Journal of Autism and Childhood Schizophrenia, 1971, 1, 1, 72–81

Biological Studies in Childhood Schizophrenia: Plasma and RBC Cholinesterase Activity

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Most investigations of possible metabolic aberrations in childhood schizophrenia failed to demonstrate differences between schizophrenic patients and control groups. Cholinesterase activity has not been previously reported in schizophrenic children. Similar studies with adult schizophrenics have not been consistent, despite some reports of abnormal cholinesterase activity. The present study reports the results of plasma (pseudo) cholinesterase and erythrocyte (true) cholinesterase activity in 16 male childhood schizophrenic patients and 16 male nonpsychotic hospitalized control patients. No significant differences were found between the two groups. An unexpected finding of high significance was the inverse correlation between acetylcholinesterase activity of erythrocytes and serotonin uptake by platelets in both the nonschizophrenic patients and the combined groups. This negative correlation was less significant in the schizophrenic patients.

While the study of childhood schizophrenia has received much attention during the middle third of the 20th century, few workers have directed their research to biochemical determinations and possible metabolic aberrations in this illness. In an earlier paper, Lucas, Warner, and Gottlieb (1970) reviewed biochemical studies in childhood schizophrenia and reported the results of a

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[3]We wish to thank Dr. Ralph Rabinovitch, Dr. Sara Dubo, and Dr. Frank Pasley of Hawthorn Center for making the subjects available for study and for their part in selecting and diagnosing the patients. Dr. James Grisell, Head of the Lafayette Clinic Computing Laboratory, analyzed the data.

study of serotonin uptake by platelets. Most of these studies have failed to demonstrate differences between schizophrenic patients and control subjects.

Domino and Krause have recently reviewed the literature on cholinesterase activity and mental disease. They report that numerous workers have demonstrated that stress and some psychiatric conditions with marked autonomic activity (anxiety, panic, acute adult schizophrenia, and depression) cause an increase in plasma cholinesterase. It has also been reported that some patients with chronic schizophrenia have decreased activity of plasma or serum cholinesterase, suggesting a possible liver, nutritional, or genetic deficiency. The findings in adult schizophrenia are controversial: Rubin (1958) suggested the presence of an acetylcholinesterase imbalance in functional psychosis. Ellman and Callaway (1961) found no difference in erythrocyte acetylcholinesterase levels between healthy and mentally ill persons. Gal (1963) reported increased blood cholinesterase activity in acute adult schizophrenic patients.

Domino and Krause have also just completed a study of cholinesterase activity in drug-free chronic adult schizophrenic patients and normals. They found decreased plasma cholinesterase activity in the adult chronic schizophrenic patients compared with normal controls.

The relationship of the childhood to the adult form of schizophrenia is still controversial. Nonetheless, it has been repeatedly documented that schizophrenic children grow up to become adult schizophrenics (Bennett & Klein, 1966; Brown, 1963; Freedman & Bender, 1957; Rabinovitch, Lucas, Ingram, et al., 1965). Cholinesterase activity has not previously been reported in schizophrenic children. The present study reports the results of plasma (pseudo) cholinesterase and erythrocyte (true) cholinesterase activity.

METHODS

Subjects

The subjects were 16 male schizophrenic patients and 16 male nonpsychotic control patients having the diagnosis of personality disorder or brain damage. The schizophrenic patients ranged in age from 8 years 10 months to 15 years 11 months (mean 12.9 years); the controls ranged in age from 9 years 4 months to 16 years 8 months (mean 12.5 years). Fourteen of the schizophrenic patients and 14 of the controls were selected from the same state hospital children's unit, where they had been hospitalized for periods of up to 4 years. They were in good physical health and all received the same well-balanced diet of 3,000 calories per day. The remaining four subjects were inpatients on the children's

unit at the Lafayette Clinic, in a short term treatment program. Final selection of all patients was made by the senior author after reviewing the records and interviewing the children to confirm their diagnosis, based upon the criteria described by Bender (1947) and Rabinovitch, Lucas, Ingram, et al. (1965). Comparison of the schizophrenic and nonschizophrenic patients by age, weight, height, and intellectual level is shown in Table 1.

Table 1
Comparison of Childhood Schizophrenic and Nonschizophrenic Patients

Factor	Schizophrenic (N = 16)	Nonschizophrenic (N = 16)	*t*	*p*
	Mean SD	Mean SD		
Age (yr)	12.9 ± 2.5	12.5 ± 2.2	0.492	n.s.
Height (in)	63.2 ± 5.8	61.1 ± 6.5	0.945	n.s.
Weight (lb)	114.8 ± 31.3	106.1 ± 34.4	0.718	n.s.
Verbal IQ	79.4 ± 15.7	89.4 ± 14.2	1.719	n.s.
Performance IQ	81.3 ± 14.5	95.3 ± 11.8	2.766	<.01
Full scale IQ	76.6 ± 12.3	91.6 ± 12.3	3.319	<.01

All but one of the schizophrenic patients and three of the controls had received psychoactive drugs (primarily phenothiazines) in moderate doses. Medication was withdrawn for at least 3 days prior to the study. The laboratory personnel were not aware of the identity of the subjects.

Cholinesterase Assay

A 5-ml fasting blood sample was drawn and placed in a heparin-containing tube (4U heparin/ml blood) which was centrifuged for 20 minutes at 3,000 rpm Plasma was separated from the packed elements and the buffy coat removed. The cells were mixed with an equal volume of cold 0.9% saline and centrifuged for 10 minutes at 3,000 rpm, after which the supernatant was removed and discarded. Plasma samples were diluted with 1 volume of cold 0.9% saline. The red cells were diluted with 9 volumes of cold saline. Samples were kept in ice until just before assaying. Assay was performed within 2 hours of the time the blood was drawn.

Cholinesterase was determined by a minor modification of the technique described by Ellman, Courtney, Andres, et al. (1961). This method utilizes acetylthiocholine (ASCh) as the substrate. Acetylthiocholine is hydrolyzed by both erythrocyte acetylcholinesterase (AChE) and plasma cholinesterase (ChE). The liberated thiocholine reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), producing a yellow anion. The intensity of the yellow color is directly proportional to enzyme concentration. Change in optical density was recorded (at 37.5°C ± 0.1°C and 405 nm) using a Beckman DB spectrophotometer equipped with a linear-log recorder. The final concentration of substrate in the cuvette was 5.0×10^{-3} moles. All enzyme activities were expressed in international units, defined as the amount of enzyme which catalyzed the cleavage of 1 μmole of substrate per ml of either plasma or packed red cells per minute at 37.5°C and pH 7.2.

Red cell count and hematocrit determinations were performed. Mean corpuscular volume (MCV) was calculated by the formula MCV = (hematocrit x 10)/RBC count expressed in millions per cubic centimeter.

Results

The result of plasma and red cell cholinesterase activity in the childhood schizophrenic and nonschizophrenic patients are summarized in Table 2, Figure 1 and Figure 2. Cholinesterase activity is expressed in millimicromoles (mμM)

Table 2

Plasma and RBC Cholinesterase Activity in Childhood Schizophrenic and Nonschizophrenic Patients

Activity (mμmole/ml/min)	Schizophrenic (N = 16)	Nonschizophrenic (N = 16)	*t*	*p*
	Mean SD	Mean SD		
Plasma cholinesterase	4651.5 ± 615.5	4434.6 ± 984.0	0.723	n.s.
RBC acetylcholinesterase	11,464.1 ± 834.5	11,399.6 ± 684.8	0.229	n.s.

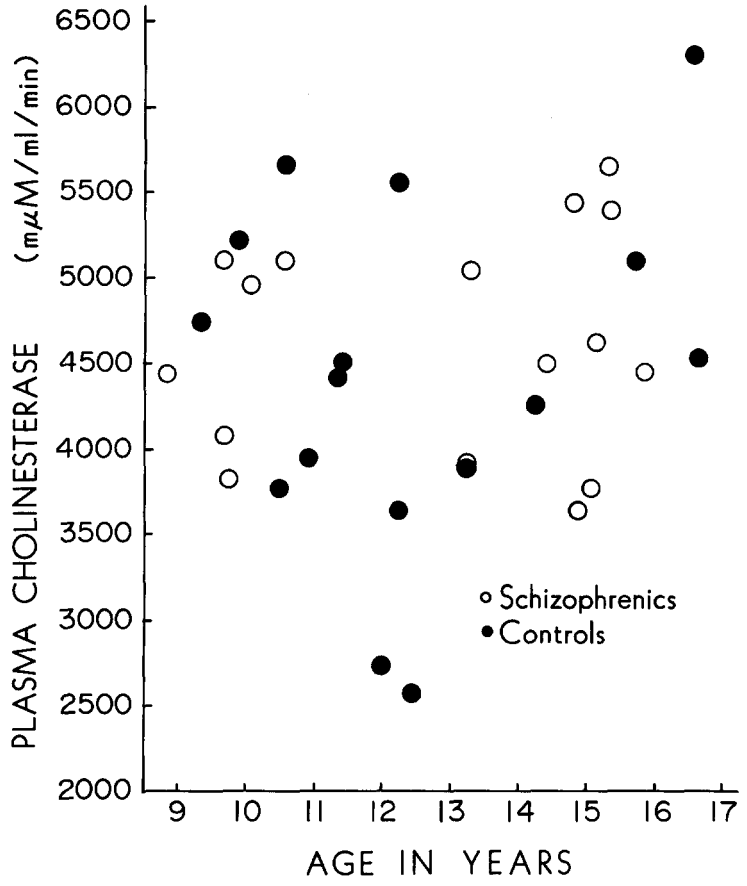

FIG. 1. Scattergram showing the relationship between plasma cholinesterase activity and age in childhood schizophrenic and nonschizophrenic patients (Mean, schizophrenics = 4,651.5 ± 615.5; mean, nonschizophrenics = 4,434.6 ± 984.0; p = n.s.).

of acetylthiocholine (ASCh) hydrolyzed per milliliter per minute at 37.5°C and pH 7.2. In the schizophrenic patients the range of plasma cholinesterase activity was from 3,609 to 5,663 (mean 4,651.5 ± 615.5) and in the nonschizophrenics from 2,579 to 6,318 (mean 4,434.6 ± 984.0). Red blood cell acetylcholinesterase ranged from 9,417 to 12,753 (mean 11,464.1 ± 834.5) in the schizophrenics and from 9,590 to 12,254 (mean 11,399.6 ± 684.8) in the nonschizophrenics. The differences were not significant.

Table 3 compares the hematocrit, red blood cell count (RBC), MCV, and red cell cholinesterase expressed as the cholinesterase activity per cubic micron

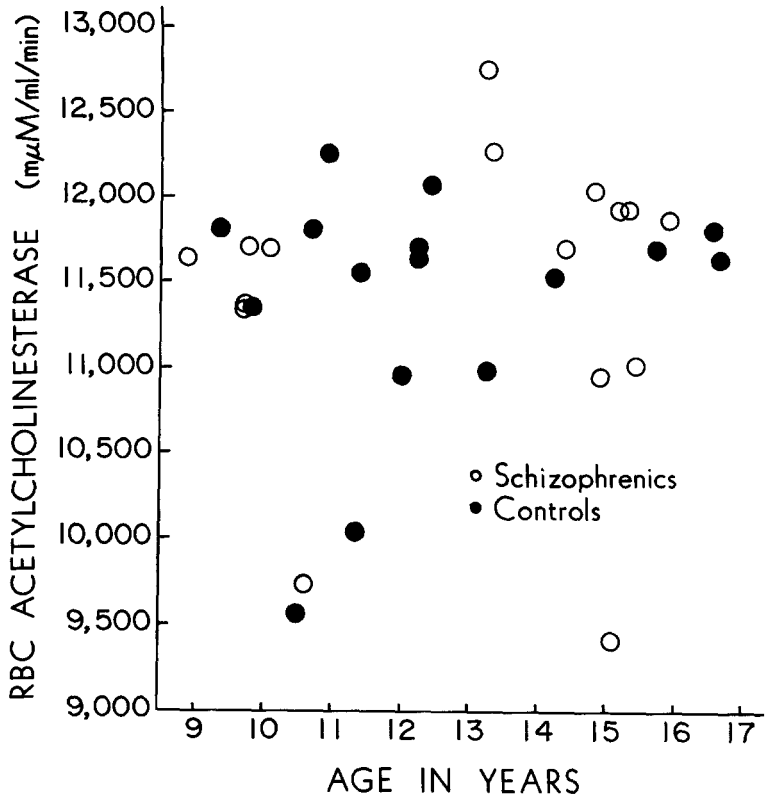

FIG. 2. Scattergram showing the relationship between RBC acetylcholinesterase activity and age in childhood schizophrenic and nonschizophrenic patients (Mean, schizophrenics = 11,464.1 ± 834.5; mean, nonschizophrenics = 11,399.6 ± 684.8; p = n.s.).

Table 3

Comparison of Blood Variables in Childhood Schizophrenic and Nonschizophrenic Patients

Variable	Schizophrenic (N = 15)	Nonschizophrenic (N = 16)	t	p
	Mean SD	Mean SD		
Hematocrit (vol. %)	46.9 ± 4.3	44.4 ± 2.7	1.877	n.s.
RBC (mil)	5.15 ± 0.54	4.89 ± 0.39	1.477	n.s.
MCV (μ^3)	91.5 ± 9.1	90.7 ± 10.7	0.223	n.s.
RBC activity/μ^3	125.9 ± 13.3	127.4 ± 15.2	0.283	n.s.

Note.—RBC = red blood cell count; MCV = mean corpuscular volume.

(RBC Act/μ^3) for the two patient groups. None of these variables differed significantly.

Correlation coefficients were determined for the above variables and for verbal, performance, and full-scale IQ scores (WISC or Stanford-Binet) as well as for serotonin uptake by platelets. The correlations with serotonin uptake have been reported elsewhere (Lucas, Warner & Gottlieb, 1970). Correlations that are of interest are summarized in Table 4. The only significant correlations were the negative ones between RBC acetylcholinesterase activity and serotonin uptake by platelets in the nonschizophrenic patients and in the combined groups.

Discussion

The activity of plasma and red cell cholinesterase varies considerably in hospitalized children and adolescents shortly after drug withdrawal and is unrelated to the diagnosis of childhood schizophrenia. Urbanek and Pietruszewska (1966) have reported changes in cholinesterase activity during phenothiazine treatment. It is possible that the drug treatment as well as some self-selection of diet and age contributed to the variability observed. In the recently completed study, Domino and Krause found much less variation in drug-free adults and generally lower values for plasma cholinesterase activity, especially in drug-free chronic schizophrenics (adults schizophrenics, mean = 3,292.0 ± 186.4; normal adults, mean = 4,073.2 ± 108.6). Red cell cholinesterase activity in adults approximated the values in children, but individual variation was not nearly so great (adult schizophrenics, mean = 10,670 ± 239.9; normal adults, mean = 11,156.3 ± 314.0).

We found no significant difference in serotonin uptake by platelets between the schizophrenic and nonschizophrenic children. However, there was a trend toward lower serotonin uptake in the older patients (Lucas, Warner & Gottlieb 1970).

The major positive finding in this study is the negative correlation between RBC acetylcholinesterase and serotonin uptake by platelets in the nonschizophrenic patients ($r = -0.531$) and in the combined groups ($r = -0.439$). This unexpected finding is of theoretical interest. It is known that in various animal species, especially in man, there is a striking inverse relationship between the acetylcholinesterase activity of red blood cells and platelets (Zajicek & Datta, 1953). In both types of cells this enzyme is associated with membrane stroma. Since membrane acetylcholinesterase has been implicated with the regulation of membrane permeability (Holland & Greig, 1950) as well as renewal of membrane phospholipids (DeSandre & Ghiotto, 1958), it is of interest to note that the uptake of serotonin into platelets may be inversely related to their acetylcholinesterase content. However, whether or not acetylcholinesterase is at all

Table 4

Correlation of Some Variables in Schizophrenic and Nonschizophrenic Patients

Variable	Schizophrenic			Nonschizophrenic			Combined		
	N	Mean SD	Correlation coefficient	N	Mean SD	Correlation coefficient	N	Mean SD	Correlation coefficient
Plasma cholinesterase *vs* age	16	4651.5 ± 615.5 12.9 ± 2.5	0.129	16	4434.6 ± 984.0 12.5 ± 2.2	0.204	32	4543.1 ± 827.8 12.7 ± 2.4	0.175
Plasma cholinesterase *vs* serotonin uptake	16	4651.5 ± 615.5 12.43 ± 2.77	0.152	16	4434.6 ± 984.0 12.85 ± 3.04	–0.189	32	4543.1 ± 827.8 12.64 ± 2.92	–0.072
RBC acetylcholinesterase *vs* age	16	11464.1 ± 834.5 12.9 ± 2.5	0.048	16	11399.6 ± 684.8 12.5 ± 2.2	0.222	32	11431.8 ± 764.0 12.7 ± 2.4	0.124
RBC acetylcholinesterase *vs* serotonin uptake	16	11464.1 ± 834.5 12.43 ± 2.77	–0.363	16	11399.6 ± 684.8 12.85 ± 3.04	–0.531*	32	11431.8 ± 764.0 12.64 ± 2.92	–0.439**
Plasma cholinesterase *vs* verbal IQ	13	4651.5 ± 615.5 79.4 ± 15.7	–0.448	16	4434.6 ± 984.0 89.4 ± 14.2	0.119	29	4543.1 ± 827.8 84.9 ± 15.7	–0.116

*Statistically significant: $p < 0.05$.
**Statistically significant: $p < 0.02$.

present in human platelets is problematical; Zajicek and Datta (1953) were able to detect none. At any rate the activity is too low to be measured by the Ellman method. We are now attempting to answer this question by utilizing a more sensitive radiochemical method of cholinesterase assay (Siakotos, Filbert, & Hester, 1970).

In view of current biological theories of mental disease involving a membrane deficit (Frohman, Warner, Yoon, Arthur, & Gottlieb, 1969) it is also of interest that the schizophrenic children show a less significant negative correlation (r = −0.363) between serotonin uptake and acetylcholinesterase activity than the controls. The reasons are unknown. Obviously mental illness involves a brain dysfunction rather than disturbances of red cell, platelet, or plasma. Further study of these variables in cerebrospinal fluid and brain tissue is therefore clearly indicated in the search for biological correlates of mental disease.

Summary

Plasma and RBC cholinesterase activity was determined in 16 male childhood schizophrenic patients and 16 male nonpsychotic hospitalized control patients. No significant differences were found between the two groups. An unexpected but highly significant finding was the inverse correlation between acetylcholinesterase activity of erythrocytes and serotonin uptake by platelets in both the nonschizophrenic patients and the combined groups. This negative correlation was less significant in the schizophrenic patients.

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