Reports

Presence of Toxic Factors in Urine from Schizophrenic Subjects

Abstract. Mice injected intraventricularly with toxic substances extracted from urine exhibited differential behavioral changes of novel character. Extracts from schizophrenics showed significantly more toxicity than those from nonschizophrenics. Further purification by a chromatographic procedure indicated that at least two currently unidentified active components are present.

This study is part of a larger coordinated program in which the clinical, psychological, biochemical, and biological aspects of schizophrenia are being investigated. Among tests used in this program to detect and evaluate characteristics of this illness, many bioassays have been explored. The one reported here seems especially promising as an objective criterion of schizophrenia. The study under discussion is an extension of the work of Wada and Gibson (see *1*); the chemical extraction methods differed from theirs, and the mouse was substituted as the experimental animal.

Urine was collected from 44 male patients, 18 to 50 years old, who had been kept for at least 4 weeks under controlled conditions, which included a uniform diet, uniform ward routines, and absence of drugs. The hospital population included 23 schizophrenic patients, roughly equal numbers of chronic undifferentiated and paranoid patients, and 21 nonschizophrenic patients, mainly sociopaths, some with alcoholism. The volume of urine for 24 hours was 20 to 25 percent lower in the schizophrenic than in the nonschizophrenic patients.

Twenty-five percent of the fresh 24-hour collection from each subject was stirred with 20 g of activated charcoal (Norit) (*2*) for 1 hour and then filtered. (The wet charcoal was washed with 50 ml of water and filtered; this step was found unnecessary and was discontinued.) The active components were extracted from the charcoal with a mixture of 50 ml of water (*p*H 11.9 to 12.0) and 70 ml of acetone. The mixture was stirred for 1 hour, and again the extract was collected by filtration. The charcoal was transferred to a beaker, 10 ml of water (*p*H 11.9 to 12.0) plus 50 ml of acetone was added, the mixture was stirred for 30 minutes, and the washing was collected by the same method. The two extracts were combined and lyophilized to dryness. The dried gummy residue was dissolved in 10 ml of water to give the "stock" solution. This was diluted fourfold with Tyrode solution, and 0.02 ml was injected into the brains of mice (strain C. F. No. 1, male, 40 to 50 days old) (*3*) by the standard technique used by bacteriologists (*4*). An India ink injection was found, in most cases, in the lateral ventricles. After injection the animals were observed over a period of several hours for changes in gross behavior. Control injections of Ringer's solution or Tyrode solution produced no changes.

Each diluted stock solution was administered to ten mice; behavioral changes, if they occurred, regularly appeared in seven or more of these. Nineteen of 23 samples from schizophrenic subjects produced abnormal behavior; 16 of 21 samples from nonschizophrenic subjects produced only slight effects (Table 1). The abnormal responses were as follows. In most cases, immediately after the injection the mice assumed a hunched-up position, with the hind legs spread far apart; the tails were elevated perpendicular to the body (Straub's tail phenomenon), and the mice were oversensitive to both sound and touch. This state lasted for 1 to 5 minutes and was followed by a second phase, which lasted a few minutes to several hours. Seven patterns (Table 1) were distinguished, from maximal excitation to maximal depression. Pattern 1 showed running movements followed by clonic and tonic convulsions; death occurred in 5 to 30 minutes. The survivors (pattern 2) remained immobile for several hours and appeared normal 24 hours later. Pattern 3 showed a novel behavioral phenomenon (*5*)—abnormal jumping, with crying, at the rate of 40 to 100 jumps per minute, lasting for 10 to 20 minutes and followed by immobility for another 100 to 160 minutes and then a return to normal. Pattern 4 was a stupor lasting for 40 to 120 minutes, during which time the mice were immobile and unresponsive to sound or touch. Animals could be left in an abnormal position during this period, but prodding evoked movement. Pattern 5 differed from pattern 4 in that the mice could not be placed in an abnormal position. Pattern 6 was a decrease in activity and playfulness, lasting for 20 to 30 minutes. Pattern 7, a quieting for 20 to 40 seconds after injection and full return to normal, was the "control" response to Ringer's or Tyrode solution and to some extracts from nonschizophrenic subjects. For six samples, selected at random for each group, the LD_{50} values (*6*) for urinary extracts from schizophrenic and nonschizophrenic subjects were found to be 35- and 80-percent dilutions, respectively, of the pooled stock solutions. The ED_{50} values for convulsions and/or abnormal jumping with crying-behavior response were

Table 1. Effects of "intracerebral" injection in mice of urinary extracts from schizophrenic and nonschizophrenic subjects.

Behavioral changes	Schizophrenic (23 samples)	Nonschizophrenic (21 samples)
Pattern No. 1		
Convulsion to death	7	0
Pattern No. 2		
Convulsion to coma	4	2
Pattern No. 3		
Jump with cry to stupor*	4	3
Pattern No. 4		
Stupor*	4	0
Pattern No. 5		
Unresponsiveness	3	2
Pattern No. 6		
Depression	1	10
Pattern No. 7		
No change	0	4

* See text.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

9.4- and 36-percent dilutions for the extracts from schizophrenic and nonschizophrenic patients, respectively.

These values strongly indicate that the observed differences are due to quantitative rather than qualitative factors.

One-dimensional descending chromatograms of the concentrated stock solution [Whatman No. 1 paper; 24° to 27°C; solvent: isopropyl alcohol, ammonium hydroxide, and water (40:1:9, vol/vol)] revealed several isolated spots under ultraviolet light of short wavelength. The volume of the water extracts for each of these spots was reduced to a proper amount and the extracts were injected into mice. The substance (or substances) from a blue spot (approximate R_F value, 0.69) produced pattern 4; the substance (or substances) from a purple spot (R_F value, 0.56) produced patterns 1, 2, and mainly 3, but with more frequent jumps (80 to 100 per minute) and a longer period of jumping behavior (20 to 30 minutes). From another area of the chromatograms, substances were obtained that produced violent scratching for 15 to 30 minutes, or Straub's tail phenomenon, lasting 20 to 40 minutes in the mild case. Water extracts from other areas produced only slight effects. The chromatographic separations were confirmed by several replicate runs.

Work is continuing on the identification of the active substances, which seem to be relatively stable and simple molecules, and on their significance in schizophrenia (*7*).

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References and Notes

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Epileptogenic Cerebral Electrical Activity and Serotonin Levels

Abstract. Chronically epileptic cats and monkeys showed marked activation of paroxysmal electrographic abnormalities both in the original focus and in a number of structures with secondarily altered functional states after intraperitoneal injection of the serotonin precursor 5-hydroxytryptophan (10 to 25 mg/kg) plus vitamin B_6. Much less 5-hydroxytryptophan was required to produce such epileptogenic activation if the animals had previously been treated with Marsilid, a monoamine oxidase inhibitor. Marked paroxysmal activity in epileptic animals was also produced by injection of Marsilid alone or of Marsilid in combination with reserpine. Since all of the activating agents used have been shown by others to elevate brain serotonin levels, the epileptogenic activation may be correlated with such high levels. Since the effects were at least partially blocked by atropine, such "serotonin-induced" activation may possibly involve some cholinergic mechanism.

During recent years, increasing significance has been attached to the alteration of the brain levels of neurohumoral agents such as serotonin and the catecholamines in the modification and organization of behavior. However, very little attention has been paid to the possible effect of such neurohumoral agents on epileptogenic electrical activity. In a recent study it was concluded that elevation of the serotonin level was responsible for the decrease in electroencephalographic abnormalities in epileptic patients after administration of Marsilid (*1*).

It is the purpose of this study (*2*) to show that, on the contrary, measures which are known to elevate serotonin levels cause increased electroencephalographic abnormalities in epileptic animals.

Three monkeys (two normal, one epileptic) and six cats (two normal, four epileptic) were used. All the animals had 42 to 50 electrodes permanently implanted in both the cortical and the deep structures (*3*). The epileptic animals had been made epileptic by placing aluminum hydroxide in one sensorimotor or frontal polar cortex at least 3 to 4 years prior to the experiment under discussion (*4*). A number of agents, or combinations of agents, which have been shown by others to increase the brain levels of physiologically active serotonin were used. These were (i) the serotonin precursor DL-5-hydroxytryptophan (5-HTP) (10 to 50 mg/kg), plus the decarboxylase coenzyme vitamin B_6 (50 mg/kg) (*5*); (ii) the monoamine oxidase inhibitor Marsilid (100 mg/kg), alone (*6*) or with 5-HTP (*7*); and (iii) Marsilid plus reserpine (0.1 mg/kg) (*7*). All the agents were given intraperitoneally. The electroencephalographic recording was made at least once every hour up to 24 hours after administration of any single agent. The time sequence of the administration, when more than one agent was given, is specified in the legend to Fig. 1.

None of the measures used had a notable effect on either the behavior or the electroencephalogram of the normal animals, except that some drowsiness and electrographic slowing was noted in normal animals given the larger doses of 5-HTP (25 mg/kg) or the smaller doses of 5-HTP (15 mg/kg) 17 to 20 hours after administration of Marsilid.

In epileptic animals, on the other hand, all of these agents produced marked activation of spontaneously occurring epileptic abnormality. Augmented paroxysmal discharges, of identical morphology and localization of spontaneous abnormality, occurred almost continuously up to 8 hours after administration of 5-HTP (15 to 25 mg/kg) plus vitamin B_6 (50 mg/kg) (Fig. 1, *d–f*). However, such electrographic evidence of epileptogenic activity was not accompanied by any behavioral seizure phenomena. When the animal was injected with Marsilid 17 to 20 hours before the administration of 5-HTP, as little as 10 mg of the latter per kilogram produced a similar but greatly intensified effect (Fig. 1*j*). Electrographic abnormalities, somewhat less marked but of almost identical morphology and localization, were produced in epileptic animals by administration of Marsilid or reserpine alone (Fig. 1*i, k, l*), or, more strongly, by Marsilid plus reserpine (Fig. 1, *m,n*). While the early effects of reserpine alone (Fig. 1*k*) and of 3,4-dihydroxyphenylalaine (DOPA) plus 5-HTP (Fig. 1*g*) were very similar, later, at 24 hours (Fig. 1*l*), the effect of 5-HTP was clearly predominant (Fig. 1, *d–f* and *h*). This finding is compatible with the view (*8*) that reserpine simultaneously releases physiologically active serotonin and catecholamines in the brain but that, because of the very rapid synthesis of serotonin (*9*), the effect of free serotonin is predominant in later reserpine action. No such epileptogenic-activating effect was noted when vitamin B_6 was given, either alone or with DOPA (25 to 100 mg/kg), the precursor of the catecholamines. The latter had eliminated all the spontaneously occurring epileptogenic ab-