*INDOLURIA IN SCHIZOPHRENIA:

I. STATISTICAL STUDY AND INVESTIGATION OF HEPATIC FUNCTION

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INTRODUCTION

INVESTIGATION of the urinary excretion of indoles in psychotic illnesses has a certain historical precedent. Thus Townsend (1905) and Bruce (1906) reported excess of indican in the urine of melancholic patients. Bruce correlated this finding with the recognized tendency to constipation in depressives and treated his patients with enemata. Ross (1913) reported that schizophrenic subjects excreted an excess of indole-acetic acid in the urine. De Jong (1945) reported the experimental induction of catalepsy in cats by the intravenous administration of indolethylamine and noted in his monograph that this substance, if perfused *in vitro* through the liver, is converted to indole-acetic acid. Buscaino (1952) reported finding abnormal amounts of primary and secondary amines in the urine of schizophrenics. More recently Riegelhaupt (1956, 1958) has reported an excess of tryptophan metabolites in schizophrenic urine while McGeer and co-workers (1957) have reported a difference in the excretion of aromatic substances as between normals and schizophrenics. Though these authors do not make the point clear, yet in fact indoles come under the general heading of aromatic substances.

There are currently three main chemical concepts of schizophrenia. The first theory, that an abnormal oxidation product of adrenaline might be the endogenously produced toxic substance involved, was promulgated by Osmond and Smythies (1952). A further report (Hoffer, Osmond and Smythies, 1954) described experiments with adrenochrome. However, their results were not confirmed (Rinkel *et al.*, 1957). Further reports by Hoffer (1957a, 1957b) on the hallucinogenic effects of adrenolutine were somewhat equivocal.

The second theory, proposed by Woolley and Shaw, that schizophrenia might result from an excess or a deficiency of the neurohormone serotonin (5 hydroxy tryptamine) remains a hypothesis but has resulted in considerable research into serotonin and other tryptophan metabolites.

The third concept, that of Heath and his co-workers (1958), implicates a circulating toxic substance which they have named Taraxein. They isolated this substance from schizophrenic serum and found that they could produce schizophrenic symptoms by injecting the extract into healthy volunteers. It has been suggested that the substance may be a globulin. As yet there has been no confirmation of their findings by other workers.

This present investigation is based on somewhat different ideas. It seems to the author that a specific endogenously produced toxic factor in schizophrenia would be biologically improbable. An analogy with diabetes mellitus

* Based on work already submitted for the Degree of M.D. at the University of St. Andrews.

https://doi.org/10.1192/bjp.105.440.674 Published online by Cambridge University Press

might be illustrative. With the discovery of insulin the aetiology of diabetes seemed clear but in fact this has proved to be far from the case. A defect in the secretion of insulin by the Beta cells of the pancreas is certainly an important factor but only one factor in a complex system for maintaining this particular aspect of homeostasis. Similarly in schizophrenia, if we are in fact dealing with a biochemical disorder, it seems likely that the defect will be a complex one and one involving more than one anatomical system.

There are certain theoretical grounds for believing that the current interest in tryptophan and its metabolites may not be misplaced. Tryptophan has, of course, an indole nucleus and shares this feature with the majority of known hallucinogenic substances. Serotonin is synthesized in the body from ingested tryptophan and is known to have some role in brain function (Brodie *et al.*, 1955). In fact serotonin shares with noradrenaline the function of synaptic inhibition in experimental preparations, measuring impulse transmission across the transcallosal synapse in the cat (Marrazzi and Hart, 1955). These "neurohormones" also share another feature, that of being inactivated by the same enzyme, monoamine oxidase. It may also be relevant that reserpine, one of the most powerful neuroleptics used in the treatment of schizophrenia, appears to work through the release of serotonin from its usual "bound" form (Pletscher *et al.*, 1956).

As mentioned earlier, there are historical grounds for this re-emergent interest in tryptophan metabolites. However, the work of Bruce and Townsend was severely criticized by Easterbrook (1906) and, in fact, the theory of autointoxication fell into disrepute until resuscitated in sophisticated form in relation to hepatic encephalopathy (McDermott and Adams, 1954). Recent work in this field (Dawson *et al.*, 1957; Phear *et al.*, 1956) suggests that hepatic coma may be due to the absorption of toxic substances from the small intestine, the result of bacterial action on ingested protein. In the patients under discussion there is, of course, the particular factor of a portal-systemic shunt so that blood can travel straight to the cerebral circulation without passing through the liver.

A recent paper by Summerskill (1958) advocates the use of neomycin to depress the activity of the bacterial flora, plus a low protein intake and purgation. It should be made clear that constipation *per se* is irrelevant. The site of the bacterial action is the small gut where the absorption of toxic protein breakdown products also occurs. It could be objected that hepatic encephalopathy bears little clinical or symptomatic relationship to schizophrenia. This is, of course, true but the question of hepatic dysfunction in schizophrenia has in fact been the subject of extensive research. The Quick Test, in which administered benzoic acid is coupled with glycine to form hippuric acid which is then excreted in the urine, was employed by Quastel and Wales (1940). These authors found a low excretion rate in 100 per cent. of 18 catatonic patients and 15 per cent. of other schizophrenics studied. However, their findings were not confirmed (Greville, 1945). The Cephalin-Cholesterol Flocculation Test (C.C.F.T.) was found by Buscaino (1952), De Jong (1945) and Shattock (1950) to give a high proportion of positive results in schizophrenia, especially the catatonic subgroup. However, Zimmermann and co-workers (1948) and Smith (1954) reported essentially negative results. Of interest in this connection was the report by Patzig and Block (1953), These workers injected mice with mescaline labelled with ^{14}C and found that the mescaline was rapidly bound to liver protein. After a time interval, which would correspond to the delay before mental changes (i.e. hallucinations) are experienced in man, they

sacrificed some of their animals and found that the brain content of mescaline was surprisingly low. They suggested that the protein-mescaline complex might be the cause of the mental symptoms or alternatively that the adsorption of mescaline on to liver protein might block sites normally available for the detoxication of endogenous toxins. It is relevant also that the first stage in the metabolism of tryptophan, namely that of ring substitution at position 5, giving 5-hydroxy tryptophan, is effected in the liver. The enzymatic processes involved have been investigated in animals (Udenfriend *et al.*, 1956).

The oxidative catabolism of tryptophan can proceed along the aromatic or the quinolic pathways. The former is initiated, again in the liver, by a labile enzyme system which is composed of tryptophan peroxidase and an oxidase which produces hydrogen peroxide as a product of its activity (Knox and Meyler, 1950). The content of the tryptophan oxidizing system present in liver is commonly low. It can be increased by feeding tryptophan (Williams and Elvehjem, 1950) and by administering adrenaline, which is believed to act via the pituitary-adrenal axis (Knox, 1951).

The author believes that the observations outlined above provide a basis for thinking that tryptophan metabolism may be implicated in the aetiology of schizophrenia or more particularly some schizophrenics. He suggests that once again a complex series of interlocking mechanisms will be involved. At the very least dietary differences, intestinal function and flora, hepatic function and probably adrenal function will be implicated and require study.

This paper describes a statistical comparison of indoluria in schizophrenics and other groups and a re-investigation of hepatic function in schizophrenia using the Cephalin-Cholesterol Flocculation Test.

Experimental Observations

Part 1. Statistical Study

The object of this part of the study was to compare the amount of indole substances excreted in the urine by different "populations". While it was appreciated that there is likely to be considerable day to day variation in such excretion patterns, it was only practicable to examine one specimen from each subject. The specimen in each case was the "night urine", that is the first urine passed on waking in the morning. Initially it was planned to collect all the urine passed between 8 p.m. and 8 a.m. However, so many of the early specimens had to be discarded as being incomplete that it was decided merely to examine the first morning specimen. It should be noted that the different "populations" were not comparable as to situation, diet or fluid intake. The specific gravity was measured on the first fifty specimens and was found to range from 1012 to 1020. However, as there appeared to be no correlation between the specific gravity of the urine and the indole content, this practice was discontinued.

Materials

The "normal population" consisted of 25 male and 20 female doctors and nurses. The mean age was $32 \cdot 6 \pm 9 \cdot 2$ years.

The "Female schizophrenic population" consisted of 34 patients resident in the wards of the Royal Edinburgh Hospital. The mean age was $57 \cdot 2 \pm 10 \cdot 5$ years.

The "Female non-schizophrenic psychotic population" consisted of 20 patients also in the hospital. The mean age was $55 \cdot 85 \pm 12 \cdot 8$ years.

The diagnosis in these 54 patients was confirmed by study of the case notes and discussion with Dr. S. Gray.

The "Mental defective population" consisted of 25 female patients in Gogarburn Hospital. The mean age was $38 \cdot 7 \pm 14 \cdot 5$ years. The diagnosis in each case was supplied by courtesy of Dr. R. Bailey, Physician Superintendent.

The "Male schizophrenic population" consisted of 61 patients resident in the wards of the Royal Edinburgh Hospital. The mean age of this group was $47 \cdot 4 \pm 11 \cdot 6$ years.

The "Male non-schizophrenic psychotic group" consisted of 50 patients also resident in the Royal Edinburgh Hospital. The mean age of this group was $52 \cdot 8 \pm 16 \cdot 5$ years. These patients were all under my care at the time of the observations and the diagnosis in each case was checked by personal observation and reference to the case notes.

The "Medical population" consisted of 25 male and 25 female patients under treatment at Bangour General Hospital. The diagnoses were supplied to me by courtesy of Dr. Wright under whose care these patients were at that time (1956). The mean age of this group was $50 \cdot 4 \pm 12 \cdot 6$ years.

Methods

The two-way ascending chromatographic method employed was based on that described by Jepson (1955). As a matter of convenience a smaller tank and aluminium frame were employed, made by Messrs. Shandon Ltd. This frame carried square papers (20 cm. $\times$ 20 cm.) which did not permit of a very wide separation of the "spots". The difficulty was overcome to some extent by using Whatman No. 1 paper.

The solvent used for the first run was Isopropanol-ammonia (Isopropanol 200 ml. + water 20 ml. and ammonia 10 ml.). For the second run Butanol-acetic acid was employed (n-butanol 120 ml. + water 50 ml. + glacial acetic acid 30 ml.).

The origin was marked in pencil 2 cm. from each edge in the lower right-hand corner of each paper. The patient's name and the date were recorded on the top right-hand corner and the paper was then fitted on to the frame. Filtered urine was then "spotted" on to the origin using a micrometer pipette (Messrs. Shandon's) in 5–10 microlitre portions. The spot was dried between each application using an electric hair drier. A total of 100 microlitres was applied. The frame employed carried twelve papers, the last one in each run being a "reference paper". On to this paper had been spotted 10 microlitres of a mixture containing urea, tryptamine hydrochloride, serotonin creatinine sulphate, indolyl acetic acid and tryptophan (1 mg. per ml. of each substance).

The first solvent (Isopropanol-ammonia) was then poured into the aluminium tray, the frame placed in the tray and the lid replaced. The "run" was allowed to proceed overnight (10 hours) the frame was removed and the solvent blown off using a hair drier. The tray and tank were then washed, the tray filled with butanol-acetic acid and the frame replaced but at right angles to the first position. This run was permitted to continue for 6–7 hours. This solvent was then blown off and the papers, still on the frame, were thoroughly dried.

The papers were then removed from the frame and were dipped through the Ehrlich location reagent. This was made from a 10 per cent. w/v solution of p-dimethylaminobenzaldehyde in concentrated hydrochloric acid. Just before use 10 ml. of this solution was mixed with acetone 40 ml. (this amount

lasted 4–5 papers). The reagent was poured into a polythene tray and the papers were rapidly drawn through the liquid. The papers were then dried by means of the hair drier (though latterly facilities became available in the Royal Edinburgh Hospital Pathology Laboratory, including the use of a fume cupboard) and examined flat on pieces of white paper. The "spots" were outlined in lead pencil as they appeared.

The Rf. values, as Jepson remarked in his paper, are not very constant using the technique but the relative positions of the indole substances are very constant. It was found that a large amount of urea in the urine caused a spreading "spot" which pushed indoxyl-sulphate ahead of it and thus displaced indoxyl-sulphate beyond its expected Rf. position. This point is also noted by Ivor Smith (1958). Also as a matter of economy the same solvent was used for two "runs". Such "old" solvents appeared to alter the Rf. values but the relative positions remained unchanged.

Rf. values were measured using a perspex device described by Bloch *et al.* (1958) and constructed according to their instructions by the author.

TABLE I

Substance	Range of Rf. (× 100)	
	IPr. Am.	Bu. A.
Urea	40–48	45–55
Tryptamine hydrochloride	72–80	60–70
Serotonin creatinine sulphate	42–50	48–57
Tryptophan	20–30	45–55
Indolyl-acetic acid	35–40	86–92

The colour reactions given by the Ehrlich dip are quite characteristic and taken in conjunction with the Rf. values give a fair idea of the probable nature of the "spots" found. In addition the time of appearance was helpful. Thus urea always appeared first and its central position provided a good "marker". Next a blue-purple spot with slightly greater Rf. values than urea appeared; this quickly faded to grey (later referred to as spot QFB). Meanwhile the characteristic orange of indoxyl sulphate appeared, then the slow purple of tryptophan and indolyl-acetic acid. Tryptamine was only seen on the reference papers. It appears about the same time as indoxyl sulphate.

It should be made clear that in this part of the project no attempt was made to identify spots. It was decided somewhat arbitrarily (on the basis of Jepson's paper) that normal urine may contain urea, indoxyl sulphate and tryptophan. These "spots" bear a fairly constant relationship to each other. Any other "spots" showing a reaction in the blue-purple-red range to Ehrlich's reagent constituted the grounds for allocation to the "excess indole excretion" group. This criterion permitted the separation of all the urine examined into the two categories "normal excretion" and "excess indole excretion".

PART 2. INVESTIGATION OF LIVER FUNCTION USING CEPHALIN-CHOLESTEROL FLOCCULATION TEST

Material

This consisted of 29 male and 19 female schizophrenic patients resident in the wards of the Royal Edinburgh Hospital. There is considerable overlap between this group and that used for the indoluria study. The new patients used in this group were either under the immediate care of the author or of

Dr. S. Gray and the diagnosis of schizophrenia was confirmed. There were 12 catatonics, 22 hebephrenics and 14 paranoid schizophrenics in this group. The mean age was $41 \cdot 4 \pm 7 \cdot 1$ years and the mean length of illness was $15 \cdot 6 \pm 11 \cdot 2$ years.

Methods

1. Five ml. of venous blood was withdrawn from the arm veins at approximately the same time each day (10–11.30 a.m.) from each patient. The blood was collected in a plain tube, allowed to clot and then centrifuged for five minutes.

2. Five ml. of anaesthetic ether was added to a bottle of cephalin-cholesterol antigen (prepared by Difco Laboratories, Detroit, Michigan). This was then kept in the refrigerator.

3. Thirty-five ml. of distilled water was brought to the boil in a 100 ml. beaker. To this was added, in parts and with stirring, 1·0 ml. of the ether-antigen mixture. The beaker was then kept on the electric hot-plate with further stirring of the mixture until the volume had been reduced to 25 ml. This constituted the "working solution".

4. To 4 ml. of 0·85 per cent. saline in a test-tube was added 0·2 ml. of patient's serum and 1 ml. of "working solution". The test-tube was inverted twice to ensure thorough mixing and was then left, uncorked, in a rack in a dark cupboard.

5. Readings were taken at 24 and 48 hours. If the emulsion remained as a homogeneous suspension the result was negative. In the case of a positive reaction the lipoid tended to flocculate and precipitate to the bottom of the tube. Complete precipitation leaving a clear supernatant fluid was classed as 4 plus. Lesser degrees were classed as 1 plus, 2 plus, 3 plus. The results were checked by consultations with Miss J. A. B. Darling, a biochemist working in the laboratory and accustomed to the details of the test.

RESULTS

Part 1

As explained under "Methods", night urine samples were examined from 95 schizophrenic subjects, 70 non-schizophrenic psychotic subjects, 50 "medical subjects", 25 mental defectives, and 45 "normals".

The chromatographic results were classified, according to criteria already described, into those showing Excess Indole Excretion (E.I.E.) and those not showing E.I.E. Fifteen out of the 50 "medical subjects" showed E.I.E. (30 per cent.) and 7 out of the 25 mental defectives (28 per cent.). The results for the other groups are shown in Table II.

TABLE II

Incidence in Different Groups of Excess Indole Excretion

Population	No. of Subjects	Mean Age	S.D.	No. with E.I.E.	Per cent. with E.I.E.
Schizophrenic males	61	47·4	11·6	32	52·5
Non-schizophrenic psychotic males ..	50	52·8	16·5	22	44
"Normal" males	25	35·9	9·9	2	8
Schizophrenic females	34	57·2	10·5	12	35·3
Non-schizophrenic psychotic females ..	20	55·85	12·8	4	20
"Normal" females	20	29·2	9·4	5	25

Applying the χ^2 formula to the figures for the male schizophrenic and male non-schizophrenic psychotic groups (using Yates correction) gives a value of 0·4929, N=1. This is not significant at the 5 per cent. level.

On the other hand the difference between the "normal" males and the other two male groups is significant at the 5 per cent. level (Table III).

TABLE III

Observed Frequencies: Male Groups

	Normal	Schizophrenic	Non-Schizophrenic Psychotic	Total
With E.I.E. ..	2	32	22	56
No E.I.E. ..	23	29	28	80
Total	25	61	50	136

Expected Frequencies: Male Groups

	Normal	Schizophrenic	Non-Schizophrenic Psychotic	Total
With E.I.E. ..	10·3	25·1	20·6	56
No E.I.E. ..	14·7	35·9	29·4	80
Total	25	61	50	136

χ^2=8·68. N=2.

Applying the χ^2 formula to the figures for the female schizophrenic and female non-schizophrenic psychotic group (again using Yates correction) gives a value of 0·9475, N=1. This is not significant at the 5 per cent. level. Similarly the difference between the "normal" and other two female groups is not significant at the 5 per cent. level (Table IV).

TABLE IV

Observed Frequencies: Female Groups

	Normal	Schizophrenic	Non-Schizophrenic Psychotic	Total
With E.I.E. ..	5	12	4	21
No E.I.E. ..	15	22	16	53
Total	20	34	20	74

Expected Frequencies: Female Groups

	Normal	Schizophrenic	Non-Schizophrenic Psychotic	Total
With E.I.E. ..	5·7	9·6	5·7	21
No E.I.E. ..	14·3	24·4	14·3	53
Total	20	34	20	74

χ^2=1·06. N=2.

There is an apparent difference in incidence of E.I.E., as between the male and female populations. The difference is not significant at the 5 per cent. level (Table V).

TABLE V

Observed Frequencies: Male and Female Populations

	Male	Female	Total
With E.I.E.	56	21	77
No E.I.E.	80	53	133
Total	136	74	210

Expected Frequencies: Male and Female Populations

	Male	Female	Total
With E.I.E.	49·8	27·2	77
No E.I.E.	86·2	46·8	133
Total	136	74	210

$\chi^2=2{\cdot}15$. N=1.

Finally the question of the relationship between age and E.I.E. has been considered (Table VI).

TABLE VI

Showing Per cent. Incidence of E.I.E., in 5 Year Age Groups

Age Groups	No. in Age Group	No. with E.I.E.	Per cent. Incidence E.I.E.
20–24	13	1	7·7
25–29	18	3	16·7
30–34	15	6	40
35–39	23	12	52·2
40–44	22	9	40·9
45–49	19	9	47·4
50–54	19	6	31·6
55–59	26	11	42·3
60–64	18	6	33·3
65–69	12	5	41·7
70–74	12	5	41·7
75–79	7	2	28·6
80–84	4	2	50
85–89	2	0	0
Totals	210	77	0

It is likely that the 50 per cent. incidence of E.I.E., for the age group 80–84 is a distortion due to the few subjects (4) in the group. The two other "peaks" at 35–39 and 45–49 probably reflect the age distribution among the male schizophrenics. On the whole the table suggests that there is little correlation between age and E.I.E., in the subjects studied.

Part 2. Cephalin Cholesterol Flocculation Tests

In accordance with the criteria for positive reactions, as outlined under Methods, four positive results were observed. The serum from two patients gave results rated as 2 plus and serum from a further two patients gave results rated as 1 plus. Thus the per cent. incidence of positive results in these 48 patients was 8·3 per cent. Two of the patients giving positive results were from the catatonic subgroup and two from the hebephrenic subgroup, representing a percentage incidence for the two groups of 16·7 per cent. and 9 per cent. respectively. It should be noted that when this flocculation test is used for

suspected liver disease a 1 plus result is often disregarded. If this practice were applied to the present findings the incidence over the series of 48 subjects would fall to 4·15 per cent.

Discussion

The statistical study reported here, involving 285 subjects, confirms that more subjects excreting excess indoles were found among the schizophrenics than among the other group studied. Only in one case, however, was the difference in incidence significant at the 5 per cent. level, namely the difference between the "normal" male, male psychotic and male schizophrenic groups. Another way of interpreting the figures would be to say that whereas there are marked differences between different members of the population regarding the amount of indolic substances excreted in the urine, yet no very marked correlation appeared between excess indole excretion and age, sex or diagnostic category. This might alternatively indicate that the schizophrenic group is heterogeneous from the aetiological standpoint.

Or it might reflect defects in the experimental methods. Admittedly the psychiatric diagnoses were only checked personally in the case of the male schizophrenic and male non-schizophrenic psychotic groups. The patients in the female groups were under the immediate care of Dr. S. Gray and the diagnosis in each case was discussed by the author with Dr. Gray. The diet differed as between the psychiatric, medical "normal" and defective subjects. Many of the patients were on neuroleptic drugs. However, there is evidence that reserpine by mouth in the usual therapeutic doses has no effect on indole excretion (Forrest, 1957). Todrich and co-workers (1958) have shown a rise in the rate of excretion of 5 H.I.A.A., after I.M. reserpine (5 mg.) but this only lasted a matter of 4–8 hours. In regard to chlorpromazine the author has studied five schizophrenics before and during treatment (300 mg. per day by mouth) and found no change in the pattern of excreted indoles. This has been confirmed by Buscaino and Stefanachi (1958) and could have been predicted from the work of Brodie *et al.* (1956). These workers found that serotonin-releasing activity was limited to certain tranquillizers belonging to the Rauwolfia group.

Notwithstanding these objections the author feels that the results of this study indicate that within the group designated schizophrenic there is a sub-group excreting an excess of indole substances. These findings are in keeping with those of Riegelhaupt (1958) and McGeer and co-workers (1957). A more recent paper by Rodnight and Aves (1958) reported that the mentally ill subjects studied showed a higher total of indole spots on their urine chromatograms than did the "normal" or "medical" subjects. Whether this suggested difference in indole excretion bears any aetiological relationship to mental illness requires more elaborate evidence. As Horwitt (1956) has pointed out, many of the supposed differences between schizophrenic and non-schizophrenic subjects may be due to environmental artifacts. The investigation of hepatic function in 48 schizophrenic subjects using the Cephalin-Cholesterol Flocculation Test gave essentially negative results. Hanger (1939) who introduced this test examined 900 normal subjects and got only one positive result. The test has been extensively used in schizophrenia. De Jong (1945) reported 46·8 per cent. of positive results in catatonics and lower figures for other schizophrenics. He also reported 6·9 per cent. of positive results in his controls. Negative results were, however, reported by Zimmermann *et al.* (1948) and Smith (1954).

These differences may reflect the improved dietary and physical health of hospitalized schizophrenics over the past 15 years. This is the sort of environmental artifact to which Horwitt referred in his paper. However, Richter (1957), reviewing the work on liver dysfunction in schizophrenia, concluded that whereas there is no evidence that hepatic dysfunction is an obligatory concomitant of schizophrenia yet we are not in a position to exclude the possibility of disturbance at an enzymatic level.

Summary

1. A chromatographic study of urine samples from 285 subjects revealed a higher incidence of subjects excreting excess indoles amongst the schizophrenics.

2. The differences in incidence were only significant at the 5 per cent. level as between the male "normals", male schizophrenics and male non-schizophrenic psychotics.

3. Cephalin-Cholesterol Flocculation Tests were performed on 48 schizophrenic subjects. Positive results were obtained in 8·3 per cent. of cases. This incidence is not considered to be significant.

Acknowledgments

I would like to express my thanks to Professor A. Kennedy, Drs. T. A. Munro, A. K. Macrae, R. Bailley and A. W. Wright for access to patients; Dr. D. Crombie for laboratory facilities and technical advice; Mr. J. Hodgson and Dr. K. Fotherby for helpful criticism.

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