CORRELATION BETWEEN PSYCHOPATHOLOGY AND URINARY STEROID AND BIOGENIC AMINE METABOLITES IN MALE SCHIZOPHRENICS

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SUMMARY

In eight normals, eight anxiety neurotics and eight patients of acute schizophrenic episode, twenty-four-hour urinary excretion of each of the following was measured: (1) 17-ketosteroids (17-KS)—total, glucuronides and sulphates, (2) 17-hydroxycorticosteroids (17-HS)—total, conjugated and free, (3) Vanillmandelic acid (VMA), and (4) total indoles. In case of schizophrenics, such measurements were made before starting treatment, and after 30 days of chlorpromazine therapy. The psychopathology of schizophrenic subjects were rated on Rockland and Pollin (R. P.) scale on both occasions. Correlating the psychopathology with the biochemical values, amongst schizophrenics, R. P. score was found to show significant positive correlation with VMA and, to a lesser extent with total indoles. Schizophrenics excreted greater amount of VMA than normals and this returned to near normal levels with treatment. On the other hand, schizophrenics excreted lower amounts of most steroid fractions than normals, but amongst schizophrenics, there was no significant correlation between R. P. score and steroid excretion.

During the last three decades, tremendous advances have been made towards the understanding of the biological substratum of schizophrenia. Unfortunately, it is still not fully elucidated which may have been due to the complexity of the illness or problems in identification and measurement of its biological correlates.

The substances which have been extensively studied include steroids and biogenic amines. Most of the studies reported, however, have been concerned with only the global endocrinial function and have neglected to study the conjugation patterns of the steroids. On serial studies, Matsumoto et al. (1966) and Sachar et al. (1963) have reported increase in 17-ketosteroid and 17-hydroxycorticosteroid excretion during exacerbation of symptoms of anxiety and motor unrest and periods of increased turmoil in schizophrenics. Michael and Gibbons (1963) reported that correlation of increased adrenocortical activity appear to be with the disturbances of affect rather than the schizophrenic illness per se.

The biogenic amines, especially non-epinephrine and serotonin, because of their high concentration in the limbic cortex and their important role in neuronal transmission, have also been suspected to be implicated in schizophrenia and have been extensively investigated since the pioneering work of Osmond and Smythies (1952).
who, on the basis of chemical relationship between mescaline and norepinephrine, suggested that schizophrenia may be associated with disturbance in catecholamine metabolism, and of Woolley and Shaw (1954) who, on the basis of pharmacological antagonism between psychotomimetics like LSD and serotonin, suggested that schizophrenia may be associated with some abnormality in serotonin metabolism. As regards the relationship between the urinary excretion of biogenic amine metabolites and manifest psychopathology of schizophrenia, Berlet and co-workers (1964, 1965) reported that increase in urinary catecholamines usually accompanied aggravation of psychotic symptoms and that there was marked increase in tryptamine urinary excretion preceding peak of psychotic syndrome. Himwich (1965) found striking agreement between the degree of psychotic activity and daily output of tryptamine, 3-indole acetic acid and 5-hydroxy-indole acetic acid. On the basis of available evidence, Himwich and Himwich (1967) have summarized that the rise in urinary catechols and steroids accompanies increased motor activity or motor restlessness, heightened tension and anxiety and rise in urinary indoles is associated with intensification of hallucinatory and delusional experiences, usually with mounting hostility. The indoles begin increasing before a sudden flare-up of symptoms and continue at high levels during psychotic activation. On the other hand, epinephrine and norepinephrine begin rising simultaneously with the increase of psychotic behaviour.

The aetiology of schizophrenia is more likely to be multi-factorial and more than one biochemical factor may be implicated, hence the need to simultaneously study several such biochemical substrates. Also, in cases of steroids, both the free and conjugated fractions (in urine) need to be studied to get a composite picture of disturbances in its metabolism, if any.

One strategy to elucidate the relationship between the bio-chemical substrates and schizophrenia, if any, could be to study the correlation between the clinical disturbance and biochemical factors which can be a way to get around the problem. The present paper is related to the correlation between the psychopathology of acute schizophrenics as measured by the Rockland and Pollin scale (R. P. scale) with the urinary excretion of (a) total 17-ketosteroids (17-KS) and their glucuronides and sulfates, (b) 17-hydroxycorticosteroids (17-HS) : total, conjugated and free, (c) vanilmandelic acid (VMA), a metabolite of catecholamines, and (a) total 5-hydroxy indoles, a metabolite of serotonin.

The above biochemical values obtained in schizophrenics were also compared with those in control groups of normals and anxiety neurotics and among the schizophrenics, the effect on these values of treatment was studied which has been reported in detail elsewhere (Ghosh et al., 1976).

**METHOD**

1. Selection of subjects

The following groups of subjects were selected for the study:

(a) Eight patients diagnosed to be suffering from acute schizophrenic episode (AS). The diagnosis of schizophrenia was made according to the criteria of Breakey and Goodell (1972). The illness was considered acute when the time interval between the onset of symptoms to a full-blown picture of psychosis was less than one month. The duration of illness at the time of the study was less than three months.

(b) Two control groups:

(i) Eight psychiatrically normal subjects (N), and

(ii) Eight patients diagnosed to be suffering from Anxiety Neurosis.
(AN) according to the usual clinical criteria.

All subjects were males in the age range of 18 to 30 years. All subjects were subjected to routine psychiatric and physical evaluation to ascertain the diagnosis. Those suffering from detectable organic illness were excluded from the study. Relevant investigations were conducted wherever necessary to rule out organic illness.

2. Collection of data

Assessment of psychopathology:

Schizophrenic subjects were rated on the Rockland and Pollin scale (1965) (R. P. scale) so as to correlate it with the biochemical variables studied. Assessments were made the day before collection of the first urine sample and after 30 days of treatment.

Anxiety neurotic subjects were rated on Hamilton's anxiety rating scale on the day before the collection of urine sample for biochemical estimations.

All patients of acute schizophrenic episode were hospitalised at the psychiatry ward for the full duration of the study. They were administered a standard hospital diet throughout the period of the study. The substances which are known to interfere with or falsify biochemical estimations involved, viz. coffee, cocoa, cheese, fruits especially bananas, soft drinks, spinach and ice cream were excluded from the diet of the subjects. They were asked to engage themselves only in a specified moderate amount of physical activity.

On intake, one 24-hour urine sample was collected from 8 a.m. to 8 a.m. next day, from all subjects. Thereafter, in case of schizophrenics, they were put on chlorpromazine in a clinically optimal dose and additional 24-hour urine samples were collected after 30 days of treatment. The acute schizophrenic patients were not given any other drugs, (other than non-barbiturate hypnotics, e.g., chloral hydrate and paraledehdye) till after the collection of first sample of urine, nor were these administered during the period of the study.

No special preservative was used during the collection. Later, the urine was stored (in no case for more than 24 hours) in glass bottles at 4°C, using thymol for steroids and glacial acetic acid for indoles and VMA as preservatives.

All urine samples were subjected to a preliminary washing with carbon tetrachloride.

For estimation of 17-KS, the urine sample was hydrolysed with hydrochloric acid (Enriori, 1965). The 17-KS was extracted with petroleum ether-benzene and estimated by Zimmerman reaction (Dorfman, 1962). The 17-KS glucuronide fraction was determined after the urine was digested with B-glucuronidase, whereas the sulphate conjugates were estimated in the aqueous phase left, after the removal of steroids liberated by B-glucuronidase, following acid hydrolysis.

For estimation of free 17-HS, the urine was extracted with methylene chloride and assayed by the tetrazolium blue method (Peron, 1962). For estimation of conjugated 17-HS, the urine aliquot left after extraction of free 17-HS was subjected to B-glucuronidase extraction. The liberated steroids were, thereafter, measured as in the case of free 17-HS, after a preliminary purification by Silica-gel G thin layer chromatography (Lisboa, 1969). Total 17-HS was estimated separately by the same method as in the case of conjugated 17-HS, without pre-treatment with methylene chloride.

VMA was estimated by the method of Pisano et al. and the total hydroxy-indoles by that of Udenfrined et al., (Oser, 1965).

Analysis of data

In schizophrenics, the 24-hour urinary excretion of each biochemical fraction was correlated with the total and sub-category scores of the R. P. scale using rank-order correlation. Similarly, the values of each
biochemical constituents studied were correlated with the anxiety scores obtained by anxiety neurotics on Hamilton's anxiety scale.

RESULTS

Table 1 shows the correlation between R. P. scores (Total and its three subcategories, viz. general appearance, effect and thinking process) and biochemical values in acute schizophrenics expressed in mg/100 mg of creatinine. Significant relationships were noted between the clinical and biochemical parameters.

**Table 1—Correlation between R-P scores and biochemical values (expressed in mg/100 mg of creatinine) in schizophrenics. (N=8)**

|                | Total rating score | General appearance score | Affect score | Thinking process score |
|----------------|--------------------|--------------------------|--------------|-----------------------|
| 17-KS (Total)  | -0.095             | -0.071                   | -0.185       | -0.167                |
| 17-KS (Glucuronides) | -0.071          | +0.036                   | -0.220       | -0.238                |
| 17-KS (Sulphates) | +0.018            | +0.107                   | -0.238       | -0.030                |
| 17-HS (Total)  | -0.048             | +0.250                   | -0.351       | -0.167                |
| 17-HS (Conjugated) | +0.190            | +0.345                   | -0.220       | 0.000                 |
| 17-HS (Free)   | -0.291             | -0.095                   | -0.271       | -0.018                |
| VMA            | +0.950**           | +0.821*                  | +0.518       | +0.786*               |
| Total indoles  | +0.816*            | +0.667                   | +0.661       | +0.381                |

*p<0.05, **p<0.01.

The anxiety rating (Hamilton's anxiety rating scale) in case of anxiety neurotics was found to be significantly related with total and conjugated 17-hydroxy corticosteroids when expressed in terms of 100 mg of creatinine.

**Table 2—Correlation between anxiety score and biochemical values in anxiety neurotics (N=8)**

|                | in mg/100 mg of creatinine |
|----------------|-----------------------------|
| 17-KS (Total)  | +0.565                      |
| 17-KS (Glucuronides) | +0.589                  |
| 17-KS (Sulphates) | +0.054                      |
| 17-HS (Total)  | +0.780*                     |
| 17-HS (Conjugated) | +0.780*                   |
| 17-HS (Free)   | +0.125                      |
| VMA            | -0.018                      |
| Total indoles  | +0.387                      |

*p<0.05, **p<0.01.

DISCUSSION

The most noteworthy finding of this study was a significant positive correlation between psychopathology of schizophrenia as measured by the R. P. score and the urinary excretion of VMA. This was true for the total R. P. score as well as for its three components separately (the correlation with effect score, although statistically not significant, was approaching significance and was in the same direction). This indicates that within the schizophrenia group, psychopathology as measured by R. P. score correlates positively with VMA excretion. As earlier reported (Ghosh et al., 1976), comparing schizophrenics with normals and anxiety neurotics (Table 3), considerable difference was found between normals and schizophrenics on urinary excretion of VMA (this difference, however, was not significant on analysis of variance apparently on account of large variability within the diagnostic groups). There was
**Table 3—Urinary excretion of steroids and bioamines (mean values ± S.D., in mg/100 mg of creatinine)**

|                | N  | 17-KS | 17-KS | 17-KS | 17-HS | 17-HS | 17-HS | Total |
|----------------|----|-------|-------|-------|-------|-------|-------|-------|
|                |    | Total | Gluc. | Sulph.| Total | Conj. | Free  | VMA   | Indoles |
| Normals        | 8  | 0.751 | 0.551 | 0.200 | 2.032 | 1.793 | 0.252 | 0.184 | 1.869   |
|                |    | ±0.135| ±0.095| ±0.048| ±0.472| ±0.437| ±0.042| ±0.09  | ±1.31    |
| Anxiety neurotics | 8  | 0.520* | 0.382* | 0.137* | 1.212* | 1.051* | 0.161* | 0.288  | 1.116   |
|                |    | ±0.096| ±0.076| ±0.023| ±0.233| ±0.23  | ±0.03  | ±0.125 | ±0.69    |
| Schizophrenics (pre-treatment) | 8  | 0.626* | 0.449* | 0.176* | 1.723* | 1.509* | 0.216  | 0.428  | 1.660   |
|                |    | ±0.558| ±0.073| ±0.031| ±0.235| ±0.195 | ±0.071 | ±0.29  | ±0.936   |

The three groups were compared with one another by one-way analysis of variance using SED derived from analysis of variance to compute the 't' ratios.

*Significantly different from normals
+Significantly different from anxiety neurotics.

A statistically significant reduction in the level of VMA in schizophrenics with treatment to normal or near normal levels (Mean reduced from .428 to .210 mg/100 mg of creatinine). This seems to indicate that VMA excretion may be related to the manifest psychopathology of schizophrenia, as evidenced by the correlation co-efficient between the two and reduction in VMA level with treatment. Indoles also showed positive correlation to R. P. score, similar to but less pronounced than in case of VMA. However, as opposed to VMA, schizophrenics excreted lower amounts of indoles than the normals.

The present study did not find consistent significant relationship, amongst the schizophrenics, between R. P. score and steroid excretion. This is surprising considering that there was a significant difference between normals and schizophrenics on most of the steroid fractions (Table 3).

In this study as well as in another similar study of ours (Varma et al., 1978), the schizophrenics were found to excrete lower amounts of 17-KS as well as its glucuronide and sulphate fractions than normals. Hence, although, the schizophrenics differed from normals on most of the steroid values, amongst schizophrenics, there was no significant correlation between psychopathology as measured by the R. P. score and the steroid values. Therefore, it may be said that although, the steroid distinguished schizophrenics from normals, steroid excretion may not be a sensitive index of psychopathology of schizophrenics themselves. Also in this study, the changes in the steroid values amongst schizophrenics with treatment were significant only for 17-KS, total and glucuronides (reported in detail in Ghosh et al., 1976). Hence, by and large, most steroid values were apparently related to the diagnosis and not to the severity of symptomatology or changes in it with treatment. The relationship of schizophrenia with steroids and VMA suggests that whereas VMA may be more sensitive index of psychopathology, steroids may be more related to the diagnosis of schizophrenia per se.

In case of anxiety neurotics, significant positive correlation was found between the anxiety scores and 17-HS, total and conjugated, expressed in mg/100 mg of creatinine. It may suggest that these values
are more related to anxiety per se. The anxiety neurotics did not show a consistent correlation between anxiety scores and steroids. They also did not show a significant correlation between anxiety scores and VMA excretion, whereas the schizophrenics did.

To conclude, it may be said that VMA excretion may be more significantly related to the manifest psychopathology in schizophrenia and may be related to the level of psychotic anxiety, whereas steroid excretion especially 17-KS excretion may be more related to the basic schizophrenic process per se.

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