BODY FLUID INDOLES OF NORMAL AND MENTALLY-ILL SUBJECTS

I. PRELIMINARY SURVEY OF THE OCCURRENCE OF SOME URINARY INDOLES

By

R. RODNIGHT

With the technical assistance of

E. K. AVES

Department of Biochemistry, Institute of Psychiatry
Maudsley Hospital, S.E.5

A ROLE in mental illness for an aberrant metabolism of indole compounds was first advanced some sixty years ago, when their alleged toxicity to the nervous system figured prominently in the then current theory of auto-intoxication by bacterial action in the bowel (Herter, 1898, 1907). Although in a modified form it continues to receive the support of Buscaino (1958), auto-intoxication of this nature is now generally rejected as a factor in mental illness (see particularly Alvarez, 1924) and the renewed interest in indoles and brain function of recent years has come from a different standpoint. Thus, study of the central nervous system effects of indoles such as 5-hydroxytryptamine (serotonin) and bufotenin, and of compounds containing an indole group such as lysergic acid and reserpine, has inspired several new theories concerning normal and abnormal roles for indoles in the brain (Woolley and Shaw, 1957; Brodie and Shore, 1957; Hoffer, 1957).

Reports of body fluid abnormalities involving indoles in groups of mentally-ill subjects have occasionally appeared in the literature. Many of the earlier papers are listed and briefly reviewed by McGeer, McNair, McGeer and Gibson (1957). In our opinion it is difficult to assess the significance of most of these studies, as the methods used were usually relatively non-specific for indoles and the experiments often inadequately controlled, but the general impression emerges that indoles may be concerned in the increased concentration of aromatic compounds often observed in the urine of acutely disturbed patients, particularly schizophrenics. Well-defined disturbances of indole metabolism, however, have been found in two rare hereditary conditions exhibiting mental abnormalities: in phenylketonuria (Armstrong and Robinson, 1954; Pare, Sandler and Stacey, 1957) and in the pellagra-like syndrome known as Hartnup disease (Baron, Dent, Harris, Hart and Jepson, 1956; Rodnight and McIlwain, 1955; Jepson, 1956; Hersov and Rodnight, in preparation); and also in a group of schizophrenics, who were found by Zeller, Bernsohn, Inskip and Lauer (1957) to metabolize large doses of tryptophan differently from normal subjects.

It is clear, therefore, that much scope exists for further work in the subject. In the research now described aspects of body fluid indoles have been studied and compared in normal and mentally-ill subjects by chromatographic methods. The present paper, which is in the nature of a preliminary survey, reports on

the occurrence of the main urinary indoles in 156 mental hospital patients, 63 normal subjects and 35 general hospital patients. Subsequent papers will be concerned with the urinary excretion in mental disease of serotonin, tryptamine and some indole acids, and observations on indoles in blood.

SUBJECTS

The 254 subjects were divided into the 5 groups described below. Details of their sex and age are given in Table I. About three-quarters of the mentally-ill subjects were receiving some form of treatment, mainly electro-shock therapy,

TABLE I

Classification of Subjects: Sex and Age

Group	Description	Sex	Number Examined	Age in Years Followed by S.D. and Range
I	Normals	Male	37	33·1± 9·4 (19–61)
		Female	26	30·6±10·6 (18–52)
II	Schizophrenics in hospital	Male	27	34·0± 9·6 (22–56)
		Female	38	33·1±10·8 (18–61)
III	Other mental illnesses in hospital ..	Male	20	55·1±12·3 (30–71)
		Female	40	49·2±14·3 (16–69)
IV	New admissions to Observation Ward	Male	17	34·4± 8·6 (22–53)
		Female	14	40·1±13·0 (22–60)
V	Physical illnesses in General Hospital	Male	19	46·5±18·6 (15–79)
		Female	16	36·9±18·9 (13–67)
	Total		254	

or sedation with chlorpromazine or barbiturates. Treatment was not suspended before collection of the specimens, but a record of its nature was kept for each patient.

Group I. A total of 63 healthy normal subjects from the staff of a mental hospital and their friends and relatives volunteered specimens. No special dietary instructions were given.

Group II. This group comprised 65 patients suffering from schizophrenia and drawn from three distinct mental hospital populations. All of them had been detained in hospital for longer than three weeks.

Group III. Miscellaneous mental illnesses other than schizophrenia formed this group. A total of 60 patients were chosen from the same three mental hospitals as was the case with Group II subjects, and are roughly classified as follows: 20 depressive illnesses, 17 involutional and senile states including organic illnesses, 7 manic psychoses, 5 cases of psychoneurosis, 3 epileptics and 3 psychopaths.

Group IV. Acutely-ill subjects were obtained from amongst new admissions to the psychiatric observation ward of a general hospital. There were 31 subjects; the provisional diagnosis of 25 of them was schizophrenia; for the remainder it was depression or hypomania.

Group V. The final group consisted of 35 patients with physical illnesses drawn from a different general medical hospital to the one referred to above. They comprised 7 patients from whom the specimen was obtained 1–3 days

after a major operation, 6 cardiovascular diseases, 6 patients with neoplasms, 6 endocrine disorders, 4 toxaemias from various causes, 4 inflammatory conditions and 2 blood diseases.

METHODS

Specimens. Urine was collected before breakfast in glass or polythene containers without the addition of preservative. The great majority of specimens were analysed on the day of their collection in batches of 12 or more; in a few instances where immediate analysis was not possible they were stored at −20° C.

Chromatography. The method employed was similar in principle to that described by Jepson (1955). The urine spot was placed 5·5 cm. along the diagonal of a 9 cm. square sheet of Whatman No. 20 chromatography paper having holes punched in each corner. The use of Whatman No. 20 paper is an important technical detail, as unsatisfactory results were obtained with faster running papers such as Whatman No. 1. To apply the spot an "Agla" micrometer syringe (Messrs. Burroughs Wellcome Ltd.) was used: the syringe was clamped so that the needle just touched the paper, the urine added in 5 μl. portions and after each addition the spot was dried with air at 40° C. from an electric blower. The maximum volume of urine commensurate with the production of undistorted chromatograms was applied. With practice its approach could be judged firstly by the rate at which each successive 5 μl. of urine soaked into the paper, and secondly by the appearance of the dried spot, whose circumference became crinkly and distorted when the critical volume was reached. The volume of urine used with each specimen was noted and the mean value calculated for each group (Table II).

TABLE II

Mean Volume of Urine used for Chromatography in each Group

	Mean Volume in μl.				
Group	I	II	III	IV	V
First specimens ..	95·5	96·3	97·4	110·0	108·0
Second specimens ..	104·3	106·1	101·6	—	—

The paper squares were fitted on to a chromatography frame of the same basic design as that described by Datta, Dent and Harris (1950), but constructed from $\frac{1}{8}$ inch diameter stainless steel rod and $\frac{1}{4}$ inch thick sheet polythene, instead of aluminium alloy. Short lengths of glass tubing were used as spacing washers. The frame, which accommodated 12 papers, was placed in a polythene tray containing the first solvent, which in turn rested in a glass tank of dimensions 16×11×11 inches, and covered with a sheet of glass.

The papers were first irrigated overnight with a n-propanol/water ammonia mixture of proportions 80:15:5, and the next day for 6 hours with the n-butanol/acetic acid/water solvent of Jepson (1955). Before developing the indole spots with Ehrlich's reagent, the dried papers were viewed in ultra-violet light and the fluorescent spots outlined in pencil.

Indoles and other compounds reacting with *p*-dimethylamino-benzaldehyde were located with the Ehrlich reagent of Jepson (1955). The papers were dipped in the reagent and examined at intervals of 5 minutes for about 20 minutes and again after 24 hours. The blue and purple spots of the indoles were marked with pencil as they appeared; several of the fainter spots faded rapidly.

According to their maximum intensity they were given a rating of faint, weak, medium or strong. By examining in a few instances a series of dilutions of the same urine the quantitative relation between the faint to weak and the weak to medium ratings was very roughly estimated as twofold.

The reproducibility of the method from day to day was high: on repeatedly analysing a single specimen, the same spots were noted with the same rating. However, it was considered inevitable that over a period of months minor variations would occur in the conditions under which the chromatography was carried on. To reduce the effect of these, abnormal specimens were usually analysed in parallel with some normal specimens collected during the same period. Nearly all of the specimens from Groups I to III, about three-quarters of those from Group IV and one-third from Group V, were dealt with in this way.

Comment on Method of Preparing Chromatograms. The early morning specimens varied considerably in concentration and in preparing the chromatograms it clearly would have been unsatisfactory to use a standard volume of urine. The method described above of applying to the paper the maximum volume of urine was found to compensate approximately for differences in urinary concentration. since the required volume was roughly inversely proportional to the specific gravity. The procedure was further investigated as follows: from a random selection of 16 urines, spots were prepared on tarred squares of paper. These were dried in a vacuum desiccator, weighed, and the weight of the urinary solutes deposited determined in each case. The mean weight was $4 \cdot 46 \pm 0 \cdot 92$ mg. (standard deviation) and the mean volume applied was 105 ± 33 μl.; the correlation coefficient of the two sets of figures was $-0 \cdot 2$, revealing no tendency for the urinary concentration to influence the quantity of urinary solutes deposited on the paper.

Results

Pattern of Indole Spots in Normal Subjects

The indoles were identified by their position on the chromatograms relative to themselves, to the fluorescent spots of other compounds and to the large urea spot which was coloured yellow by the Ehrlich reagent. The approximate positions occupied by the indoles are illustrated in Figure 1. Further clues as to their identity were given by the characteristics of their reaction with Ehrlich's reagent, details of which are given in Table III.

Table III

Details of Indole Spots Observed on Chromatograms of Normal Urine Specimens

Description of Spot	Colour with Ehrlich's Reagent	Approximate Time for Appearance of Weak Spot (minutes)
Indican	Brown	3
Tryptophan	Purple	4
Indole acetic acid	Red-purple	3
"Unknown 1"	Red-purple	5
"Unknown 2"	Purple to blue	1 or less
"Unknown 3"	Blue-purple to blue	6
"Unknown 4"	Red-purple	3
"Unknown 5"	Purple	3

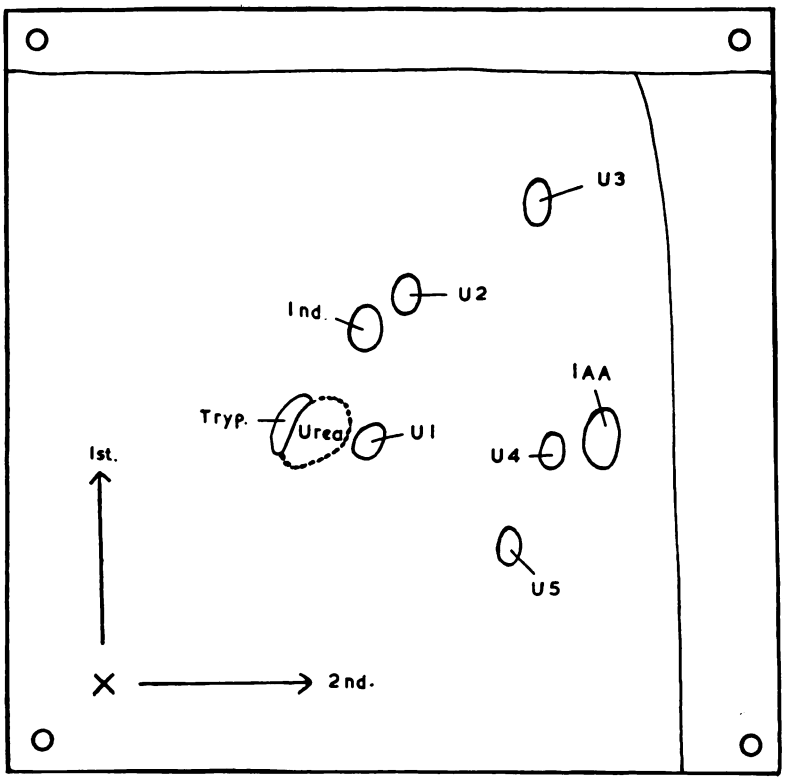

FIG. 1.—Diagram of two-dimensional chromatogram of 100 μl. urine, stained with *p*-dimethylaminobenzaldehyde (Ehrlich's reagent) and showing all the observed blue and purple spots and the yellow spot of urea. 1st solvent: n-propanol/water/ammonia, 80:15:5; 2nd solvent: n-butanol/acetic acid/water (Jepson, 1955). "Tryp.": Tryptophan; "Ind.": Indican; "I.A.A.": Indole acetic acid; "U 1–5": Unknowns 1–5.

From the Figure and Table III it can be seen that the indican spot was clearly differentiated from the other indoles by its distinctive brown colour. Two other spots were identified with reasonable certainty as tryptophan (purple) and indole acetic acid (red-purple). Faint spots of tryptophan were readily masked by the yellow colour of the closely adjacent urea. The remaining 5 blue or purple spots could not be definitely recognized as known indoles, although in some cases their identity was suspected. The only chemical evidence for their indolic nature is the colour they gave with Ehrlich's reagent, which is not known generally to produce blue or purple hues with other classes of compounds. They are referred to as "unknowns 1–5"; some of their properties and suggestions for their identity are given below.

"*Unknown 1*". This spot very probably corresponds to indole acetyl glutamine, a compound found in much higher concentration in urine from cases of Hartnup syndrome (Jepson, 1956). An authentic specimen for comparison was not available, but the chromatographic behaviour of the spot in several solvent systems was identical with the indole acetyl glutamine spot in urine from a known case of Hartnup disease.

"*Unknown 2*". The spot reacted very rapidly with the Ehrlich reagent, the colour often appearing immediately on dipping the paper. The initial colour was purple, turning within 15–30 minutes to a pure cobalt blue, which

with strong spots was permanent for weeks. The substance was not extracted from salt-saturated urine by benzene or ether at either acid or alkaline pH's, but was partially extracted by n-butanol after saturation of the urine with potassium carbonate. On paper it gave no colour with ninhydrin, diazotized sulphanilic acid or ammoniacal silver nitrate. In R_f and reaction to Ehrlich's reagent it is very similar to the unknown spot No. 2 described in urine extracts by Decker (1954), who considers it a derivative of skatol. We have confirmed that this is indeed likely by feeding skatol to rats and observing a great increase in the amount of "unknown 2" in the urine; other new indoles also appeared. We were not, however, able to demonstrate on the present spot, either in human urine or in rat urine after feeding skatol, the "fluorindal" reaction, typical according to Decker (1955) of his substance.

"*Unknown 3*", also gave a spot initially purple, changing to a semi-permanent grey-blue colour, but it developed more slowly than was the case with "unknown 2". It gave no reaction to reagents other than Ehrlich's and was apparently acidic in character since, from salt-saturated urine at pH 2, it was completely extracted by n-butanol and partially extracted by ether. Its position on the chromatogram was very close to that occupied by tryptamine. The latter, however, is definitely excluded as it only occurs in urine in quantities of the order of 0·1 μg./ml. and requires for its demonstration special methods (Rodnight, 1956).

"*Unknown 4 and 5*" were less frequently seen and then only as faint spots. They probably correspond to the compounds indole lactic acid and 5-hydroxy-indole acetic acid respectively. Thus both were quantitatively extracted from salt-saturated urine at pH 2 by ether and on chromatograms of the ether extract they gave identity with authentic samples of the two indole acids.

Pattern of Indole Spots in Abnormal Subjects

Chromatograms of the abnormal urines showed no blue or purple spots other than those noted in normal specimens and described above.

The frequencies with which the normal spots occurred, expressed as percentages of the number of subjects in each group, are given in Table IV.

TABLE IV

Frequency of Occurrence of Indole Spots on Chromatograms

Results are quoted as percentages of the total number of subjects in the group; those which differ significantly from the normal (Group I) percentage are marked with an asterisk ($P<0·01$). I: 63 normal subjects. II: 65 schizophrenics. III: 60 miscellaneous mental illnesses. IV: 31 new admissions to a psychiatric observation ward (mainly schizophrenics). V: 35 physical illnesses from a general hospital. For further details see text.

Description of Spot	No Spot (%)					Low Intensity Spot (%)					High Intensity Spot (%)				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Indican	0	0	2	0	0	73	46(*)	67	52	80	27	54(*)	31	48	20
Tryptophan	19	27	28	19	14	73	62	60	81	69	8	11	12	0	17
Indole acetic acid	8	9	17	6	14	86	83	78	77	83	6	8	5	17	3
"Unknown 1"	24	35	50(*)	61(*)	77(*)	74	62	50(*)	36(*)	23(*)	2	3	0	3	0
"Unknown 2"	64	55	46	29*	74	27	26	28	32	23	9	19	26	39*	3
"Unknown 3"	34	51	45	42	71*	63	43	52	55	23*	3	6	3	3	6
"Unknown 4"	98	94	88	90	92	2	6	12	10	8	0	0	0	0	0
"Unknown 5"	83	59*	73	74	89	17	38*	25	23	11	0	3	2	2	0
Total spots (of possible 800)	330	330	349	321	431	415	366	372	366	320	55	104	79	113	49

Here it was found more satisfactory to condense the five ratings of spot intensity to three by combining the faint and weak spots and the medium and strong spots into low and high intensity ratings respectively. The condensation was probably necessary because the subjective error involved in judging the spot

intensities was too great to allow more than two levels to be used in groups of the present size; other groupings of the original ratings gave no more information.

In order to determine the reliability of observed differences of frequency between the groups, it was necessary to obtain a measure of the degree to which the indole excretion pattern was reproducible. Ideally this would be done by examining a series of specimens from each subject, but in practice this course was not possible. An approximate estimate of overall reproducibility was accordingly made by analysing a second specimen of urine, one to three weeks after the first, from just over one-third of the subjects in Groups I–III. With one exception the resulting percentages agreed with those from the first specimens to within 20 per cent. To estimate reproducibility in individual subjects, the results from the second specimens were combined with those from the corresponding first specimens and the percentage frequency for each spot re-calculated; for convenience this is referred to as the "combined percentage". The upper and lower limits of the "combined percentage" were obtained by comparing each pair of specimens, scoring the discordant results as agreements in each direction and then re-calculating the percentage. These data are given in Table V, where it can be seen that reproducibility is highest

TABLE V

Frequency of Occurrence of Indoles in Combined Groups of First and Second Specimens

Description of Spot	No Spot (%)			Low Intensity Spot (%)			High Intensity Spot (%)		
	I	II	III	I	II	III	I	II	III
Indican	0	0	0	79±17	63±21	63±17	21±17	37±21	37±17
Tryptophan	14±10	15± 4	25±25	79±12	75±10	65±27	7± 2	10± 6	10±10
Indole acetic acid ..	14± 7	10±10	15±10	72± 9	90±10	81±15	14± 9	0	4± 4
"Unknown 1" ..	19±14	19±12	33±17	81±14	79±13	65±19	0	2± 2	2± 2
"Unknown 2" ..	67±14	56±17	54± 8	24±14	21±21	29±13	9± 5	23±12	17± 8
"Unknown 3" ..	36±17	58±15	65±19	64±17	42±15	35±19	0	0	0
"Unknown 4" ..	100	87±13	73±23	0	13±13	27±23	0	0	0
"Unknown 5" ..	93± 7	54±19	71±17	7± 7	46±19	27±19	0	0	2± 2

I: 21 pairs of specimens from subjects in Group I. II: 26 pairs from Group II. III: 24 pairs from Group III For method of calculating the limits of the percentages see text.

in the normal group. For the abnormal groups it is considerably lower and for some spots it is very low indeed. This is possibly due to the fact that the two specimens may have been collected during different phases of the subjects' illnesses; it may also reflect a higher incidence of somatic disorders in patients than normals, and for Group III subjects a higher average age.

In interpreting the main group differences, significance was first calculated in each case by a "t" test on the standard error of the difference between the percentages observed in the normal and abnormal groups. A strict test of significance was employed; only differences with a level of probability of chance less than 0·01 were accepted. These are marked with an asterisk in Table IV or with an asterisk in parentheses where a significant result was considered unreliable for special reasons stated below. The significant results were then assessed on their individual merits, where possible taking into account the results of the second specimens.

Indican. A significantly raised percentage of high intensity spots occurred from Group II subjects. Although the same tendency is evident in the "combined percentages" (Table V), the degree of reproducibility for Group II indican spots is very low and for this reason the result was not accepted as reliably significant.

"*Unknown 1*". Significantly fewer spots of low intensity occurred in Groups III, IV and V, and in the case of Group III a difference in the same direction is present in the "combined percentages". The reliability of this result is also in doubt, because the "unknown 1" spot, which more often than not was classed as faint rather than weak on the original intensity scale, was frequently partially obscured by the yellow colour of the closely adjacent urea. The difference, moreover, was not confined to mentally-ill subjects, but also occurred in the physically-ill subjects of Group V.

"*Unknown 2*". The three groups of mentally-ill subjects all showed an increased percentage of high intensity spots of this substance, although only in Group IV was the difference significant. It is noteworthy that the increase is only evident at the high intensity level; there is little variation in the percentage of low intensity spots occurring. The "combined percentages" from subjects in Groups II and III also gave a result in the same direction.

The significant percentage occurred in Group IV subjects and this suggested that a correlation might be found between its occurrence and the acuteness of the illness. In Group II, 39 of the subjects were acutely disturbed at the time the specimens were obtained. Out of these, 9 or 23 per cent. of the specimens showed a high intensity "unknown 2" spot; in the remaining 26 more chronic patients the figures were 3 or 12 per cent. Although the figure for the acutely-ill subjects is higher it is still not significantly different from the normal value of 9 per cent. (Table IV, $P \simeq 0 \cdot 1$). In the heterogeneous Group III a correlation with diagnosis was sought. Here 16 high intensity "unknown 2" spots were observed; 13 of these occurred in patients with a diagnosis of depression (including 2 involutional melancholias), 2 in cases of senile dementia and one in a patient with psychoneurosis (chronic anxiety). Together with the involutional melancholias the depressive illnesses in the Group totalled 27; amongst these therefore the percentage of strong reactors for "unknown 2" was a significant 48, whilst for the rest of the Group it equalled the normal percentage of 9.

"*Unknown 3*". No explanation is offered for the significantly lower percentage of this spot noted in Group V subjects. Its absence could not be correlated with diagnosis.

"*Unknown 5*". The significantly raised percentage of weak spots in Group II subjects and a trend in the same direction in Groups III and IV, is well reflected in the "combined percentages". There appears therefore no reason to doubt its reliability.

"*Total indole spots.*" The individual percentages were totalled for each Group for the main investigation (Table IV). The differences are not significant, but it is noteworthy that the three groups of mentally-ill subjects all show an increase in the percentage of high intensity spots.

Discussion

Experimental Factors

In planning the present investigation it was considered desirable to examine a wide spectrum of mental illnesses in subjects living under several different hospital regimes. For this reason, and also because the number of subjects was large, it was impossible to control strictly all the experimental variables. The most pertinent of these would appear to be the following:

Diet: No control or records were practicable, but by analysing early morning specimens the influence of diet was reduced to the minimum possible without control. Thus the great majority of the subjects had eaten their last meal some 12 hours before the time of the early morning urine collection. *Drugs and treatment:* About three-quarters of the subjects in Groups II and III were receiving treatment in the form of electro-convulsive therapy, chlorpromazine or barbiturates at the time the specimens were obtained. No significant correlation between treatment and the occurrence of any of the indole spots was found, but there was a tendency for more high intensity spots of "unknown 2" to occur in untreated than in treated subjects; the respective values were 28 per cent. and 17 per cent. *Urinary concentration:* As was mentioned in the Methods section, variations in this were largely compensated for by the procedure for preparing the chromatograms, where the volume of urine was roughly inversely proportional to its specific gravity. However, it is possible that the method would have been adequate if the mean specific gravities varied greatly between the groups; in fact this did not occur, since the mean volumes of urine used in each group agreed closely (Table II). *Fluctuations in level of indole excretion:* The degree to which indole excretion varies from hour to hour is not known. In theory, such short term fluctuations could have been allowed for by pooling all the urine passed over a period of time (e.g. 24 hours), but in practice this was not possible. Some reliance may be placed on the fact that the specimens were obtained from all the subjects at the same time of day, and at rest.

In the absence of experiments in which the above factors were standardized, it is not possible to determine precisely their influence on the present results. It seems very unlikely, however, that the significant differences can be wholly ascribed to chance variations in the experiments circumstances. This is concluded because (*a*) the total number of subjects was relatively large, (*b*) a strict test of significance ($P < 0{\cdot}01$) was applied to the results, (*c*) the specimens were obtained from virtually fasting subjects in the early morning and (*d*) in a proportion of subjects the significant difference was approximately confirmed by analyses of second specimens.

Pattern of Indole Spots

The most interesting result concerned the substance labelled "unknown 2", in which a significantly raised percentage of high intensity spots were observed in urines from acutely-ill new admissions to a psychiatric observation ward, and in urines from nearly half the depressed patients detained in hospital. Results from the schizophrenic subjects were suggestive, although not statistically significant, of an increase in acutely-ill patients. However, a raised excretion of the substance was not confined to the mentally-ill subjects, since in a few normal subjects high intensity spots were also noted. No correlation between age or sex and the occurrence of high intensity spots of "unknown 2" was found.

The substance appears to be derived from skatole, which in turn almost certainly arises from the action of bacteria on tryptophan in the intestine. Probably, as Decker (1955) suggests, it represents a product of the detoxication of skatol by the liver, as does indican of indole. It would be particularly interesting to know whether the excretion of the substance varies from day to day, or whether it is relatively constant and thus part of the concept of biochemical individuality, as has been observed for amino acids (Dent, 1954;

Williams, 1956). In the event of the former it could be postulated that excretion is influenced by factors such as digestion or bowel function, which are occasionally disturbed in normal subjects and more often so in the mentally ill. A persistently raised excretion, on the other hand, would require a different interpretation; it is tempting to suggest that it might prove a metabolic pointer to a higher than normal susceptibility to certain forms of mental illness, just as recent work has shown that a persistently raised level of indican in the urine may indicate a susceptibility to the pellagra-like attacks observed in Hartnup disease (Rodnight and McIlwain, 1955; Jepson, 1956; Hersov and Rodnight, in preparation). Evidence for this cannot be derived from the chronically-ill subjects of the present investigation (who differed little from normal in their excretion of the substance), since they were drawn from a different population to the acutely-ill subjects. Longitudinal studies with a fully quantitative method on patients and normals showing a raised excretion are required.

Stress, using the term in its widest and non-specific sense, does not appear to influence the excretion of "unknown 2", since the lowest percentage of high intensity spots of all was found in the physically-ill patients from a general hospital, including some examined very soon after a major operation.

A further significant difference was found for "unknown 5", where more low intensity spots were observed in schizophrenic subjects. Since this spot is believed to be 5-hydroxyindole acetic acid and is the subject of a quantitative study in a later paper, discussion of the result will be deferred until then. Buscaino and Stefanachi (1957) and Sano (1957) were unable to find any abnormality in the urinary excretion of 5-hydroxyindole acetic acid in schizophrenia.

The absence of a reliably significant difference in the percentage of indican spots observed is in accord with the early, but excellent, study of Borden (1906), who measured by a quantitative adaptation of the Obermayer reaction the 24-hourly output of indican in normal and mentally-ill subjects. He found widely varying values in both groups, but little difference in the mean daily excretion. It would seem, therefore, that a moderate degree of indicanuria is of no significance in mental illness, and the occasional reports which have appeared contradicting this conclusion (Townsend, 1905; Gullotta, 1929; Sano, 1954) are probably explained by the use of relatively non-specific methods and the influence of diet, which is known to affect indican excretion (Borden, 1906; Underhill and Simpson, 1920). As has already been mentioned a persistently high level of indican in the urine may indicate Hartnup disease; none of the high intensity spots observed in the present study approached the intensity of the indican spot in a typical Hartnup urine.

Absence of Abnormal Indoles

Although no evidence emerges from the present work for the occurrence in the urine of mentally-ill subjects of an "indole toxin", it is necessary to point out that an abnormal indole excretion of less than about 10 mg./24 hours, would not have been detected by the method used. That such an excretion might be important is clear when one considers the potency for the nervous system of indole-containing alkaloids like lysergic acid diethylamide and reserpine which are effective in man in doses of 100 μg. and 5 mg. respectively.

Support, however, for the present negative result comes from the work of Curzon (1957), who studied indole excretion by paper chromatography in schizophrenic and non-schizophrenic (mainly neurotic) mental hospital patients,

and was unable to find any difference between the two groups. By contrast, Buscaino (1958) claims to have found qualitative differences in urinary indoles in schizophrenia.

Summary

1. Urinary indole excretion was studied by two-dimensional paper chromatography in 63 normal subjects, 65 schizophrenics, 60 cases of miscellaneous mental illness, 31 new admissions to a psychiatric observation ward and 35 cases of physical illness from a general hospital.

2. The three groups of mentally-ill subjects showed a higher total of indole spots on their urine chromatograms than did the other two groups. The increases were most marked in two unknown indoles, and they occurred most frequently in acutely ill patients.

3. No evidence was obtained for an abnormal indole excretion greater than about 10 mg./24 hours in mentally ill subjects.

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