Table 2. Analysis of Variance of Whole Blood Cholinesterase Activity

	ASCh				BuSCh				AcMSCh			
	SS	*DF*	*MS*	*F*	*SS*	*DF*	*MS*	*F*	*SS*	*DF*	*MS*	*F*
Total	15,319·0	244	—	—	18,854	244	—	—	6,747·0	206	—	—
Regression on age	31·7	49	0·65	0·014	131	49	2·68	0·036	190·0	49	3·7	0·099
Male versus female	1,107·0	1	1,107·00	23·600*	923	1	923·00	12·400*	279·0	1	279·0	7·500*
Control versus exp.	4,950·0	1	4,950·00	105·000*	3,302	1	3,302·00	44·300*	491·0	1	491·0	13·100*
Between races	218·0	1	218·00	4·600	171	1	171·00	2·300	22·4	1	22·4	0·600
Error	9,012·0	192	47·00	—	14,327	192	74·00	—	5,765·0	154	37·4	—

Substrates: acetyl-, butyryl-, acetyl-β-methyl-thiocholine respectively.
*Significant at the 0·01 level of confidence.

results on serum cholinesterase activity in mental diseases are equally at variance with one another[5,6]. Of interest is the extensive work of Kalow *et al.*[7] on a genetic approach to the correlation of human serum cholinesterase to various factors.

A population consisting of 321 samples has been analysed. Of this population, 169 employees of the Hospital (clerks, nurses and labourers), 50 per cent of them males, were designated on the basis of their available medical records as 'healthy', and 152 mental patients (90 per cent males) made up the schizophrenic group. All blood samples were collected 1 h before the mid-day meal and in the case of the patients, at the day of admission, only individuals without previous medication were considered for analysis. At the time of the assay the psychiatric diagnosis was not known to the laboratory. However, after the psychiatric record became available only 76 mental patients (89 per cent male), all clear-cut cases of schizophrenia uncomplicated by epilepsy, early meningo-encephalomyelitis, alcoholism, barbiturate addiction, liver damage, etc., were considered for statistical analysis. Samples of 0·01 ml. of blood withdrawn from finger-tip puncture were immediately pipetted into 2 ml. of 1:1 mixture (kept at 25° C) of 0·1 M potassium phosphate buffer *p*H 7·4 and salt solution containing 0·04 M magnesium chloride and 0·1 M sodium chloride. Samples were also taken for hæmatocrit. For analysis of cholinesterase activity 0·2 ml. of the foregoing solution was added to the assay system as described elsewhere[8]. The spectrophotometric method[8] was used throughout the analyses. The substrates in the assays were acetyl (ASCh), butyryl (BuSCh), and acetyl-β-methyl(AcMSCh)-thiocholine iodides respectively.

The results given in Table 1 show the levels of cholinesterase activity of whole blood as measured by the hydrolysis rate of three substrates, that is, acetyl, butyryl, and acetyl-β-methyl-thiocholine iodides respectively. It was anticipated that these three substrates would be helpful in revealing differences, if any, with respect to the presence and function of the different types of cholinesterases in the blood of healthy and schizophrenic individuals. In applying Student's *t* test to the data obtained from cholinesterase activity of the whole blood of healthy and schizophrenic individuals, irrespective of age, sex, and race, one is led to the conclusion that there is a significant increase of hydrolysis ($P < 0{\cdot}001$) of each substrate by blood cholinesterase in schizophrenia. Indication of this difference is already intimated by the work of Early *et al.*[9] on serum cholinesterase activity of a small number of psychotic patients.

In Table 2 an analysis of variance is given for a total of 245 patients for two substrates (ASCh and BuSCh) and of 207 for acetyl-β-methyl-thiocholine, according to the technique indicated by Snedecor[10]. The results show that the increase of cholinesterase activity of the whole blood in schizophrenic individuals is significant, the confidence level being better than 0·01. Furthermore, the differences between the sexes are equally significant, with the males having higher cholinesterase activity if the groups are analysed without regard to the state of health. If, however, the effect of sex is considered with the state of health in view, then in the schizophrenics, in contrast to the healthy population, the females will show high values of cholinesterase activity. Other factors according to our results, such as age and race (Negro versus Caucasian) do not show correlation to differences in cholinesterase activity in the whole blood. It is to be noted, however, that unfortunately there were no samples available of Negro female in the schizophrenic population. Therefore, further assays would be desirable to establish whether race is another differentiating factor of cholinesterase activity in the whole blood.

These results, while they are in apparent support of earlier observation[1] on increased blood cholinesterase activity in schizophrenics, ought not to be construed as proof in assigning a direct responsibility to cholinesterase as a causative factor for the behaviour characteristics of schizophrenics. The scope of this communication precludes an extensively cogent discussion of the many variables contributing to contradictory results in similar work on interpretation and technique. Such surveys have been presented by others[11,12].

I thank all the blood donors and the management of the Veterans Administration Hospital for their collaboration. These statements and conclusions are the results of my own work and do not necessarily reflect the opinion or policy of the Veterans Administration.

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1 Rubin, L. S., *Science*, **128**, 254 (1958).
2 Krech, D., Rosenzweig, M. R., and Bennett, E. L., *Science*, **128**, 1176 (1958).
3 Rubin, L. S., *Science*, **128**, 1176 (1958).
4 Ellman, G. L., *Nature*, **192**, 1216 (1961).
5 Ravin, H. A., and Altschule, M. D., *Amer. Med. Assoc. Arch. Neurol. and Psych.*, **68**, 645 (1952).
6 Plum, C. M., *Clin. Chem.*, **6**, 332 (1960).
7 Kalow, W., and Staron, N., *Canad. J. Biochem. and Physiol.*, **35**, 1305 (1957).
8 Gal, E. M., and Roth, E., *Clin. Chim. Acta*, **2**, 316 (1957).
9 Early, D. F., Hemphill, R. E., Reiss, M., and Brummel, E., *Biochem. J.*, **45**, 552 (1949).
10 Snedecor, G. W., *Statistical Methods*, fifth ed. (Iowa State Univ. Press, Ames, Iowa, 1961).
11 Kety, S. S., *Science*, **129**, 1528 (1958).
12 Augustinsson, K. B., in *Methods of Biochemical Analysis*, **5** (Interscience Publishers, New York, 1957).

Effects of Lysergic Acid Diethylamide on Urinary 17-Ketosteroid and 17-OH Corticosteroid Levels of Female Rats

The marked aberrations in mental functions produced by minute doses of lysergic acid diethylamide (LSD-25) resemble aspects peculiar to schizophrenia[1,2]. As a result the drug has been suggested to be, and used as, an effective tool for the exploration of mechanisms and pathophysiology common to schizophrenia and LSD-25 intoxication[2,3].

Various investigators have explored the effects of LSD-25 on the pituitary-adrenal axis via alterations in adrenocortical output[4–6]. It has been reported that LSD-25 appeared to stimulate the pituitary-adrenal system as indicated by increased urinary 17-ketosteroid levels in man[4]. Bliss *et al.*[5] similarly reported elevated 17-OH corticosteroid levels in the peripheral blood of subjects who reacted to the drug with significant emotional disturbances. Nadel *et al.*[6], however, investigating urinary corticosteroid levels, claimed that LSD-25 did

not significantly alter the activity of the pituitary-adrenal axis in treated guinea pigs.

The present investigation sought to determine the effects of prolonged and intermittent administration of LSD-25 on adrenal function by evaluating alterations in urinary 17-ketosteroid and 17-OH corticosteroid levels. The excretion of urinary 17-ketosteroids[7,8] and 17-OH corticosteroids[5,9] have been frequently used as indices of adrenocortical activity.

Forty-eight female Wistar rats averaging 100 g in body-weight were divided equally into 3 groups. (All rats, including controls, were housed singly in metabolism cages. The room temperature maintained by an air-conditioning system ranged from 22° to 24° C. The average level of the laboratory background noise during the period of experimentation was 68 decibels during the daylight hours[20,21]. The laboratory recognizes, moreover, that isolation *per se*, by its neurotic-inducing tendencies, may counteract or minimize the endocrinological effects of an experimental situation. Previous investigations of isolated versus paired mice indicated significant increases in adrenocortical function[22].) Group *A* and *B* rats were injected subcutaneously with 50 and 75 μg doses of LSD-25 dissolved in normal saline. Group *C* control animals received an equivalent 1 ml. volume of saline. To diminish the development of tolerance[10,11], six injections were administered on alternate days. Urine samples were collected on the twelfth day for the 24-h period following the sixth injection. One ml. volumes were subjected to 17-ketosteroid analyses by the method of Rappaport *et al.*[12]. Similar volumes were used for free 17-OH corticosteroid analyses[13]. Urine volumes measuring less than 2 ml. were discarded.

Table 1. Effect of LSD-25 on Urinary 17-Ketosteroid and 17-OH Corticosteroid Production in Female Rats

Group	*n*	Dose (μg)	17-Keto (μg/day)	17-OH (μg/day)
Group *A* ± *S.E.*	14	50	55·0 ± 6·2	7·3 ± 1·5
Group *B* ± *S.E.*	14	75	58·4 ± 7·4	11·1 ± 4·4
Group *C* ± *S.E.*	15	—	45·9 ± 5·3	4·2 ± 0·6
% difference between *A* and *C*			+ 19·8	+ 73·8
P value			0·28	0·06
% difference between *B* and *C*			+ 27·2	± 164·3
P value			0·18	0·12
% difference between *A* and *B*			+ 6·2	+ 52·1
P value			0·73	0·42

Table 1 lists the alterations induced by LSD-25. It is apparent that although the 50 and 75 μg doses induced 19·8 per cent and 27·2 per cent increases in 17-ketosteroid output, these increases were not statistically significant[14]. Analyses of the 17-OH corticosteroid data revealed 73·8 and 164·3 per cent increases in group *A* and *B* values which approached the 0·06 and 0·12 levels of significance. While the differences between group *A* and *B* reactions were not statistically significant, the results demonstrated that the higher dose produced somewhat greater alterations in the steroid levels.

It is of interest that Bliss *et al.*[5] reported raised 17-OH corticosteroid levels in subjects displaying significant emotional disturbances but normal diurnal response patterns in subjects remaining emotionally calm after administration of LSD-25. Although the psychological response patterns of the various individual rats were not observed sufficiently to permit correlation of emotionality responses with the corticosteroid levels, the highest 17-OH corticosteroid value observed in group *C* rats was 8·6 μg. This value was exceeded by 4 rats in group *A* and 5 rats in group *B*, which attained levels as high as 52 μg. This suggests the importance of comparing graded psychological response patterns of emotionality against the biochemical parameter being investigated.

In general, the results presented here agree with claims reporting stimulated adrenal activity[4,5]. Additional evidence of alterations in eosinophilic[11,15] and leukocytic[15] frequencies similarly support this premise. Although Nadel *et al.*[6] reported no significant alterations in pituitary-adrenal activity, their data, nevertheless, indicated that LSD-25 caused a 19·2 per cent increase in urinary corticosteroids. The use of pooled guinea pig urine volumes in the Nadel *et al.* investigation would tend to neutralize and limit the observation of individual variations.

In conclusion, the work recorded here indicates that LSD-25 induced pronounced increases in free 17-OH corticosteroid levels and moderate increases in the 17-ketosteroid levels. This suggests increased pituitary-adrenocortical activity effects in the female rat. It is of interest that the LSD-25-induced hormonal alterations resemble pathophysiological patterns noted in certain schizophrenias. This indicates a possible relationship of LSD-25 intoxication responses to schizophrenia which may go beyond mere hallucinogenic similarities. To illustrate, Sackler *et al.*[16] present evidence indicative of elevated adrenocorticoid effects in schizophrenic patients and counter evidence of an improvement of psychosis following massive sex steroid therapy. These improvements were likewise associated with increases in eosinophil levels. Bliss *et al.*[5] reported increases in 17-OH corticosteroid output of disturbed schizophrenics and Altschule[17] similarly noted increased urinary 17-ketosteroid excretion in schizophrenics. Thus, the present findings of a stimulatory effect on the pituitary-adrenal system parallels conditions observed in schizophrenia. This lends support to the Sackler *et al.* hypothesis that relative and operative hyperadrenocorticalism and related concomitant endocrine deficiencies, such as relative or absolute hypothyroidism and/or hypogonadism, may be a possible ætiological and pathogenetical factor in at least one subgroup of schizophrenias[18,19].

We thank Sandoz Pharmaceuticals, Hanover, New Jersey, for supplying the LSD-25.

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[1] Forrer, G. R., and Goldner, R. D., *Arch. Neurol. and Psychiat.*, **65**, 581 (1951).
[2] Lingjaerde, P., and Skaug, O. E., *J. Nerv. and Ment. Dis.*, **124**, 578 (1956).
[3] Sackler, A. M., Sackler, R. R., Marti-Ibanez, F., and Sackler, M. D., *J. Clin. and Exp. Psychopath.*, **15**, 382 (1954).
[4] Hoagland, H., Rinkel, M., and Hyde, R. W., *Arch. Neurol. and Psychiat.*, 73, 100 (1955).
[5] Bliss, E. L., Migeon, C. J., Branch, C. H. H., and Samuels, L. T., *Psychosom. Med.*, **18**, 56 (1956).
[6] Nadel, E., Burstein, S., and Hoagland, H., *Amer. J. Physiol.*, **189**, 73 (1957).
[7] Herrmann, W. L., Schindl, I. K., and Bondy, P. K., *Proc. Soc. Exp. Biol. and Med.*, **103**, 103 (1960).
[8] Munday, K. A., and Blane, G. F., *J. Endocrinol.*, **20**, 266 (1960).
[9] Glenn, E. M., and Nelson, D. H., *J. Clin. Endocrinol.*, **13**, 911 (1953).
[10] Isbell, H., Belleville, R. E., Fraser, H. F., Wikler, A., and Logan, C. R., *Arch. Neurol. and Psychiat.*, **76**, 468 (1956).
[11] Feld, M., Goodman, J. R., and Giudo, J. A., *J. Nerv. and Ment. Dis.*, 126, 176 (1958).
[12] Rappaport, F., Fischl, F., and Pinto, N., *Clin. Chem.*, **6**, 16 (1960).
[13] Liddle, G. W., Richard, J. E., and Peterson, R. E., *Endocrinol.*, **57**, 594 (1955).
[14] Snedecor, G. W., *Statistical Methods*, fourth ed. (Iowa State College Press, Ames, 1946).
[15] Sackler, A. M., Weltman, A. S., and Sparber, S. B., *Effects of Lysergic Acid Diethylamide (LSD-25) on the Total Leukocytes and Eosinophils of the Female Rat* (in the press).
[16] Sackler, M. D., Sackler, R. R., Sackler, A. M., and van Ophuijsen, J. H. W., *J. Clin. Psychopath.*, **11**, 15 (1950).
[17] Altschule, M. D., *New England J. Med.*, **257**, 919 (1957).
[18] Sackler, A. M., Sackler, R. R., Marti-Ibanez, F., and Sackler, M. D., *J. Clin. and Exp. Psychopath.*, **15**, 382 (1954).
[19] Sackler, M. D., Sackler, R. R., Marti-Ibanez, F., and Sackler, A. M., *Quart. Rev. Psychiat. and Neurol.*, **17**, 297 (1956).
[20] Sackler, A. M., Weltman, A. S., and Jurtshuk, jun., P., *Aerospace Medicine*, 31, 749 (1960).
[21] Weltman, A. S., Sackler, A. M., and Gennis, J., *J. App. Physiol.*, **16**, 587 (1961).
[22] Weltman, A. S., Sackler, A. M., Sparber, S. B., and Opert, S., *Fed. Proc.*, 21, 184 (1962).