THE LIPIDS OF THE BLOOD PLASMA IN EPILEPSY

I. A Statistical Study of Single Determinations in 100 Epileptic and 32 "Normal" Subjects [1, 2]

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Since the underlying pathogenesis of epilepsy is still obscure after many years of investigation by ordinary technique, a physicochemical approach to the problem now appears to offer the greatest hope for its ultimate solution. The view, early expressed by Kussmaul and Tenner (1) and by Gowers (2), that the convulsive tendency which characterizes the disorder may have its basis in a subtle disturbance in the cellular physiology of the brain rather than in some gross localized lesion, has gained ground in recent years (3). Inasmuch as there is no procedure available for directly investigating the chemical physiology of the brain in the living subject, various attempts have been made in recent years to obtain indirect information regarding it by determining changes in the constituents of the blood and by studying various phases of the body metabolism.

The present study on the blood lipids was undertaken because a high percentage of children with severe epilepsy have been observed to be strikingly benefited by fasting or by a diet high in fat and low in carbohydrate (4), procedures which are now known to effect changes in the levels of the various blood lipids (5). Presumably, this type of regimen exerts a favorable influence on the activity of the brain cells in those patients who show improvement under such treatment. Because of the recognized importance of the physiologically antagonistic substances, lecithin and cholesterol, in the life processes of all cells (6) and because of the peculiar richness of brain tissue in these compounds, our attention has centered largely on them. It has been tentatively assumed that any alteration in their quantitative relationships in the blood might be indicative of a disturbance of their relationships in the fixed tissues (brain?).

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A survey of the literature shows that a number of workers have studied the blood cholesterol in epilepsy, but very little attention has been given to the other lipids. Even in the case of cholesterol there has been no agreement of opinion regarding its status in the disease. In small series of cases Pighini (7) and Parhon, Urechia and Popea (8) reported normal values. DeCrinis (9), Jacobi (10), Targowla, Badonnel and Berman (11), and Goebel (12) reported a tendency to hypercholesterinemia in small groups of cases, while Pezzali (13), Popea and Vicol (14), Robinson, Brain and Kay (15) and Gosden, Fox and Brain (16) found a mild degree of hypocholesterinemia. In a study of 40 cases Ornstein (17) found hypocholesterinemia in 52 per cent, hypercholesterinemia in 25 per cent and normal values in 23 per cent. Osnato and Killian (18) found a considerable degree of variation in the blood cholesterol in 22 patients. An examination of their data shows that 50 per cent of the values found by them were below the normal range claimed for the method used (Myers and Wardell (19)). The remainder were almost equally divided between normal and increased values.

In 1911 Bornstein (20) reported an increased quantity of lecithin in the blood of patients suffering from various nervous disorders, including epilepsy. He assumed this to be due to an increased breakdown of lipids in the cerebrum. Feigl (21) in a critical re-examination of Bornstein's work claimed that the increase in lecithin was not a common phenomenon but that, when it did occur, it was accompanied by other alterations in the blood lipids.

The present communication and the one following deal with the status of the plasma lecithin and total fatty acids as well as cholesterol in young epileptics under a variety of conditions. The purpose of this first study was to determine if there are any definite trends characteristic of epileptic patients as a group.

MATERIALS AND METHODS

In order that the problem might be attacked from the statistical point of view, the various lipids of the plasma have been determined in a series of 100 patients and 32 essentially normal persons in the same age range. With a few exceptions the patients and control subjects were children between the ages of 8 and 18 years. It was ascertained before blood samples were taken that none of the subjects included in the study were suffering from infectious diseases, anemia or other complicating disorders, which might be accompanied by alterations in the blood lipids. The diet of all subjects was the ordinary mixed diet, which is relatively high in carbohydrates. Although the 100 epileptic patients were institutional cases, very few of them were classed as extremely severe. Major convulsive seizures were recorded in but 11 of the 100 patients within 24 hours of the time when the blood samples were obtained. Slightly fewer than

half of them were on phenyl-ethyl-barbituric acid (luminal) or other drug therapy. No drug was given during the 12 hours prior to the time of taking the blood sample. The non-epileptic subjects were receiving no form of medication at the time. But one blood specimen was obtained from each person.

All samples were collected in specially cleaned tubes not less than 15 hours after the ingestion of food. Minimum amounts of saturated sodium citrate solution were used for preventing coagulation of the blood. Plasma was separated from cells by centrifugalization soon after the blood sample was drawn and was then immediately poured into the alcohol-ether mixture. The various chemical analyses were made according to the methods of Bloor (22).

For assistance in the statistical treatment of our data we are indebted to Dr. Edith Boyd. The particular statistical technics employed have been outlined by Dunn (23).

STATISTICAL ANALYSIS OF RESULTS

For the sake of brevity and conservation of space, the data for both the epileptic and normal subjects are recorded in Tables 1 and 2 and on Figure 1.

The average or mean values for cholesterol, lecithin, total fatty acid and the lecithin-cholesterol ratio are given in Table 1.

The current practice in statistics is to assume that there is a significant difference between two statistical constants, such as means, when the difference between the two constants is two or more times its standard error. This assumption is based on the fact that, when the ratio, R, of the difference and its standard error, is two, such a difference would occur by chance only 5 times in 100. Some authorities think that for biological data, it is advisable to assume a significant difference only after a ratio of 3 or a value for P of 0.003 (*i.e.* 3 chances in 1000) has been reached.

Following the latter interpretation of R and P, the difference between the means of cholesterol in the blood of non-epileptic and epileptic subjects is not significant. In contrast the mean value for lecithin in the blood of epileptics is significantly less than that for "normals" while that for total fatty acid is significantly greater. Moreover, the reduction in lecithin in the blood of the epileptics as a group is sufficient to lower significantly the lecithin-cholesterol ratio in spite of a "normal" mean value for cholesterol.

The differences in the constituents of the blood just described are graphically illustrated in Figure 1. The scale for the histograms of lecithin, cholesterol and total fatty acid in the first panel are so arranged that the mean values (indicated by the white square) all fall on the same perpendicular line. The frequencies for the epileptics are plotted on the same scale in the second panel. The mean value of lecithin in the blood

TABLE 1

Comparison of the mean values in milligrams per 100 cc. of cholesterol, lecithin and total fatty acid for 32 non-epileptic and 100 epileptic subjects *

	Non-epileptics			Epileptics			Difference ± Standard error	R	P
	Minimum	Maximum	Mean ± Standard error	Minimum	Maximum	Mean ± Standard error			
Cholesterol	103	257	167 ± 6.1	82	313	169 ± 4.3	+ 2 ± 7.5	0.27	.787
Lecithin	88	261	172 ± 7.7	26	283	125 ± 6.0	− 47 ± 9.8	4.80	.000004
Total fatty acid	213	463	312 ± 10.1	228	1203	471 ± 21.4	+159 ± 23.7	6.72	.000000
$\frac{\text{Lecithin}}{\text{Cholesterol}}$	0.74	1.73	1.04 ± 0.04	0.15	1.51	0.74 ± 0.03	−.30 ± 0.05	5.64	.000000

* Difference ± Standard Error equals the difference between the means of the epileptics and the means of the non-epileptics and its standard error. *R* indicates the difference divided by its standard error. *P* is the probability integral. $P \times 100$ equals the chances in 100 that the difference could be due to sampling.

of epileptic subjects falls below the line of the "normal" means, that for cholesterol on it, and that for total fatty acid above it. Moreover, the individual values for cholesterol in the blood of epileptics are evenly

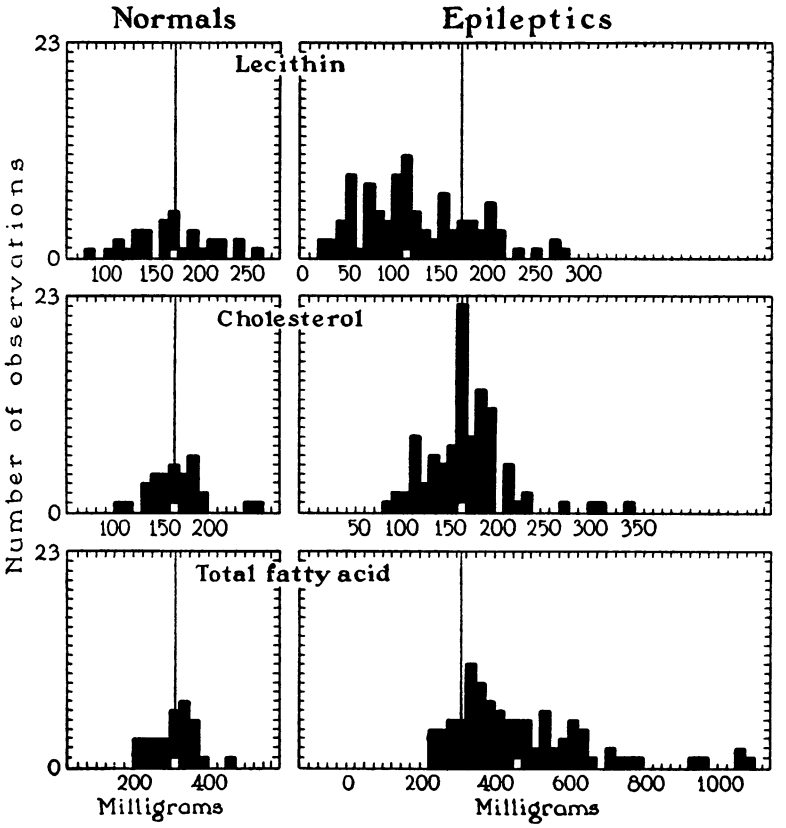

FIG. 1. HISTOGRAMS FOR THE LECITHIN, CHOLESTEROL AND FATTY ACID CONTENT OF THE BLOOD PLASMA IN "NORMAL" AND EPILEPTIC SUBJECTS

The histograms are so arranged that the perpendicular black line in both the normal and epileptic series passes through the mean value for each constituent in the plasma of normal subjects. The white square indicates the mean value for each constituent.

distributed around the "normal" mean, while most of those for lecithin fall below it and those for total fatty acid above it.

In addition the range of values for the pathological cases appears on inspection to be greater than that for the non-epileptic subjects. To test the reliability of this impression the coefficients of variation and their standard errors, and the difference and its standard error between the coefficients for "normals" and epileptics were calculated. The results are given in Table 2. The same basis of significance is used as in inter-

TABLE 2

Comparison of coefficients of variation for cholesterol, lecithin, total fatty acid, and the lecithin-cholesterol ratio for 32 non-epileptic and 100 epileptic subjects

	Non-epileptics	Epileptics	Difference ± Standard error	*R*	*P*
	C.V. ±S.E.*	C.V. ±S.E.*			
Cholesterol	20.35±2.69	25.44±1.93	+ 5.09±3.31	1.54	0.12
Lecithin	25.00±3.37	48.00±4.14	+23.00±5.34	4.31	0.01
Total fatty acid	17.94±2.35	45.22±3.83	+27.28±4.49	6.08	0.01
$\frac{\text{Lecithin}}{\text{Cholesterol}}$	23.55±3.15	38.64±3.14	+15.09±4.45	3.39	0.01

* C.V. ± S.E. is the coefficient of variation and its standard error.

preting the difference between two means. Since *R* is only 1.54 and *P* 0.12 for the difference between the coefficients of cholesterol, this constituent of the blood of epileptics is not more variable than in the blood of normal subjects. In contrast to cholesterol, total fatty acid, lecithin and the lecithin-cholesterol ratio are all significantly more variable in the epileptics than in the non-epileptics; *R* is always greater than three.

The possible significance of these results will be discussed in paper number 2.

SUMMARY

1. The lecithin, cholesterol and total fatty acid of the blood plasma have been determined in a group of 100 epileptic and in 32 non-epileptic children under essentially the same conditions.

2. No significant difference was found in the range of values for cholesterol or in its variability in the two groups.

3. The mean value for lecithin was found to be significantly lower and that for total fatty acid significantly higher in the epileptics than in the non-epileptic control subjects.

4. Both the phospholipid and the total fatty acid values showed significantly greater variability in the epileptic than in the non-epileptic subjects.

BIBLIOGRAPHY

1. Kussmaul, A., Tenner, A., and Bronner, E. On the Nature and Origin of Epileptiform Convulsions Caused by Profuse Bleeding and Also of Those of True Epilepsy. New Sydenham Society, London, 1859. Translated by E. Bronner.
2. Gowers, W. R. Epilepsy and Other Chronic Convulsive Diseases; Their Causes, Symptoms and Treatment. J. and A. Churchill, London, 1881.
3. (*a*) Lennox, W. G., and Cobb, Stanley, Medicine, 1928, vii, 105. Epilepsy from the Standpoint of Physiology and Treatment.
   (*b*) Holmes, E. G., Brit. M. J., 1929, ii, 260. The Epilepsies.

4. (*a*) Guelpa, G., and Marie, A., Bull. gen. de thérap., 1910, clx, 616. La lutte contre l'epilepsie par la désintoxication et par la rééducation alimentaire.
(*b*) Geyelin, H. R., Med. Rec. N. Y., 1921, xcix, 1037. Fasting as a Method for Treating Epilepsy.
(*c*) Wilder, R. M., Mayo Clinic Bull., 1921, ii, 307. The Effects of Ketonuria on the Course of Epilepsy.
(*d*) Petermann, M. G., Am. J. Dis. Child., 1924, xxviii, 28. The Ketogenic Diet in the Treatment of Epilepsy.
5. (*a*) Shope, R. E., J. Biol. Chem., 1927, lxxv, 101. Sugar and Cholesterol in the Blood Serum as Related to Fasting.
(*b*) Bloor, W. R., J. Biol. Chem., 1932, xcv, 633. Diet and the Blood Lipids.
6. (*a*) Leathes, J. B., Lancet, 1925, i, 803, 853, 957 and 1019. Role of Fats in Vital Phenomena.
(*b*) Hober, R. Physicalische Chemic. Zelle und der Gewebe. W. Engelmann, Leipzig, 1924, 5th ed.
(*c*) Bayliss, W. M. Principles of General Physiology. Longmans, Green and Co., New York, 1924, 4th ed.
(*d*) Degkwitz, R., Klin. Wchnschr., 1930, ix, 2336. Cholesterin und Lecithin im Wasser- und Säure-Basenhaushalt.
(*e*) Okuneff, N., Biochem. Ztschr., 1928, cxcviii, 296. Zur Frage rach der Bedeutung der Lipoidstoffe in der Zellpermeabilität.
7. Pighini, G., Arch. ital. de biol., 1910, liii, 297. Sur le contenue en cholestérine du liquide céphalo-rachidien dans quelques maladies mentales.
8. Parhon, C., Urechia, C., and Popea, A. (See S. Stenberg, Acta med. Scandinav., 1929, lxxi, 561.)
9. DeCrinis, M. Die Beteligung der humoralen Lebens vorgange des menschlichen Organismus am epileptischen Anfall. Julius Springer, Berlin, 1920.
10. Jacobi, W., Monatschr. f. Psychiat. u. Neurol., 1926, lxii, 296. Cholesterinstoffwechsel und Konstitutionspathologie der Psychosen.
11. Targowla, R., Badonnel, M., and Berman, A., L'Encephale, 1923, xviii, 138. Sur la cholestérinémie dan les maladies mentales.
12. Goebel, F., Compt. rend. Soc. de biol., 1924, xc, 1191. Cholestérine du liquide céphalorachidien et du sang dans les maladies psychiques.
13. Pezzali, G., Riforma med., 1923, xxxix, 433. Ricerche sul Contenuto del Sangue in Azoto (Residuo), Cloruri, Cholesterina, Grassi, Glucosi, Indicano, e Calcio nell' Epilessia.
14. Popea, A., and Vicol, A., Compt. rend. Soc. de biol., 1925, xciii, 749. Les Variations de la cholestérinémie durant l'accès épileptique.
15. Robinson, S. H. G., Brain, W. R., and Kay, H. D., Lancet, 1927, ii, 325. The Association of Low Blood Cholesterol with the Occurrence of Fits in Epileptics.
16. Gosden, M., Fox, J. T., and Brain, W. R., Lancet, 1929, ii, 12. The Cholesterol of the Blood Plasma in Epilepsy.
17. Ornstein, I., Compt. rend. Soc. de biol., 1926, xciii, 1622. Cholestérinémie chez les Aliénés.
18. Osnato, M., Killian, J. A., Garcia, T., and Mattice, M. R., Brain, 1927, l, 581. Comparative Chemical Studies of the Blood and Spinal Fluid in Epilepsy.
19. Myers, V. C., and Wardell, E. L., J. Biol. Chem., 1918, xxxvi, 147. The Colorimetric Estimation of Cholesterol in Blood with a Note on the Estimation of Coprosterol in Feces.

20. Bornstein, A., Ztschr. f. d. ges. Neurol. u. Psychiat., Originalien, 1911, vi, 605. Über die Lecithinämie der Geisteskranken.
21. Feigl, J., Biochem. Ztschr., 1918, lxxxvii, 237. Über das Vorkommen von Phosphaten im menschlichen Blutserum. VI.
Ibid., 1918, lxxxviii, 53. Über das Vorkommen und die Verteilung von Fetten und Lipoiden im Blute bei Geisteskrankheiten.
22. (*a*) Bloor, W. R., J. Biol. Chem., 1928, lxxvii, 53. The Determination of Small Amounts of Lipid in Blood Plasma.
(*b*) Ibid., 1929, lxxxii, 273. The Oxidative Determination of Phospholipid (Lecithin and Cephalin) in Blood and Tissues.
23. Dunn, H. L., Physiol. Rev., 1929, ix, 275. Applications of Statistical Methods in Physiology.