CHAPTER 29

Studies of Plasma Protein Factors That May Be Involved in Psychoses

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During the last five years work has been reported from four independent groups indicating the existence of a protein factor in human plasma that may be involved in psychotic behavior. In contrast to many investigations, these studies refreshingly tend to confirm each other.

REVIEW OF STUDIES: THREE GROUPS

In 1957 Heath et al.[1,2] at Tulane University reported the extraction of a protein fraction from the plasma of schizophrenic patients that, when injected into nonpsychotic volunteers, produced transient psychotic episodes. They called this substance "taraxein" and in double blind studies reported that similar fractions from nonpsychotic subjects had no effects on injection. They used an animal bioassay to test their taraxein fractions prior to tests on man, a test which consisted of observing the effects of its injection into monkeys. Active fractions were reported to produce catatonic-like states in the monkeys and also the occurrence of spiking from septal regions of the brain, as recorded from implanted electrodes in chronic preparations. The initial procedure used by the Tulane workers for the isolation of taraxein proved to be difficult to reproduce. The procedure indicated, however, the precipitability of the postulated protein by ammonium sulfate at concentrations long known to precipitate the less soluble globulins from plasma. It also showed taraxein to belong to that group of globulins which are insoluble in the absence of electrolyte, the euglobulins. The initial

steps of the Tulane procedure were virtually identical with those used for many years in the preparation of gamma globulins.

Early attempts to confirm the effects of taraxein by other workers were unsuccessful. Extraction procedures similar to those used at Tulane according to Robins [3] and Siegel et al. [4], who report negative findings from several other laboratories, yielded inactive fractions; and various criticisms of the Tulane procedures and techniques have been discussed [2]. Melander and Martens [5], however, have reported that taraxein potentiates the action of some psychoactive drugs.

Taraxein is unstable. Fractions reported active at Tulane when shipped to other laboratories for testing in monkeys were found to be inactive [2].* Some taraxein fractions from schizophrenic patients were found inactive by the Tulane group. The lability of taraxein and the uncertain reliability of its extraction procedures are factors that may well account for the difficulty in obtaining confirmatory results.

During 1956 and 1957, Winter and Flataker [6] at the laboratories of Merck Sharp & Dohme studied the effects of injecting plasma samples from hospitalized psychotic patients, physically ill non-psychotic hospitalized patients, and normal control subjects into young rats of the Holtzman strain and observed the effects on their behavior. They used for a bioassay a measure of the ability of the rats to climb a 5-ft rope from the floor to a platform containing food pellets. The rats were fasted twenty-four hours before testing and were allowed to eat only briefly on the food platform before being returned to their cages. After a period of training, the rats ran up the rope in 2 to 3 seconds with great regularity and without any indication of decrement in performance throughout the course of a day, as measured by the climb time. Drugs such as lysergic acid diethylamide (LSD) and mescaline, which in man produce psychotic episodes, increase the climbing time when injected into trained rats. Usually the increases in climbing time occur within minutes after the intraperitoneal injection of an active substance, reach a maximum within half an hour, and diminish to preinjection levels by the end of an hour. Following the injection of a drug like LSD, the rats were tested at 5, 10, 20, 30, 45, and 60 minutes post-injection or until the climb time reached its preinjection values. The climbing time in seconds was plotted against the time after

*Also, personal communication.

injection in minutes and the area under the skewed bell-shaped curve, minus that under the base line, was called the climb time delay (CTD), and reported in minute-seconds. For each determination of CTD, a group of animals, usually five, was used and the value reported as mean CTD. The area under the curve has been shown to have a linear relationship to the logarithm of the drug dosage. The injection of saline has no effect on the CTD. The validity and significance of the data were established by analysis of variance.

Winter and Flataker found that the intraperitoneal injection of 1 ml of blood plasma or serum from normal and psychotic subjects increased the CTD after the manner of LSD except that the time course for serum or plasma was longer. The injection of plasma or of serum from the majority of schizophrenic patients and other psychotic patients produced a response which averaged two to three times greater than that of plasma from normal control subjects. Comparisons of the effects of plasma from 80 psychotic patients with that of 82 nonpsychotic subjects, including both general hospital patients and normal well subjects showed a marked and highly significant difference between the two groups. The CTD for the 80 psychotic patients averaged over 400 minute-seconds, while that from both 47 normal volunteers and 35 physically ill hospitalized patients averaged about 150 minute-seconds. The difference between the psychotic subjects and the other two groups was significant with a P value of less than 0.001. The plasma samples from the psychotic subjects were obtained from chronic patients of three psychiatric hospitals and included a variety of diagnoses. There appeared to be no systematic difference in the CTD values for the various categories of psychoses among the 80 patients.

Attempts to confirm these findings have been both successful and unsuccessful. Ghent and Freedman [7] were unable to find differences when they injected serum from schizophrenic and normal control subjects into rats in a CTD study. This study involved relatively few patients and controls compared to that of Winter and Flataker. Our own study [8], however, using plasma but not serum and using the same procedure as that of Winter and Flataker, has confirmed this work.

The work of the Merck Sharp & Dohme group and our studies thus indicated the desirability of fractionation procedures to obtain more information about the possible nature of the active substance involved. Accordingly, Sanders et al. [9] fractionated human serum. Their method differed somewhat from that of the Tulane group. For

the preparation of globulins known to have solubilities similar to those described for taraxein, they chose the cold ethanol method of Cohn[10] with Lever's modification[11] for their separations and developed a further purification which gave them a fraction (Cohn Fraction III) similar in constituents to the taraxein-containing fraction of Heath. They found that this fraction produced some behavior changes in monkeys as well as marked CTD changes in rats. However, purification and concentration of this fraction yielded highly active fractions of plasma both from normal and psychotic donors. The greater the purity the greater the activity but the less the differentiation (with purification) between normal and patient plasma samples. Tests at various states of purification revealed that the activity of the fraction from normal plasma increased during the purification procedures, whereas activity of the fractions from psychotic plasma was apparent from the start. This led these workers to postulate that the active fractions occur normally in close association with an "inhibitor" which was presumably removed during the purification procedures. This postulate suggests that the inhibitor of the active substance affecting the rats is present in adequate amounts in normal individuals but in inadequate amounts in psychotic patients. We shall presently describe further tests made by the Merck Sharp & Dohme group and by our own group in an attempt to demonstrate the properties of this inhibitor, which appears to be removed in a discarded supernatant fraction during the course of extraction of the active fraction.

EXPERIENCES OF ONE GROUP OF WORKERS

We would like now to describe our own attempts to isolate and identify an active factor in human plasma. We found in our early studies of the effects of plasma on the rat CTD that the activity of the samples was lost quite rapidly on their standing and disappeared on their freezing. Pennell accordingly decided upon a more rapid and gentle method of fractionation, the zinc method 12 of Surgenor et al. [12], involving the precipitation of certain plasma protein constituents by zinc. This procedure divides the plasma into two fractions, one called the stable plasma protein solution (SPPS) containing primarily albumins and a number of other soluble protein constituents; and a second fraction precipitated by zinc and then redissolved that is called the plasma globulin precipitate (or PGP fraction). This consists primarily of beta, gamma, and some alpha

globulins and includes the fraction in which taraxein was reported by the Tulane group.

A special value of the zinc method is that fractionating requires a shorter time to perform, three days in contrast to six days necessary for the procedures of the Tulane and Merck Sharp & Dohme groups. It is important that fractionation be prompt and as short as possible to prevent loss of activity. The zinc method also involves less drastic exposures to temperature differences and effects of solvents than do other procedures. Our method permits the detection of loss of activity, as compared to the original plasma, during the preparation of the fractions, and has brought sharply into focus the great lability of the active fractions. We have, however, also used the procedures of both the Merck Sharp & Dohme and Tulane groups to compare with our results. We have been able to confirm the effects on our rats using taraxein obtained by the Tulane technique as well as fractions obtained by the Merck Sharp & Dohme method. Our PGP fraction gives CTD effects on the rats similar to those obtained with taraxein. We have found the zinc method more reliable, in that in our hands our proportion of active fractions using this method is higher as might be expected from the nature of the procedure.

There are, however, differences to be noted in comparing our tests with those of the Tulane group. Our PGP samples are active in the rats when obtained both from normal plasma and from that of the psychotics. There is about twice the average activity, however, in the samples from the psychotic patients as in those from normal control subjects, and this difference is maintained with purification and concentration. The Tulane group reports that they do not encounter any taraxein-like action from normal plasma. We also obtain, as does the Merck Sharp & Dohme group, fractions from psychoses other than schizophrenia (involutional psychoses and manic depression) with an average high activity higher than that of control samples but below that of the schizophrenic populations. While the activity of fractions from our nonschizophrenic psychotic subjects averages higher than that from plasma of nonpsychotic subjects, the differences are not statistically significant [13], as they are when we compare samples from schizophrenic subjects with those of our nonpsychotic population. According to Heath et al. [1], their taraxein is only obtained from schizophrenic and not from other psychotic patients and never from normal persons. It should be borne in mind, however, that we are using

very different bioassay methods. Heath has used monkeys and human subjects and we have used the rat CTD test for a bioassay.

In our CTD studies we have compared pooled plasma samples from two psychotic patients with a sample from one normal control subject. These samples were processed at the same time at the Protein Foundation. This matching of patient and control for each experiment is important because inevitably there are variations in batches of rats from time to time and in aspects of the extraction procedures. Blood samples of 125 ml are drawn for processing. In one study[8] we tested only patients newly admitted to the hospital and before diagnosis. Blood samples were drawn from these acute cases within a few days after admission, thus obviating effects of chronic hospitalization *per se*. Patients on ataraxic drugs were excluded. This study consists of 36 psychotic patients, 30 of whom later turned out to be schizophrenic subjects matched against 19 normal control subjects. Figure 1 shows the data of this experiment, indicating that the psychotics' samples averaged about twice the amount of substance as measured by the rat CTD as did the

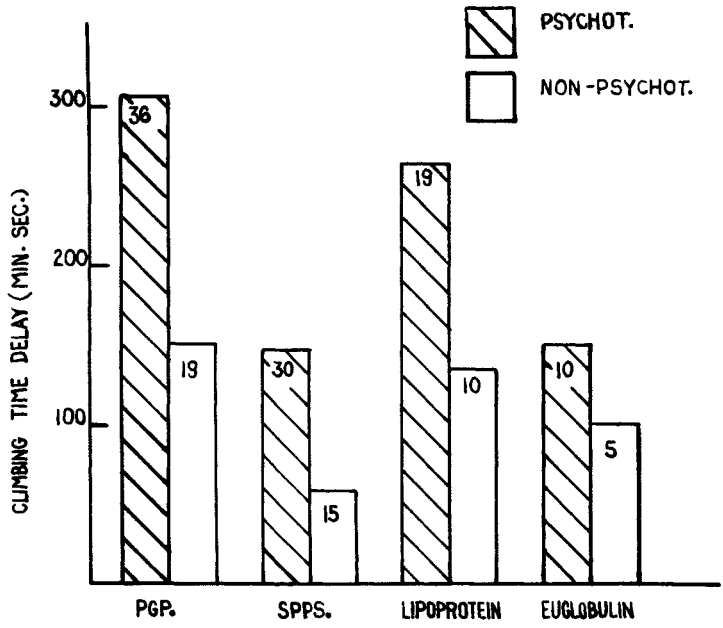

FIG. 1. Mean climbing time delay produced in trained rats by intraperitoneal injections of plasma protein solutions from psychotic and nonpsychotic persons. Thirty of the psychotic subjects were diagnosed as schizophrenic. Number of subjects tested shown by number in bar. (From A.M.A. Arch. Neurol. [8])

control samples. Maximum activity was found in the PGP fractions, but significant activity was also found in the SPPS fractions. Subsequent improvement in methods of separation, however, have shown the activity to occur, to a significant degree, only in the PGP fraction and not in the SPPS fraction. In other studies we have found no average differences in the activities of plasma PGP fractions in our rat tests between these acute cases and chronic schizophrenic patients, nor have we found significant sex differences. We have tested samples from approximately 200 psychotic patients and 100 normal control subjects. We have not as yet studied a population of psychoneurotic patients.

While not shown in the subsequent tables, an analysis of variance procedures has been applied to our data [8]. The differences between patient and control groups to be described in this paper are significant for the most part at better than the 1% level of confidence. The statistics will be published elsewhere.

As mentioned, our early studies showed that all human plasma produced a significant CTD when injected intraperitoneally into trained rats. Examination of plasma fractions prepared by standard fractionating techniques soon indicated that this activity resided in globulins easily precipitated by ammonium sulfate, cold ethanol, or zinc cations. The albumins and the less readily precipitated globulins, though comprising the bulk of the total plasma protein, were esentially devoid of the ability to delay the climbing time of the trained rats.

The fraction of plasma responsible for the delay in climbing time was extremely labile. Storage of plasma and protracted fractioning techniques resulted either in complete loss of activity or in erratic, poorly reproducible results. Prompt separation of plasma from the cellular elements of blood and prompt fractionation by the simplest fractionating techniques were therefore adopted. Even so, it was impossible to retain the activity of the plasma fractions upon storage for more than a day or two. Our investigation revealed that the activity of the fractions could be retained for weeks when they were stored under hydrogen [14]. This was not true for storage under nitrogen, oxygen, or air. Although the true meaning of this observation is not evident, it led to an investigation of the importance in maintenance of increased levels of reducing agents during the fractionation procedure. Ascorbic acid, a natural constituent of plasma, was chosen. Table I illustrates the heightened activity of the zinc-precipitated globulins (PGP) prepared in the presence of

TABLE I

Activities (Disturbance of Climbing Time) of Globulins Prepared in Presence of Varying Amounts of Ascorbic Acid

Ascorbic Acid Concentration (mM)	CTD (min-sec) Produced by Plasma Globulin Precipitate	
	Nonpsychotic	Psychotic
0.5	46	117
1.0	155	266
2.0	179	275
4.0	239	675

TABLE II

Comparison of Effect on Climbing Time of Trained Rats of PGP and SPPS Prepared from Normal Human Plasma in the Absence and Presence of Added Ascorbic Acid

Sample	No. Donors	No. Rats Injected	Mean CTD (min-sec)
		No added ascorbic acid	
PGP*	29	117	181
SPPS†	17	130	52
		Added ascorbic acid	
PGP†‡	32	115	549
SPPS†‡	9	20	45

*Each injection equivalent to amount of fraction in 1 to 2 ml plasma per 100 g rat body weight.

†Each injection equivalent to amount of fraction in 1 to 5 ml plasma per 100 g rat body weight.

‡Ascorbic acid concentration during isolation: 1 to 4 mM.

increasing concentrations of ascorbic acid. The activity of all the samples increases with increasing concentrations of ascorbic acid, and the ratio of the differences between patient and control samples is maintained.

In all subsequent fractionations a concentration of 4 mM ascorbic acid has been used throughout the procedure. Table II permits more extensive comparison of the CTD produced by PGP prepared with no added ascorbic acid with that prepared in the presence of an added reducing agent. It will be noted that the ascorbic acid has no effect on the CTD produced by SPPS. Storage under hydrogen likewise has no effect on the activity of SPPS.

Further purification of the active factor could be obtained by precipitation of the redissolved PGP at pH 5.8 followed by diethylaminoethyl cellulose (DEAE) chromatography of the resulting precipitate. The procedure in brief by which the active globulin may be prepared is as follows:

Plasma is separated promptly from the cells of whole blood by centrifugation. The plasma is diluted with an equal volume of cold (2 to 4 C) 0.15 M sodium chloride solution containing 8 mM ascorbic acid adjusted to pH 6.4. To the diluted plasma is added zinc glycinate to give a concentration of 25 mM Zn. The mixture is adjusted to pH 6.8. After 2 hours to overnight at 2 to 4C, the PGP is removed by centrifugation at 2 to 4 C. The PGP is dissolved in 0.5 M EDTA (ethylenediaminetetraacetic acid) solution, pH 7.0, a quantity of EDTA equivalent to the amount of added zinc being used. The dissolved PGP is dialyzed with stirring against large volumes of 0.15 M NaCl solution containing 4 mM ascorbic acid adjusted to pH 6.4 at 2 to 4 C overnight.

The dialyzed PGP is diluted to one-half of the plasma volume with cold 0.15 M NaCl and 4 mM ascorbic acid solution adjusted to pH 6.4. Zinc glycinate is added to a 25-mM concentration, and the mixture is adjusted to pH 5.8. After 2 hours to overnight a precipitate is collected by centrifugation at 2 to 4 C. The precipitate is dissolved as before in 0.5 M EDTA, pH 7.0, and is dialyzed overnight against large volumes of 0.15 M NaCl and 4 mM ascorbic acid solution at pH 6.4.

After dialysis, insoluble fibrinogen is removed by centrifugation, and the protein content of the solution is estimated by nitrogen determination or by optical density measurement. Final purification is achieved by chromatographic separation on DEAE cellulose. The milligrams of protein per unit of wet resin volume should not

exceed 3. The column is prepared and equilibrated in 0.02 M sodium acetate and 4 mM ascorbic acid, pH 7. Elution is by variable gradient, the column being fed from a mixing chamber which at first contains 0.02 M sodium acetate and 4 mM ascorbic acid, pH 7, with which is gradually mixed 0.2 M acetate buffer, pH 4.5, containing 4 mM ascorbic acid. Protein is precipitated from the eluates at pH 7 by 20 mM Zn. The precipitates are dissolved in EDTA as before and are dialyzed. The preparation is then ready for assay. Solutions of the active fraction may be sterilized by filtration through Millipore filters and stored under hydrogen with little loss of activity. Table III presents data obtained from normal human plasma.

From the inception of this study, plasma from schizophrenic and other psychotic subjects has been fractionated and tested simultaneously with that from normal donors.

Different amounts of an active PGP fraction have been injected into rats and the CTD values for each dose measured. A plot of PGP dose against the CTD yields good logarithmic dose response curves.

TABLE III

Precipitation of Active Globulin from PGP Prepared from Normal Human Plasma at 25 mM Zn^{++}, 1 Plasma Volume, pH 5.8, 0.15 Ionic Strength, 4 mM Ascorbic Acid

Sample	No. Donors	No. Rats Injected	Mean CTD (min-sec)	Relative Purity
Plasma*	8	55	136	1
PGP†	32	155	549	3
SPPS*	9	20	45	-
25 mM precipitate‡	25	145	393	20
25 mM supernatant‡	10	40	58	-
Chromatographed Product§	8	145	1230	500

*Each injection equivalent to amount of fraction in 1 to 2 ml plasma per 100 g body weight.

†Each injection equivalent to amount of fraction in 1 to 5 ml plasma per 100 g body weight.

‡Each injection equivalent to amount of fraction in 3 to 5 ml plasma per 100 g body weight.

§Each injection equivalent to amount of fraction in 5 ml of plasma per 100 g body weight.

It will be seen in Table IV that greater CDT consistently is produced by fractions prepared from the plasma of psychotic than by fractions prepared from normal donors. This differential is maintained at each step of the purification. The CDT differences are significant ($P<0.01$) except, as earlier mentioned, for those between the nonschizophrenic psychotic and control subjects. These findings are believed to corroborate the existence of an abnormality in the plasma protein of schizophrenic persons, as reported by Heath et al. [1, 2]. Although we prefer the zinc preparative procedure reported, because of its speed and simplicity, the procedure currently used by Heath and his associates for the isolation of taraxein produces, in our hands, fractions from both normal and psychotic subjects giving data comparable to those reported here. The present study is most readily interpretable as suggesting an alteration in schizophrenia, either of the quantity or of the quality, of a normal plasma globulin. It does not preclude, however, that the higher climb time delays produced by the globulin prepared from the plasma of psychotic subjects is due to a protein which is not present in normal plasma. Such a new protein would, however, have solu-

TABLE IV

Comparison of Fractions Prepared and Tested Simultaneously from Normal and Psychotic Donors

Sample	No. Donors	No. Rats Injected	Mean CTD (min-sec)	% CTD of Patients Over Controls
Plasma	8 Nonpsychotic	55	136	
	13 Schizophrenic	92	197	45
PGP	12 Nonpsychotic	40	818	
	13 Schizophrenic	40	1112	36
25 mM Zn^{++} ppt.	13 Nonpsychotic	65	353	
	17 Schizophrenic	85	841	136
25 mM Zn^{++} ppt.	12 Nonpsychotic	60	320	
	14 Nonschizophrenic psychotics	84	372	16

bility characteristics entirely comparable to those of a normal plasma globulin.

Our observations suggest, although they do not yet prove, that there is increased activity and stability of the globulin as purification proceeds. This might suggest the gradual elimination of an inhibitor or of a destructive agent or of both during the isolation.

The studies have led us to various attempts further to identify the active substance. Our protein extraction procedures all involve many hours of dialysis against saline solutions. One would thus expect that all dialyzable molecules would have been removed. However, in the course of a variety of experiments we dialyzed some active PGP fractions from patients against less active PGP fractions from normal controls. The dialysis was through cellophane for eighteen to twenty-four hours. To our surprise, we found invariably that the less active fractions markedly gained activity as measured by our rat tests and the active fractions correspondingly lost it [8]. These experiments have been often repeated by us and are unequivocal. The results suggest that a small molecule was trasferred across the membrane from the active globulin to receptors on the less active globulin. Such transfers did not occur with protein solutions used as controls other than the PGP moities in our experiments. However, we also found that Fraction III as isolated by the techniques of Sanders et al. [9], that contain the same active globulins as our PGP fractions, also showed this exchange of activity between active and inactive fractions. These results could be accounted for either by the transfer of a small active prosthetic group directly from active globulin fractions of psychotic subjects to less active globulins of control subjects, or, on the other hand, they might reflect the transfer of an inhibitor of the active substance from the less active samples of plasma from normal subjects to those of the more active fractions of the psychotic patients. This finding is of special interest since it suggests a possible mechanism for the transfer of activity across the blood brain barrier. This barrier would be expected to be impermeable to large globulin molecules, as is the cellophane membrane. However, a small active molecule bound to protein might be exchanged across the blood brain barrier with a corresponding globulin synthesized in the brain. We do not know what this small molecule may be. It might be an indole or a biogenic amine or something quite different. All attempts to separate and isolate it from the carrier protein have so far been unsuccessful.

We have sought indices of effects of our substance on the central nervous system other than that of confusing and slowing the rat's

ability to climb. Koella and Wells[15] have reported studies of effects of injections of the psychotomimetic drug LSD on optically evoked potentials in the nonanesthetized rabbit. Using a similar procedure Koella has studied the effects of injected PGP samples from normal subjects and schizophrenic patients on the cortical potentials evoked by flashes of light to the eyes of unanesthetized rabbits with chronically implanted electrodes [13]. It was found that active fractions from psychotic subjects produce similar effects on the evoked potentials to those of LSD. The relatively inactive PGP fractions from normal control subjects have no effect on the evoked potentials. Both PGP extracts from the patients and injections of LSD markedly decrease the variability of the amplitude and wave pattern of repetitively evoked potentials. The decrease in variability following the injection of LSD lasted for about 15 minutes and that of PGP samples for an average of 8 minutes. These effects on variability for both substances are statistically highly significant. Latency of cortical responses to the light flashes is also significantly reduced after injections of PGP samples from schizophrenic but not by samples from normal control subjects. There is also a reduction of variability of latency, but this is not as marked as is the reduction of variability of amplitude. The similarity of effects of the psychotomimetic drug LSD and the more active PGP samples from patients in these tests, as well as in the rat climb test, indicates similar actions of these substances on the brains of the animals.*

*Marrazzi et al. (in the symposium volume Inhibition of the Nervous System and Gamma Aminobutyric Acids, Pergamon Press, New York, 1960, p. 534; Fed. Proc. 18:419, 1959; and personal communication) have reported that they have demonstrated a cerebral synaptic inhibitor of transcallosal conduction in the cat present in normal human serum. Serum or serum fractions are injected into the ipsilateral artery and produce inhibition similar to that resulting from injection of the LSD, serotonin, and some other biogenic amines. Only aged and not fresh whole serum shows that effect. They have also reported that a taraxein sample sent them by Heath and Leach similarly acts as an inhibitor. They have extracted for testing, albumin and globulin fractions from normal serum and have also tested albumin and globulin fractions sent by the Protein Foundation. They found maximum inhibitory activity in the albumin fractions and less in the globulin fractions. These fractions lost activity with aging. The inhibitory action occurs so promptly (seconds) that they believe a small molecule may be involved in passing the blood brain barrier. As in our own studies, they were unable to separate this possible small molecule from the protein by ultrafiltration. They have not as yet made comparisons of serum or its fractions from schizophrenic and nonpsychotic subjects.

Those findings are both interesting and puzzling, since our active substance and taraxein are composed primarily of globulins and our albumin fractions are not active in our tests. Marrazzi finds that aging enhances the activity of whole serum in inhibiting the transcallosal response, but aging destroys the activity of plasma fractions and of taraxein. We also found this loss of activity in our tests if the samples were unprotected by hydrogen storage or ascorbic acid. Of course different bioassay tests are involved and ours are fractions of plasma rather than serum, but clearly more clarifying work is needed.

Koella has pointed out that sensory deprivation may induce psychotic symptoms such as hallucinations in man by reducing afferent impulses impinging on the central nervous system, thus reducing the irregular afferent bombardment of cortical centers. The decreased variability of cortical potentials induced by light flashes in animals following both LSD and injections of active PGP extracts from psychotic patients is highly suggestive, since presumably sensory deprivation and the actions of these substances may have similar effects on sensory receptive areas.

Ulrich Schaeppi in our laboratory has made pharmacological tests of our active fractions on rabbits and cats [13]. He finds them to have no effects as stimulants or inhibitors of the peripheral autonomic nervous system as judged by a variety of pharmacological tests.

Earlier in this paper we mentioned that Sanders et al. [9] were led to consider that an inhibitor of their active globulin might be contained in normal plasma. The evidence for this came from the finding that with increasing purification and removal of extraneous protein, differences in activity between samples from normal and psychotic subjects were lost, i.e., all purified fractions were equally high in activity. This led them to reexamine certain of their supernatant fractions that had been discarded in the course of processing in hopes of finding a solution that would inhibit their active globulin fractions [16]. In 27 experiments they injected extracts of one of their previously discarded supernatants from normal subjects into their rats 15 minutes before injecting their active fractions. In 19 of the 27 experiments they found the activities of the active fractions to be significantly reduced in the rat tests, i.e., the CTD values were reduced following the injection of the presumed inhibitor.

We also have prepared the inhibitors by the methods of Sanders et al. [16] from normal plasma and injected them into rats 15 minutes before injecting our active PGP fractions. We found (unpublished) in nine experiments a relation indicating that an inhibitor reduced the PGP response in proportion to the logarithm of the CTD activity of the particular PGP sample.* It is clearly desirable to learn more about this possible inhibitory substance because of its implications for therapeutic use in relation to the psychoses.

*The "inhibition" decreased the effect of highly active samples, but this inhibition became less marked as the original activity of the samples approached a particular lower value (CTD 550). Below this value, injections of the inhibition potentiated sample activity. Samples of lowest activity showed greatest potentiation.

OTHER STUDIES

We have reviewed studies from Tulane, from the laboratories of Merck Sharp & Dohme, and from the collaborative efforts of the Worcester Foundation with the Protein Foundation. The findings of all three groups point to a globulin that may play a role in the major psychoses. Earlier in the discussion we mentioned a fourth group who have independently produced evidence for a protein factor similar to ours involved in psychotic behavior.

Frohman et al.[17, 18] from the Lafayette Clinic in Detroit have produced evidence for the presence of a globulin in blood of schizophrenic patients very similar in properties to our purified globulin fractions. Their globulin fractions appear to modify carbohydrate metabolism of cells in characteristic ways.

The Detroit workers studied the carbohydrate metabolism of erythrocytes from normal persons and schizophrenic patients by the use of C14-labeled hexose-6-phosphate and hexose-1-phosphate as tracers. They found that when 10 units of insulin were administered as a stressor to schizophrenic patients and to normal control subjects that the production of energy-rich ATP of the cells from normal persons was increased but that of the patients' cells was not. The red blood cells of the schizophrenic subjects thus responded less effectively to this insulin challenge than did those of the controls.

There are two interrelated pathways of glucose metabolism, the Embden-Meyerhof cycle of anaerobic metabolism involving primarily hexose-1-phosphate as substrate and the hexose monophosphate shunt utilizing hexose-6-phosphate as substrate. The former pathway is primarily concerned with energy metabolism via the production and utilization of ATP and the latter with the synthesis of ribose for the production of nucleic acids necessary ultimately for protein formation. The insulin decreases the percent of carbohydrate metabolism through the shunt in erythrocytes from normal persons and favors the Embden-Meyerhof cycle. This effect does not occur in the erythrocytes of schizophrenic patients, as indicated by the C14 tracer studies.

The Detroit group found, however, that if erythrocytes of normal persons were incubated in the plasma of schizophrenic patients these cells then behaved metabolically like those from schizophrenic patients. This led them to the conclusion that there was a factor in plasma from schizophrenic patients that was responsible for this shift in metabolism. Frohman et al.[18] accordingly used chicken

erythrocytes as test preparations and studied their carbohydrate metabolism after incubation in plasma from normal persons or from schizophrenic patients. They used the ratio of the cells' lactic acid to pyruvic acid production as a measure of hydrogen transport since Stoltz et al. in 1948 had shown this to be a measure of the oxidizing activity of tissues. Incubation in plasma from schizophrenic subjects significantly increased this ratio. Thus, a factor in the plasma of schizophrenic patients accentuates the anaerobic metabolism of the bird erythrocytes.

The Detroit group has presented evidence that the factor in plasma from schizophrenic subjects altering cell metabolism is probably an alpha globulin. This appears to have properties similar to those of taraxein and to our active globulin and of the concentrated globulin fraction of the Merck Sharp & Dohme group. Frohman et al.[18] used quite different isolation procedures from those of the other three groups.

None of the factors so far discussed may be regarded as one pure protein—all are clearly mixtures despite the concentration and refinement procedures used. The active protein appears to be either an alpha or beta globulin. Frohman and co-workers also have presented evidence that a small molecule may be combined with the globulin. They regard it as probable that their globulin factor may also be present in the plasma of normal persons but with more of it in the plasma from schizophrenic patients.*

Recently two groups in the Soviet Union[19, 20] have used the procedure of Heath et al. and have obtained globulin fractions from the plasma of schizophrenic patients that modify learned behavior patterns in mice. Space limitations preclude a critical discussion of these findings, but they further substantiate the findings from the American laboratories.

DISCUSSION AND SUMMARY

We have reviewed work from four groups indicating the existence of a globulin in human blood probably associated with a tightly bound small molecule. This active complex displays, on the average, considerably greater activity by several test procedures if it is extracted from the plasma of schizophrenic patients in contrast to that from normal controls.

*Personal communication.

Is this factor causally related to psychosis in man or is it an incidental by-product of the psychosis? The only direct evidence to date for its playing a causal role is that of the Tulane group, which reports that injected taraxein produces transient psychoses in man. This is the only one of the four groups whose work is here reviewed that has administered it to man. We have wished to obtain more information about the substance before testing its possible psychotogenic effects on man.

We think it especially interesting that evidence both from the Merck Sharp & Dohme group and from our group suggests the presence of an inhibitor or regulator of the activity of these globulin fractions in normal plasma that may block the actions of the possible psychotogenic factor as indicated by our rat tests. Further investigations of this inhibitor are under way.

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