhacker and Lancaster). Curzon thought that an increase of tryptophan in urine may contribute to a positive "g.a." reaction, but we thought this to be unlikely and had considered that catalysts may be at play (Fleischhacker and Lancaster). Riegelhaupt (10) working in another laboratory and using the urines of a small number of selected Shenley patients thought that there might be a relation between a positive reaction and his spot Q.F.B. (probably our spot 5). This spot, however, occurs rather frequently in other conditions too. It has also been found in many mentally normal people by other workers, who took it as a product of hospitalization, a view not confirmed by us, or by Rodnight and Aves, in acute mental patients and some normals.

The reading of the "g.a." test was made particularly critically and many results were classified as doubtful (with four exceptions, all in the groups of schizophrenic, organic and "complicated" affective or "complicated" neurotic illnesses). No connection was found between the positive or negative result of the "g.a." test and any spot, combination of spots, or their intensity. In some cases the reaction was positive in the presence of indoxyl sulphate and tryptophan only; yet we know that in ordinary circumstances it is most unlikely that these substances give rise to a positive "g.a." reaction in urine, apart from the fact that on other urines where chromatograms gave only these 2 spots, it was negative. If one goes by the qualitative chromatographic pattern of indole-compounds as described here, there is either no relationship at all, or an extremely complicated one between this and the "g.a." reaction.

SUMMARY

1. The present paper chromatographic investigation for indole compounds in the urine of schizophrenics, and "organic" and "non-organic" control groups shows that qualitative differences in the excretory pattern between the various groups are of no definite diagnostic or pathogenetic value. This agrees with the work of some other authors. Fully quantitative examinations of the individual compounds under strictly controlled metabolic conditions will be more valuable; the present investigation does not make any contribution in this respect.

2. No connection was found between positive or negative "glyoxylic acid" reactions and any spot, combination of spots, or their intensity.

3. Previous impressions on the diagnostic significance of the "glyoxylic acid" reaction have been confirmed in principle, though it is possibly less often positive in acute mental patients than formerly thought.

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