LONGITUDINAL CLINICAL AND NEUROCHEMICAL STUDIES ON SCHIZOPHRENIC AND MANIC-DEPRESSIVE PSYCHOSES [1]

SAMUEL BOGOCH, M.D., PH.D., KARL T. DUSSIK, M.D., CHRISTA FENDER, M.D., AND PETER CONRAN, M.D.[2]

This laboratory is studying structure-function relationships in psychiatric patients with reference to the nervous system itself. Despite the inherent difficulty in doing structural studies on the nervous system *in vivo*, such studies are necessary if primary structure-function relationships in the nervous system in terms of behavior are to be approached. Evidence in recent years has emphasized the relative autonomy of the nervous system with reference to peripheral organs and fluids. This physiological autonomy, frequently referred to in terms of the Blood-Brain Barrier concept, has its basis in the fact that most substances pass in and out of the nervous system relative to the blood with difficulty. There is less likelihood therefore that biochemical correlates of either normal mental function or a disturbance thereof, central nervous system in nature, will be detected as readily in its primary form by studies on peripheral organs and fluids. With this in mind, we have been investigating the structure and function of some little-studied substances, native to the nervous system itself and relating these chemical studies to detailed longitudinal clinical investigations on the same individual psychiatric patients. This report will give only a brief outline and a few examples of the combined clinical and neurochemical approach which is being followed.

STUDIES ON BRAIN GANGLIOSIDE

The carbohydrate-containing macromolecules of the nervous system, both glycolipid and glycoprotein in nature, are being investigated. One of these substances, occurring in high concentration in grey matter of brain, called brain ganglioside, has been studied in terms of its structure, histological localization, and physiological function. By the isolation of a previously unrecognized constituent of brain ganglioside, ganglio-cerebroside, the formulation was made of the repeating unit of this substance(1). The chemical structure of brain ganglioside showed it to contain water-soluble constituents (neuraminic acid, hexosamine, and hexoses) on one surface of the molecule, and lipoid-soluble constituents (sphingosine, stearic acid) on the other surface of the molecule, suggesting to us that brain ganglioside might be a membrane substance involved in receptor and transport functions in nerve cells(1, 2). By virus studies we have shown that brain ganglioside is indeed a receptor for certain neurotoxic viruses(3, 4). By pharmacological studies with smooth muscle preparations(5) it was possible to show that brain ganglioside has marked stimulatory function in a membrane-active system, the clam heart, suggesting that brain ganglioside may be involved in transmission phenomena in the nervous system. In immunological studies, specific antibodies to brain ganglioside were prepared by us and these were used with fluorescent antibody techniques to demonstrate the nerve cell body localization of brain ganglioside(6). Thus, it has been possible, by utilizing several methodologies, to go from the determination of molecular structure, to physiological function, and then back to the histological localization for a substance native to brain. These studies provide evidence that brain ganglioside and substances chemically akin to it may be involved in important regulatory functions in terms of controlling the entry and egress of a number of important constituents in the nervous system. These functions are referred to collectively as the Barrier-Antibody System(7, 8). If such is indeed the case, the relevance of these functions to mental health and mental disorder requires careful exploration.

[1] Read at the 116th annual meeting of The American Psychiatric Association, Atlantic City, N. J., May 9-13, 1960.

[2] The Neurochemical Research Laboratory, Department of Psychiatry, Harvard Medical School, and Massachusetts Mental Health Center, Boston, Mass.

EARLIER STUDIES ON NEURAMINIC ACID IN CEREBROSPINAL FLUID

There is one compartment in the nervous system which is readily accessible to repeated sampling in both man and animals without untoward physiological effects. This compartment is the cerebrospinal fluid (CSF). Our initial studies on "total neuraminic acid" in CSF in 1957, demonstrated an accumulation of this substance in CSF with age, with the maturation being apparent by 7 or 8 years of age(7, 8). Schizophrenic adults showed lower values for this substance relative to controls and comparable only to the values found in some children under 7 years of age. One group(9) working without double-blind controls, did not observe low values as consistently in a small group of schizophrenic patients. A second study(10) was unable to observe differences but used a correction factor for glucose which we have shown to be inadequate(14). On the other hand, our finding of low values for neuraminic acid in CSF in schizophrenic patients has been independently confirmed by two other laboratories(11, 12),[3] although one of these confirmatory studies(11) also employed correction factors for glucose which we have shown to be unsatisfactory. The initial findings have also been confined in our extended series of cases now numbering 1,024.

Longitudinal studies(13) on schizophrenic and other psychiatric patients over weeks and months demonstrated that these low values were "group consistent" for all but 8% of untreated individual schizophrenic patients, and that with treatment only some 7% more demonstrated increased concentration of "total neuraminic acid" bringing the values into the normal range. By "double-blind" careful clinical evaluation it was observed that clinical change was frequently temporally coincident with and qualitatively (and occasionally quantitatively) related to the change in the concentration of "total neuraminic acid."

All the above early studies used methods which determined both bound and free neuraminic acid, together with some other substances chemically related to neuraminic acid(13, 14). By the careful quantitative fractionation procedure subsequently developed in this laboratory(14, 15, 16), it has been possible to define the relative contributions of each of these fractions. In addition, it has been possible to obtain quantitative measures of the amounts of two other substances, the hexosamines and hexoses which are bound in combination with neuraminic acid in the macromolecular glycoproteins of CSF. These new quantitative studies have, in addition to supporting the earlier findings on neuraminic acid in schizophrenics, provided data on the altered neurochemistry in other psychiatric diagnostic groups : in chronic brain syndromes, manic and depressive psychoses, as seen in the concentrations of protein-bound hexosamine and hexose. Furthermore, maturation phenomena have been shown for protein-bound neuraminic acid, hexosamine, and hexose, which are of interest in relation to the possible relevance to psychological maturation(8).

THE GLYCOPROTEINS OF CEREBROSPINAL FLUID

The quantitative fractionation procedure which has been developed for CSF(14) is briefly shown in Figure 1. Whole CSF is lyophilized and dialyzed quantitatively, the whole non-dialyzable fraction (Fraction I) is then partitioned into a water-soluble fraction (Fraction G) and a very small insoluble fraction (Fraction P). Fraction G is further partitioned by column chromatography into 6 glycoprotein fractions. Fraction II, the whole dialyzable material, is then treated on column chromatography with the resultant separation of cations, anions and neutral sugars. Free neuraminic acid from 0 to 12 μg. per cc. of CSF can be demonstrated in this way. Table 1 illustrates the large amount of quantitative data in terms of total solids, nitrogen, phosphorus, hexose, reducing sugars, hexosamine, and neuraminic acid, previously unavailable, and now available on each of these subfractions of CSF. It may be noted that the analyses indicate that Fraction G is a glycoprotein fraction particularly rich in carbohydrate components when compared with comparable glycoproteins of blood. Table 2 shows the electrophoresis and quantitative elution of hexose and neuraminic

[3] See also Christoni, G., and Zappoli, R. Am. J. Psychiat., Vol. 117, Page 246, Sept. 1960.

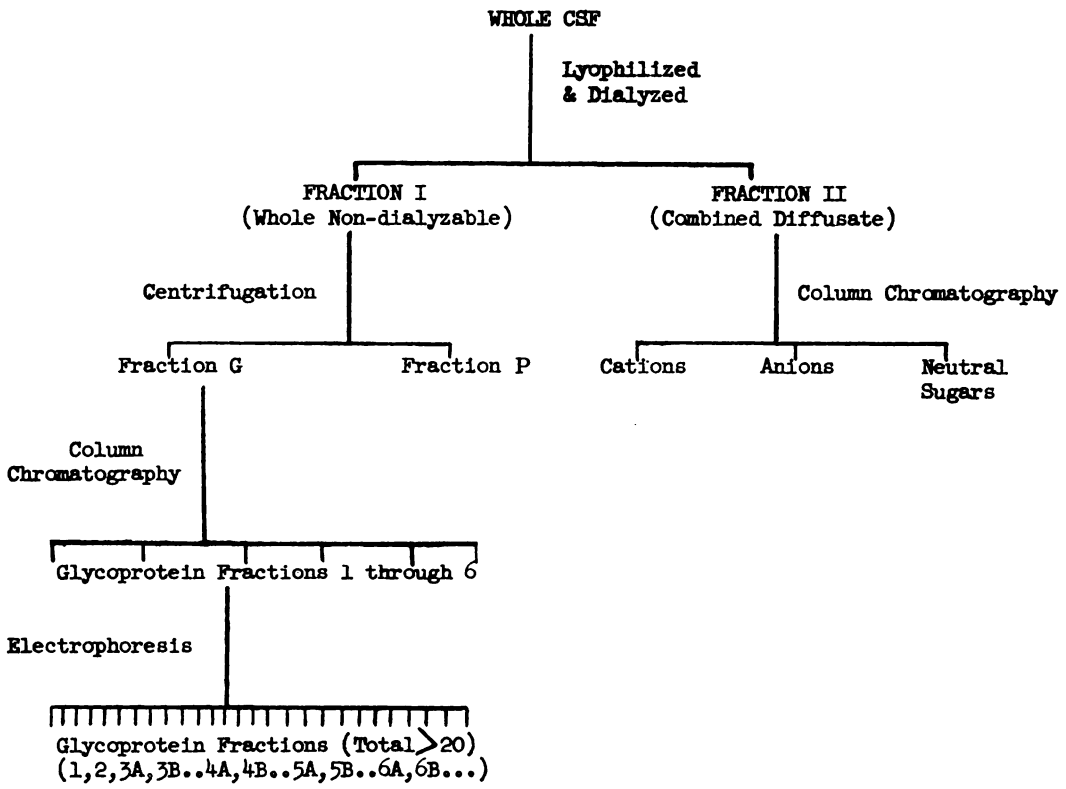

FIGURE 1
FRACTIONATION OF CSF

TABLE 1

ANALYSIS OF FRACTIONS II, I, P, AND G*

Fraction	*Total Solids mg./cc. CSF*	*% N*	*% P*	*% Hexose (as glucose)*	*% Reducing Sugars (as glucose)*	*% Hexosamine (as galactosamine)*	*% Neuraminic Acid*
II	10.58 ±1.93 N=16	1.70 ±1.39 N=11	0.18 ±0.14 N=15	5.38 ±2.63 N=11	7.16 ±2.56 N=16	0 N=17	—
I	0.442 ±0.164 N=11	13.4 ±2.6 N=3	0.57 ±0.47 N=7	5.28 ±4.97 N=8	—	3.46 ±3.30 N=8	3.12 ±1.37 N=11
P	0.029 ±0.018 N=90	—	—	14.9 ±10.0 N=90	—	3.17 ±1.56 N=80	2.43 ±1.19 N=90
G	0.355 ±0.145 N=196	14.40 ±2.99 N=64	0.51 ±0.31 N=15	6.65 ±5.07 N=165	—	3.68 ±2.40 N=170	2.20 ±0.78 N=189

* The first value listed for each parameter is the mean, the second the standard deviation of the mean, and the third (N) the number of individual cases analyzed.

acid of Fraction G. It may be seen that normal specimens show the highest concentration of these carbohydrates bound to protein in the α_2 and α_1 globulin regions, whereas schizophrenic patients and some other mental hospital patients show the highest concentration in the β-globulin zone, rather than in the α_2 and α_1 globulin zone. Table 3 shows the further partition of Fraction G by IRC-50 column chromatography(17) into 6 subfractions differing in mobility and in absolute concentration of protein-bound hexose. Each of these subfractions has been further subdivided by paper electrophoresis. By this combination of ion-exchange chromatography and electrophoresis the presence of at least 20 individual glycoproteins has been demonstrated in CSF(17). Furthermore, these fractions differ between individuals. Table

TABLE 2

ELECTROPHORESIS AND QUANTITATIVE ELUTION OF HEXOSES AND NEURAMINIC ACID OF FRACTION G.

	% OF TOTAL ELUTED				
	γ*	β	$\alpha_2+\alpha_1$	*Albumin*	*"Pre-albumin"*
			Normal Specimens		
Hexose :	13.0	0	47.4	33.4	6.4
	7.2	1.0	56.5	18.7	16.6
	29.2	8.5	37.0	21.6	37.6
	8.9	17.7	46.7	25.2	33.8
Neuraminic Acid :	30.0	0	48.5	10.5	10.9
	25.6	16.9	26.1	22.4	9.0
	17.4	10.2	42.1	26.0	4.3
			*Neuropsychiatric disorders***		
Hexose :	19.8	34.8	38.3	0.9	6.2
	11.7	75.0	4.7	6.5	2.1
	2.4	48.5	3.6	45.6	0
	23.7	25.2	8.8	19.7	22.6
	2.0	45.5	20.1	0	32.4
	41.2	45.2	2.4	8.6	4.7

* γ, β, and $\alpha_2+\alpha_1$ refer to fractions which show the same mobilities as the respective globulin fractions in human blood serum.

**Pooled samples from groups of ten acute and chronic mental hospital patients 80% of whom had the diagnosis of schizophrenia.

TABLE 3

AMBERLITE IRC-50 CHROMATOGRAPHY OF FRACTION G GLYCOPROTEINS

Fraction	*Buffer*	*pH*	*% of Nitrogen recovered*	*% Hexose of fraction*	*Electrophoretic distribution*
1	Citrate	4.25	9.3	16.3	
2	Citrate	5.2	9.9	13.1	
3	Citrate	5.72	30.2	10.3	$\alpha_2 + \alpha_1$; albumin
4	Acetate	6.15	26.4	6.7	$\alpha_2 + \alpha_1$; β ; albumin
5	Citrate-phosphate	7.0	15.3	3.4	$\alpha_2 + \alpha_1$; β ; albumin
6	Citrate-phosphate	8.0	9.1	5.5	$\alpha_2 + \alpha_1$; β ; albumin

4 shows the quantitative amount of protein-bound hexose, hexosamine, and neuraminic acid in individual specimens of CSF. The ratios of these substances in terms of molecular amounts of each vary markedly from individual to individual over the range of hexose : hexosamine concentration of less that 1 : 1 through almost 10 : 1. Most people show ratios of 1 : 1 or 2 : 1. The high degree of chemical individuation observed is of great interest since these are chemical constituents of the nervous system itself. The

TABLE 4

MOLAR QUANTITIES AND RATIOS OF HEXOSE, HEXOSAMINE AND NEURAMINIC ACID IN FRACTION G OF INDIVIDUAL SPECIMENS OF CSF

Total Solids in Fraction G mg./cc. CSF	*Molar Ratios*			*Molar Ratio Hexose: Hexosamine*	
	Hexose (as glucose)	*Hexosamine (as galactosamine)*	*Neuraminic Acid*		
0.338	1.4	5.6	1	0.25	
0.331	1.8	2.8	1	0.65	
0.490	3.5	5.2	1	0.69	
0.530	1.3	1.8	1	0.72	
0.616	2.9	3.8	1	0.76	
0.320	3.8	4.8	1	0.79	
0.658	2.7	3.0	1	0.90	
0.450	3.2	3.6	1	0.91	
0.314	2.2	2.2	1	1.00	(1:1)
0.734	2.7	2.7	1	1.00	
0.292	3.7	3.5	1	1.06	
0.323	3.4	3.1	1	1.10	
0.275	4.7	4.1	1	1.15	
0.335	3.8	3.2	1	1.19	
0.159	4.6	3.9	1	1.18	
0.371	3.6	3.0	1	1.20	
0.499	4.2	3.4	1	1.24	
0.150	4.5	3.6	1	1.25	
0.274	5.0	3.8	1	1.32	
0.178	2.9	2.0	1	1.45	
0.278	7.4	4.2	1	1.76	
0.428	6.9	3.9	1	1.76	
0.405	5.4	2.8	1	1.93	(2:1)
0.192	5.0	2.3	1	2.17	
0.308	5.7	2.6	1	2.19	
0.424	6.2	2.8	1	2.22	
0.179	3.2	1.4	1	2.28	
0.345	4.3	1.9	1	2.30	
0.354	14.4	6.2	1	2.32	
0.434	6.1	2.6	1	2.34	
0.403	6.6	2.7	1	2.44	
0.374	10.3	3.6	1	2.80	
0.187	5.0	1.7	1	2.94	
0.425	9.9	3.3	1	3.00	(3:1)
0.543	7.7	2.4	1	3.20	
0.296	3.3	1.0	1	3.30	
0.278	34.4	9.9	1	3.48	
0.538	5.3	1.3	1	4.07	(4:1)
0.142	8.7	2.1	1	4.20	
0.480	6.1	1.1	1	5.55	
0.276	6.7	1.1	1	6.09	(6:1)
0.428	13.6	2.2	1	6.18	
0.594	6.7	0.7	1	9.58	(10:1)

possibility that this potential for chemical individuation is related to chemical bases of individuality must now be considered.

GLYCOPROTEINS IN PSYCHIATRIC DISORDERS

Following the observations of 1958, when the absolute amounts of these substances are compared in groups of psychiatric disorder, certain unique patterns emerge. Table 5 shows the absolute concentrations of protein-bound hexose, hexosamine, and neuraminic acid in individual psychiatric patients and general hospital controls. It may be seen that patients with manic psychoses demonstrate very high values for macromolecular hexose. Macromolecular hexosamine is elevated in both chronic brain syndromes and in manic psychoses, but is significantly lower from the control group in untreated schizophrenic patients. Macromolecular neuraminic acid is also low in untreated schizophrenic patients. With treatment, schizophrenic patients synthesize (or release) glycoproteins with normal or even greater than normal amounts of hexosamine, but containing still significantly lower levels of neuraminic acid(18).

Longitudinal careful clinical follow-ups in double-blind studies with neurochemical studies have now been in progress for as long as 2½ years on approximately 150 patients. Figure 2 shows an example of the relative constancy of these constituents in a single depressed patient in an 11-month period and the marked change which occurred accompanying gross functional change, in this case the change from marked depression to the normal affective or slightly elated state. This change was accompanied by a 500% increase in the absolute amount of protein-bound hexose despite the fact that there was no change in the total amount of glycoprotein present per cc. of CSF. Thus, it is not that more

TABLE 5

CONCENTRATIONS OF MACROMOLECULAR (BOUND) HEXOSE, HEXOSAMINE, AND NEURAMINIC ACID IN CSF OF PSYCHIATRIC AND GENERAL HOSPITAL PATIENTS

Diagnosis	*Fraction G*								
	Hexose, μg./cc. CSF (as glucose)			*Hexosamine, μg./cc. CSF (as galactosamine)*			*Neuraminic Acid μg./cc. CSF*		
	Mean	*Range*	*N*	*Mean*	*Range*	*N*	*Mean*	*Range*	*N*
1. Schizophrenia untreated	17.8	(4.0-50.5)	49	8.0***	(1.8-24.2)	57	5.8***	(2.3-9.4)	44
2. Schizophrenia, treated	18.0	(7.0-54.5)	38	14.0	(3.2-44.6)	60	6.7***	(2.7-10.1)	65
3. Other (than 4, 5 and 6) Mental Hospital	26.8	(5.5-81.2)	9	8.5***	(1.6-15.0)	18	7.8***	(3.3-17.2)	26
4. Chronic Brain Syndromes	20.5	(11.0-31.0)	22	23.1**	(5.1-60.0)	21	8.0***	(4.4-11.9)	23
5. Manic Psychoses	72.2***	(51.6-88.0)	4	29.0*	(9.0-57.0)	5	10.6	(10.3-11.1)	3
6. Depressive Psychoses	19.2	(14.5-31.0)	18	10.8	(3.9-18.8)	14	11.0*	(5.5-15.1)	13
7. General Hospital	22.5	(7.0-55.0)	11	13.5	(8.3-17.8)	16	10.3	(9.0-13.4)	9

a- N=Number of patients.

Symbol Next to Mean	*Level of Significance of Difference of Means Relative to "General Hospital" Group*
***	P = < 0.001
**	P = 0.001
*	P = 0.05
None	P = > 0.05

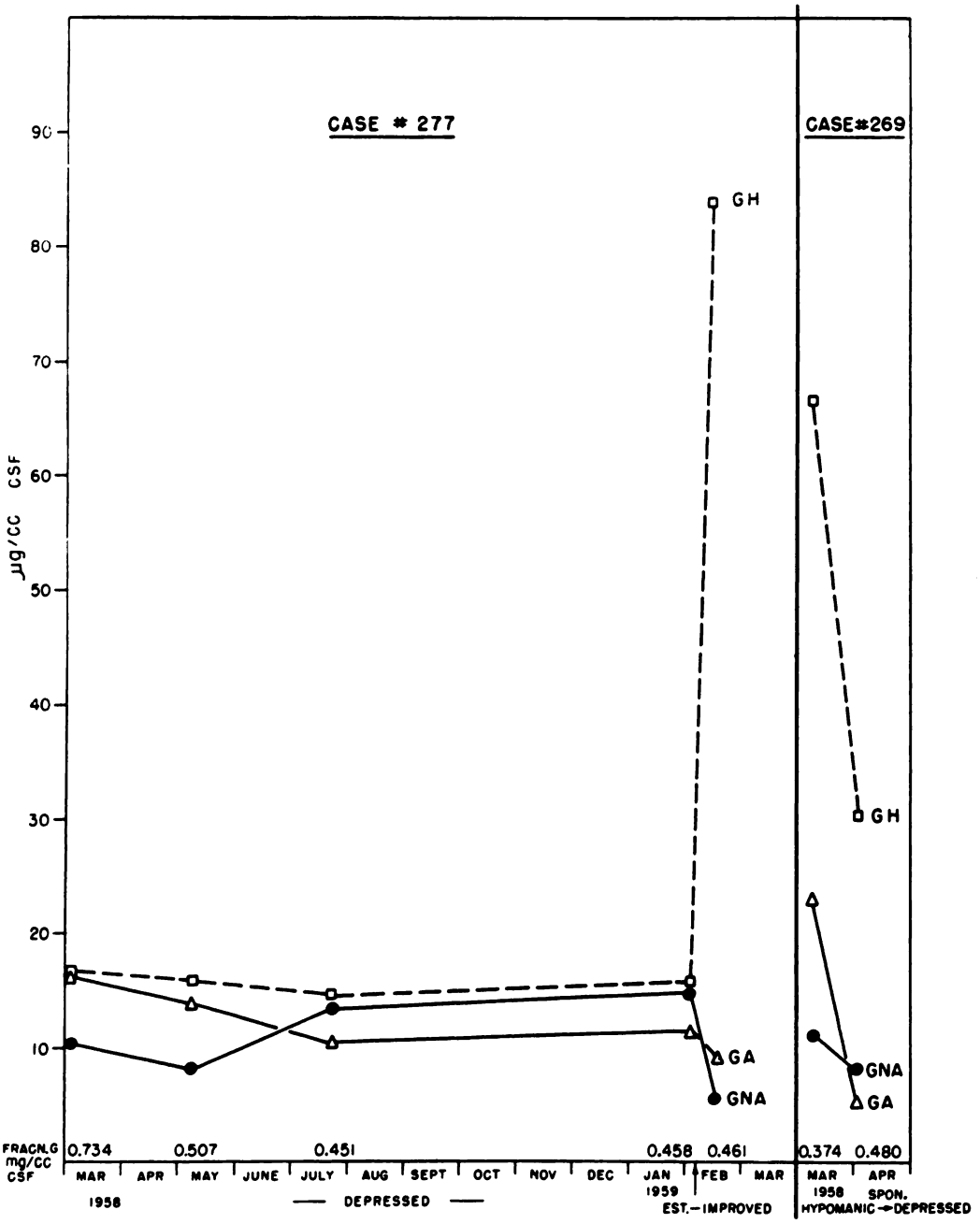

FIGURE 2

THE CHANGE IN ABSOLUTE CONCENTRATION OF MACROMOLECULAR (BOUND) HEXOSE (GH), HEXOSAMINE (GA), AND NEURAMINIC ACID (GNA) OF FRACTION G WITH TIME. CASE 277 IS A PATIENT WITH THE DIAGNOSIS OF PSYCHOTIC DEPRESSION; TREATED WITH ELECTROSHOCK THERAPY AND IMPROVED. CASE NO. 269, PATIENT WITH THE DIAGNOSIS OF HYPOMANIC STATE WHO PASSED SPONTANEOUSLY INTO A SEVERELY DEPRESSED STATE WITHOUT ANY SPECIFIC TREATMENT

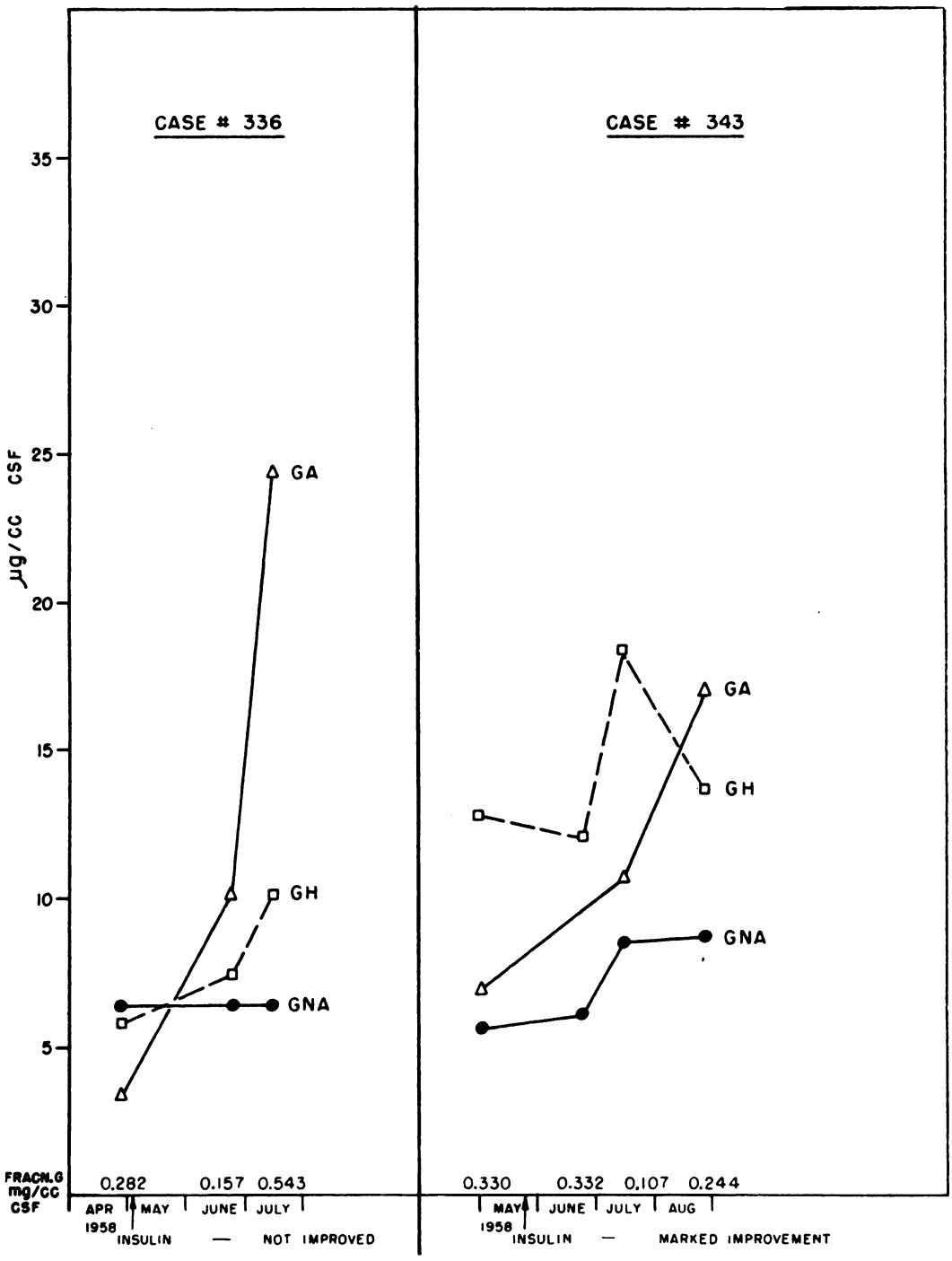

FIGURE 3

THE CHANGE IN ABSOLUTE CONCENTRATION OF MACROMOLECULAR (BOUND) HEXOSE, HEXOSAMINE, AND NEURAMINIC ACID OF FRACTION G WITH TIME IN TWO PATIENTS WITH THE DIAGNOSIS OF PARANOID SCHIZOPHRENIA WHO RECEIVED INSULIN THERAPY. THE SYMBOLS ARE AS IN FIGURE 2.

glycoprotein is present, but that a very different glycoprotein appears accompanying this functional change. The reverse change is seen also: that is, a drop in hexose accompanying the reverse functional change from a hypomanic to a depressed state in another patient who received no specific therapy. The changes in protein-bound hexose and hexosamine appear to relate to mood and other secondary symptomatology and have occurred with drug therapy as well as with electroshock therapy and spontaneously. On the other hand, levels of protein-bound neuraminic acid do not vary with secondary symptomatology but appear to be related to the level of maturity in terms of classical psychological and psychoanalytic periods of development and other more primary personality characteristics. Figure 3 shows two cases with the diagnosis of paranoid schizophrenia treated with insulin therapy. It may be seen that whereas both began with typically low amounts of protein-bound neuraminic acid and hexosamine, the changes in these glycoprotein patterns with treatment were quite different in the two cases. Thus, the first case, representing 85% (Table 5) of schizophrenic patients thus far studied, showed a 700% increase in the amount of hexosamine synthesized in protein-bound form, but no change in neuraminic acid. This patient did not improve. The second case, representing about 15% (Table 5) only of schizophrenic patients studied, showed a somewhat lesser increase in hexosamine, but there was in addition a marked increase in the neuraminic acid bringing it into the normal range. This patient showed a marked clinical improvement.

WORKING HYPOTHESES

These preliminary studies suggest the working hypothesis that there is a disturbance in the synthesis or maintenance of both macromolecular hexosamine and neuraminic acid in schizophrenia and that the disturbance in hexosamine can be overcome (indeed "overcompensated for") with treatment, but that the disturbance in neuraminic acid is most often refractory to therapies presently available. Since hexosamine is a synthetic precursor of neuraminic acid, this accumulation of a precursor, without the accumulation of its derivative, suggests the possibility of an enzymatic block.

It must be emphasized that these are neither more nor less than working hypotheses and their theoretical nature must clearly be distinguished from the quantitative nature of the experimental data from which the hypotheses arise. Most important, these examples are given in order to demonstrate that these new methods indicate a highly individual extensive chemical geography of native central nervous system constituents and provide a unique opportunity to study primary correlations between neurochemical and clinical events in individual patients over long periods of time.

SUMMARY

Macromolecular glycolipids and glycoproteins of the nervous system itself are being investigated in terms of their chemical structure, histological localization, and physiological function. Parallel "double-blind" clinical and quantitative neurochemical studies in psychiatric patients and controls have indicated a high degree of chemical individuation of these nervous system constituents, which may have relevance to individuality. In addition, distinctive patterns of these constituents have been observed in chronic brain syndrome, manic, depressive, and schizophrenic patients, and controls. These new quantitative methods provide a unique opportunity to study longitudinally primary correlations between neurochemical and clinical events in individual patients.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the chemical technical assistance in these studies of Mr. Peter Belval, Mrs. Claire Grunberg, Miss Dorothy Winer, Mrs. Barbara Schupak, Mrs. Judith Davidson, and Mrs. Annette Klein. CSF samples were also obtained with the assistance of Dr. A. Crocker and Dr. R. C. Payne of the Children's Medical Center, Dr. M. Nicholson, Dr. J. Crehan, and Dr. C. A. Fager of the New England Deaconess Hospital, and the members of the staff of the Massachusetts Mental Health Center. This work was done with the support of United States Public Health Service grants and the National Association for Mental Health through the Scottish Rite Research Committee.

BIBLIOGRAPHY

1. Bogoch, S. : Biochem. J., **68** : 319, 1958.
2. Bogoch, S. : Nature, **180** : 197, 1957.
3. Bogoch, S. : Virology, **4** : 458, 1957.
4. Bogoch, S. : Virology, **7** : 161, 1959.
5. Bogoch, S., and Bogoch, E. S. : Nature, **183** : 53, 1959.
6. Bogoch, S. : Nature, **185** : 392, 1960.
7. Bogoch, S. : Am. J. Psychiat., **114** : 172, 1957.
8. Bogoch, S. : A.M.A. Arch. Neurol. Psychiat., **80** : 221, 1958.
9. Atwood, R. P., and Green, J. P. : Federation Proc., **18** : 364, 1959.
10. Robins, E., and Marx, F. O. : Proc. IV Int. Cong. Biochem., Vienna. London : Pergamon Press, Ltd., 1958. Sec. 13, No. 94.
11. Papadopoulos, N. M., McLane, J. E., O'Doherty, D., and Hess, W. C. : J. Nerv. Ment. Dis., **128** : 450, 1959.
12. Chistoni, G., and Zappoli, R. : Riv. Sper. Freniatria, **83** : 1, 1959.
13. Bogoch, S., Dussik, K. T., and Lever, P. G. : A.M.A. Arch. Gen. Psychiat., **1** : 441, 1959.
14. Bogoch, S. : J. Biol. Chem., **235** : 16, 1960.
15. Bogoch, S. : Am. J. Psychiat., **114** : 1028, 1958.
16. Bogoch, S. : Proc. IV Int. Cong. Biochem., Vienna, ed. F. Brücke. Pergamon, 1959. p. 196.
17. Bogoch, S., and Appleton, W. G. : To be published.
18. Bogoch, S. : Am. J. Psychiat., **116** : 743, 1960.

DISCUSSION

R. A. CLEGHORN, M.D. (Montreal, P. Q.). —These three papers demonstrate the critical influence of method on the progress of research. The development of new techniques in biochemistry in the past 15 years permits the measurement of organic substances in minute amounts, which was inconceivable prior to that time. This has led to a variety of renewed attacks on problems of mental illness. Frequently the concept underlying these approaches is not new, as has been pointed out with respect to the possibility of a toxic metabolic factor in schizophrenia.

The utilization of such techniques as have been described in these papers makes certain demands of clinical investigators. It is fair to expect that they will be applied with a rigour no less exacting than that exercised in other biological fields, and presented in as comprehensible a form as possible. An audience composed of psychiatrists cannot be expected to be as familiar as yet with the intricacies of modern biochemistry as applied to its discipline, or with many of the newer terms in common parlance among biochemists. To be frank, I do not feel that certain of the three papers just read gave sufficient thought to the process of lucid communication of material which might well be expected to be somewhat unfamiliar to a large section of today's audience. Doubtless these are difficult data to present simply, but even in reading the manuscripts I found the going heavy at times. Furthermore, viewers cannot be expected to extract significance from columns of data. If any of the authors be even slightly offended by my somewhat sententious and gloomy preamble, I will not be upset if they put this down to the discussant's halting understanding.

My interpretation of a discussant's duty is that he should provide critical appraisal and, where possible, an assessment of implications of the work under consideration.

Dr. Frohman spoke in his introductory paragraph of the use of psychotomimetic drugs in "physiologic amounts." In order to be precise it is appropriate to point out that no dose of these drugs constitutes a physiologic amount. They produce effects in minimal or greater pharmacologic amounts only. A few lines later, these authors refer to Woolley and Shaw as having established : "models suggesting that a central disturbance in synaptic transmitter substances, such as catecholamines, may be present in schizophrenia." Actually, the Woolley-Shaw reference is to serotonin, an indole ethylamine, which is not a catecholamine. These are perhaps simple errors in exposition, but not happy examples of rigour.

In discussing the putative role of abnormal indole compounds from schizophrenic patients, the authors mention McGeer, *et al.*, who reported unusual amines in the urine. Unhappily, they seem to be unaware that a later paper from McGeer's laboratory back tracks on their previous position. They state in 1959 (Canad. J. Biochem Physiol., 37, pg. 1493) that : "The differences found between the schizophrenic and normal extracts have not been stressed. Difficulties in quantitation and the uncertain origin of the spots, coupled with the relatively small number of extracts studied, make us feel that significant conclusions can be drawn only after further work." Acheson, working in the same laboratory, was unable to confirm McGeer's original observations on urine. By dealing in such detail with controversial work the Lafayette group have not obtained support for their own endeavours which should, of course, be judged on its own merits. In previous work along the lines of that reported

today, Dr. Gottlieb and his associates have reported that a chronic schizophrenic population has alterations in certain aspects of carbohydrate metabolism. This seemed to be associated with an unknown component in their plasma.

The present study represents an attempt to isolate this factor, using recognized techniques which they describe. It appears from their investigations that there may be a substance, either an alpha globulin or something associated with this protein, which alters the ratio of lactate to pyruvate produced by the chicken's erythrocyte. They say that it appears to be related to previously described metabolic defects in schizophrenia. Unfortunately, as their own review emphasizes, these "defects" are still highly controversial, with various findings denied by apparently equally reliable workers. I would personally be happier if they had demonstrated that the plasma from patients with a variety of recognized infectious and metabolic diseases had been examined for this abnormal protein. There are also criticisms which might be levelled at their interpretation of the fractionation of proteins, *e.g.*, the graphs submitted do not always bear out the direction the authors are pointing to. The five runs in Fig. 1 are five quite different patterns, and it requires a great deal of intuition to find a common point among them. The situation is somewhat better with the Spinco electrophoretic separation (Fig. 3). One might have been better pleased with a larger number of cases.

One great difficulty in assessing the results is the authors' use of ratios of *numbers*; indeed, also ratios of ratios. It is necessary to have some indication of the absolute figures and the normal variation also.

Finally, the *numbers* are the amounts of lactic and pyruvic acids in chicken erythrocytes under specified conditions. According to the authors, the ratio of these indicates "the degree of oxidation within the cell (*i.e.*, the rate of function of hydrogen transport)." The lactic/pyruvic ratio simply does not measure this, and saying it does not make it so. At best, the lactic and pyruvic acid content of the cells incubated with glucose represents a balance between a great many cell constituents which basic research is constantly exploring further.

Research in psychiatry cannot make advances on the basis of rough-and-ready approximated definitions, so that, suggestive though this work may be, one cannot say that it yet means that there is an established metabolic fault in schizophrenia.

I am most unhappy with the authors' inference that the active principle they find in schizophrenic plasma, "may reflect a disturbed mechanism for adapting to stress." As long as that word "stress" is used indiscriminately, the actual mechanisms may not be examined properly.

The experimental findings of Dr. Frohman and his group are undoubtedly interesting but they have lost emphasis in being too closely associated with speculative explanations.

Controversy is a characteristic of science and one depending on a variety of factors such as subtleties of technique, accuracy of performance, and the intrusion of the investigator's emotional investment, usually in an hypothesis. The paper read by Dr. Cole demonstrates one way of dealing with conflicting reports. The introductory paragraph refers to 37 relevant papers, ignoring detail but highlighting points pertinent to the study to be reported. This is entirely legitimate and leaves the audience free to contemplate the results of the studies presented. The examination of the patients and controls by a "blind" procedure inspires confidence. The replication of the original finding supplied support for the initial data which indicated that one of the indoles in the urine was present more often in patients than in controls. Then the biochemists' regard for exactitude spoiled this promising picture. By extracting the urine at the demonstrated optimum pH, many of the previously indole negative controls became positive. Thus the difference between patients and controls disappeared. It must have been disappointing but it was science. In the exercise of another control measure it was shown that an antibiotic with activity on intestinal flora, where indole producing bacteria flourish, caused a decrease in the incidence of indole positive urines in both patients and normal individuals. The conclusion that the source of the indole studied is from the gut is modest but it puts a nail in the coffin of an elusive red herring. In this presentation of negative results one sees the operation of a highly important facet to progress in research.

Exciting though the third paper is, I confess to some blocking at my blood-brain barrier. The concatenation of unfamiliar and complicated chemical terms makes the ego and the id sound like H_2O. However, we must open our intellectual maws and get on with the job of incorporating this novel biochemical bolus.

The findings reported by Dr. Bogoch are based on studies of well over 1,000 cases. This is a reassuring population sample. To summarize his results briefly we see that :

1. The value for total neuraminic acid in the C.S.F. was low in cases of schizophrenia and chronic brain syndrome. This low value increased with improvement in a few cases.

2. The hexosamine content of C.S.F. was also lower in untreated schizophrenics than in controls. It rose with treatment, both in cases which improved clinically and those which did not. Contrary to the low level found in schizophrenics, hexosamine was elevated in both manic psychoses and chronic brain syndromes.

3. Cases of manic psychoses showed high values for C.S.F. macromolecular hexoses. This value decreased with the alleviation of the hypomanic and the onset of a depressed state.

4. The authors relate the level of protein bound neuraminic acid more closely to the level of "maturity" than to what they call secondary symptomatology and mood. In the cases studied, they point out that of the schizophrenic patients improving there was a marked increase in the neuraminic acid, bringing it within normal range. Contrariwise, the changes in protein bound hexose and hexosamine appeared to relate more closely to mood and other secondary symptoms, and might change with treatment despite the absence of improvement.

Under the label "Working Hypotheses," Dr. Bogoch and his associates suggest that an enzymatic block occurs in schizophrenia, preventing the elaboration of neuraminic acid from its precursor hexosamine in certain cases. Hence, while this constituent may accumulate, it does not necessarily follow that its derivative, neuraminic acid, will increase. They urge a clear distinction be maintained between their quantitative data and their theory. This is wise counsel for all workers and might be summarized as maintaining the distinction between content and concept.

In conclusion, I would like to express the hope that, as these biochemical studies get on to firmer ground, a closer association be made with clinical assessment of mental status. In initial stages, this is understandably subordinated, but ultimately much will be gained by keeping the patient in the picture.