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# Phenothiazine Effect on Human Antibody Synthesis

By JOHN C. SAUNDERS and ELIZABETH MUCHMORE

## Introduction

Early in our clinical studies with the phrenotropic drugs, reserpine and chlorpromazine, we observed that psychotic patients receiving them were more resistant to treat for infectious processes that occurred during their hospitalization (Saunders, 1961). We also observed that in experimental animals, the $LD_{50}$ of a standardized dose of bacteria was significantly increased if the rats had been previously treated with a phenothiazine. Preliminary observations led us to consider the role of these drugs in protein synthesis, with specific interest in the gamma globulin, since antibodies are associated almost exclusively with this fraction. Further interest in the problem of protein synthesis was stimulated by the reduced albumin and increased globulin levels found in most chronic psychoses, as the phrenotropic drugs are capable of significantly shifting this ratio toward normal. The question of phrenotropic drug action on antibody synthesis was asked at the Second International Congress of Psychiatry in September, 1957, and no specific information was known to the participants (Saunders, 1958). With this background, the question warranted further study in man. We designed our experiment to measure antibody response to oral poliovirus vaccine, Cox strains types 1 and 3, during phenothiazine therapy.

## Method

The selection of patients for any project in a neuropsychiatric hospital requires considerable effort and this was no exception. It was necessary to screen the individual case histories of not less than 1,200 patients aged 40 years or under for this study. Our objective was to study antibody synthesis in psychotic patients and to observe the action of phrenotropic drugs on this process. We were unable to explore all the anticipated variables of this study because the patient population with an antibody titre of less than 1 : 4 was very limited. We found only 345 patients with a history of not more than one Salk polio vaccination, and serum antibody titres for poliovirus types 1, 2 and 3 were obtained on this group by the metabolic inhibition test. Of these, 61 patients were found vulnerable to one or more types of the virus, but attrition reduced this number to 21; of these 4 had negative titres for the two types of the virus we studied, therefore our data are based on 25. See Table I.

The patients were divided into two groups after pharmacological therapy had been discontinued in all for 26–46 days and specimens for a second antibody titre were obtained before the vaccines were given. On zero day, all patients were started on phenothiazine therapy and one group of 12 patients received either type 1 or type 3 oral poliovirus vaccine.* Twenty-eight days after the initiation of phenothiazine therapy the second group received vaccine for the type 3 virus. Blood specimens for antibody titre were obtained 21 days after each vaccination, and phrenotropic therapy was continued throughout the 49 days the second group was being studied. The 4 patients with negative titres for both types 1 and 3 poliovirus are included in both groups, receiving the type 1 vaccine on zero day and type 3 vaccine 28 days later.

The phrenotropic drugs tested in this study were limited to chlorpromazine and perphenazine. Adults received either 4 mg. perphenazine t.i.d. or chlorpromazine, 100 mg. t.i.d., and children 2 mg. or 25 mg. t.i.d. respectively.

* Supplied by Lederle Laboratories, Pearl River, N.Y.

TABLE I

*Effect of Phenothiazines on Poliovirus Antibody Synthesis*

| Regimen | Significant Titres=or>1 : 16 | Non-significant Titres<1 : 16 | Total Patients |
|---|---|---|---|
| Phenothiazines 28 days prior to vaccine .. .. | 7 | 6 | 13 |
| Phenothiazines and vaccine given simultaneously | 11 | 1 | 12 |
| Total .. .. .. .. .. .. .. | 18 | 7 | 25 |

Our drug regimen was designed to test for time lag significance as well as differences between the two phenothiazines. Statistical analysis was made by means of the exact or binomial test, and the significance of difference between proportions from the theory of sampling because of the limitations and assumptions involved in the chi-square formula corrected for continuity (Siegel, 1956; Fisher, 1932). However, the chi-square test was applied to our data, using Yates' correction factor (Snedecor, 1946), and a clinical frequency of therapeutic response as established by the Lederle study in New York as a basis for calculating expected frequencies (Cabasso *et al.*, 1960).

## RESULTS

There have been many broad generalizations about the role of the central nervous system as a regulator of immune response (Zilber, 1958). Our determinations of the antibody titres to poliovirus types 1, 2 and 3 indicate that protective levels are present or can be produced if adequate stimulus is administered to neuropsychiatric patients. We found only 61 patients vulnerable to types 1, 2 or 3 poliovirus in the group of 345 psychotics after the three antibody titres were measured. This incidence of immune response is comparable to that found in a non-psychotic population (Ruegsegger and Delery, 1960).

Our requirement for an adequate antibody response to oral polio vaccine was that specified by the U.S. Public Health Service, Department of Health, Education and Welfare for a prophylactic vaccine, i.e., the antibody titre must be 1 : 16 or greater. Seven of the 13 patients receiving a phenothiazine prior to the oral vaccine developed a significant antibody titre, and 6 failed to respond. The remaining 12 patients were given the oral polio vaccine simultaneously with phenothiazine therapy, and 11 responded with significant titres. (See Table I for data.) Application of the exact or binomial test to this data gives a p value of 0·042837; the significance of difference between proportions gives a p value of 0·0357. A chi-square value of 21·94 with a p of $<0·001$ is obtained using one degree of freedom and Yates correction factor; the clinically established frequency of 93 per cent. is used for determining expected frequencies. These probabilities are all within the accepted $<5$ per cent. levels for biological significance. The chi-square test, using marginal totals for calculating expected frequencies, gives a value of 2·75 which falls into the almost significant 5–10 per cent. range. See Table III. This indicates the probability that phenothiazine drugs interfere with the synthesis of poliovirus antibodies in man. It also indicates that there is a time sequence correlation between the ability to produce antibodies and previously observed shifts in the serum albumin-globulin fractions of patients receiving phenothiazine therapy.

We found a significant difference between the antibody inhibitory action of chlorpromazine and perphenazine. The perphenazine treatment of 8 patients for 28 days prior to vaccination allowed only 2 to develop a significant antibody titre, while all 5 receiving chlorpromazine developed significant titres to the polio vaccine. The exact test applied to the

data as shown in Table II gives a p of 0·016317 and the significance of difference between proportions gives a p of 0·00932. Chi-square analysis using Yates correction factor and marginal totals produces a chi-square of 4·1 which gives a p of <0·05 with one degree of freedom. The results of the statistical analyses are presented in Table III.

## Discussion

The importance of the variable, time, in designing any biological study with a phrenotropic drug is re-emphasized by analysis of our data; previous studies on the clinical efficacy of these drugs made clear the need for therapy to extend for several months if optimum therapeutic results were to be obtained. Many exponents of the controlled double-blind clinical studies have failed to design their procedures to include this phenomenon. The effect of the time sequence of phenothiazine and vaccine administration on antibody synthesis may be correlated with the time of the observed shifts in serum albumin-globulin equilibrium in patients receiving these drugs. Psychotic patients have been shown to have lower serum albumin and higher serum globulin than normal. Studies indicate that during early phenothiazine therapy there is frequently an increase of albumin and a decrease in globulin which is significant by the 4th or 5th week and continues to the 9th or 10th week before arriving at normal levels which are maintained during further treatment. Clinically there is a 21-day lag in producing a protective antibody level. It was our objective to time the vaccination and antibody synthesis with regard to the phenothiazine reduced globulin levels and this normal physiological lag period.

While plasma proteins cannot be considered as direct precursors of tissue proteins, there are convincing *in vivo* experiments which indicate that serum albumin is possibly converted to globulin fractions (Maurer and Muller, 1955) and others that show its breakdown to amino acids. Many specific problems of plasma protein synthesis have been elucidated sufficiently for a reasonable understanding of the processes involved, but the mechanism of antibody synthesis awaits a description and understanding of the unique sequence of reactions in biochemical terms. The significance of chemical structures of protein molecules as

Table II

*Comparison of Activity of Chlorpromazine* vs. *Perphenazine on Antibody Synthesis*

| Drug 28 Days Prior to Poliovirus Vaccine | Significant Titres=or>1 : 16 | Non-significant Titres<1 : 16 | Total Patients |
|---|---|---|---|
| Chlorpromazine | 5 | 0 | 5 |
| Perphenazine | 2 | 6 | 8 |
| Total | 7 | 6 | 13 |

Table III

*Results of Statistical Analyses*

| Groups | Exact Test | Difference Between Proportions | Chi-Square: Expected Frequency | Chi-Square: Marginal Totals |
|---|---|---|---|---|
| Pre-treatment *vs.* simultaneous treatments | p=0·042 | p=0·035 | $\chi^2$=21·94 p=<0·01 | $\chi^2$=2·75 0·10<p>0·05 |
| Pre-treatment with chlorpromazine *vs.* perphenazine | p=0·016 | p=0·009 | | $\chi^2$=4·1 p=<0·05 |

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they relate to antibody synthesis remains to be determined. The long latent period in antibody synthesis must be related to some process that is specific to the formation of a protein not normally found in man. It has been shown by Peters (1957) that the synthesis of a protein takes at most a few minutes, and even the phenomenon of secretion of a protein by the cell is completed within less than an hour (Green and Anker, 1955; Simkin, 1958). One can conclude, therefore, that the time required for the synthesis or secretion of proteins is not responsible for the latent period, especially since it also has been shown *in vivo* that at least the major portion of the antibody is synthesized after it begins to appear in the blood (Green and Anker, 1954). By use of tagged antigen, evidence is available that the nucleus of a cell is entered on initial exposure to an antigen which is rapidly taken up by the microsomes; therefore it is unlikely that the antigen requires several days for interaction with antibody-producing cells (Crampton *et al.*, 1953; Haurowitz, 1955). These are all immature plasma cells, and the appearance of antibody in the serum follows differentiation of these plasma cells. It now appears that this first exposure to antigen is one of differentiation, while later stimulus establishes the cellular proliferative phase with elevated serum antibody. The delay, therefore, must be related in some way to establishment of the pattern for synthesis of a new protein.

The template theory of antibody formation envisages direct intervention of the antigen in such a way that its determinant groups act as the model from which antibody-combining sites can be synthesized. Schweet and Owen (1957) suggest that the antigen acts at two sites for antibody formation; the formation of a new deoxyribonucleic acid (DNA) template in the antibody-forming cell and then as an inducer, acting to influence the synthesis of ribonucleic acid (R.N.A.)-containing templates. Burnet (1956) proposes a specifically induced change of cytoplasmic template system in which the RNA-containing granules of the cytoplasm and nucleus are also capable of replication. The elective theory of antibody synthesis proposes hypermutability of DNA, and DNA through RNA as a code for amino acid sequence and the reaction between antibody and antigen (Lederberg, 1959). The vital role of nucleic acids or the ribose nucleic acid-amino acid derivatives in protein synthesis is well accepted (Gale, 1955; Gale and Folkes, 1954; Potters and Dounce, 1956). Thus, with present knowledge indicating that protein synthesis occurs at the RNA sites in the cytoplasm and that RNA controls the amino-acid sequence in the synthesis of a protein, it is logical to conclude that factors influencing generalized protein and RNA production or metabolism consequently affect antibody synthesis.

The mode of action of the phenothiazines remains unknown. By their hypothalamic action they exert a controlling influence on the anterior pituitary, which, according to experiments of White and Dougherty (1944) governs blood globulins. The tranquillizers induce a significant (30–50 per cent.) reduction in serum amino-acid nitrogen and a five-fold increase in the highly polymerized ribose nucleic acid (Donnelly and Gordon, 1958). Hormones influence enzymatic activities and protein metabolism, e.g. epinephrine, cortisone, testosterone and thyroid stimulate increments of the ribose nucleic acid-amino acid derivatives. Cortisone is known to lead to an involution of the lymphatic tissues with suppression of antibody formation along with a reduction in the rate of degradation of plasma protein. One may conclude, therefore, that the influence of hormones and phrenotropic drugs on the activity of enzymes is consistent with available data (Kochakian, 1951; Knox, 1951). The synthesis of specific proteins and antibodies depends on which of the above substances are in sufficient concentration to maintain a dynamic equilibrium, for a competitive disadvantage will promote catabolic effects on that particular protein. Have phenothiazines changed the supply of amino-acids, nucleic acids, and even energy sources for the synthesis of proteins and antibodies? There are numerous reports that the phenothiazines, both *in vitro* and *in vivo* act as inhibitors of oxidative phosphorylation essential for the respiratory chain, and therefore part of their effect could be through the mechanism of shifting the avail-

ability of high-energy nucleotides essential to protein synthesis from the cells to the serum.

The synthesis of proteins in the central nervous system is most interesting, because of the high content and role of RNA in the hypothalamus and its control in hormonal polypeptide production. Now data reveal that phrenotropic drugs not only have direct pharmacological activity in the hypothalamus, but modify the RNA-amino acid derivatives essential for protein synthesis. It is of considerable interest that there appears the possibility of a common protein complex which serves as a memory protein for the synthesis of antibodies as well as the chemical basis for storage of essential units of thought.

Our preliminary studies on the effect of phenothiazines on antibody production indicate that these drugs significantly reduce their synthesis as shown by serum titres. Antibody production involves a marked increase in the ribonucleoprotein content of the antibody-forming organ. The protein moiety of this compound contains antibodies which are released in an active form during the destruction of the RNA moiety (Feldman *et al.*, 1960). These observations are consistent with the concept that the antibody molecule is part of a ribonucleoprotein complex within the antibody-synthesizing cell, which complex may be the site of its formation. One could believe that the phenothiazines deplete the antibody-producing organ of the RNA essential for synthesis in a manner analogous to the reserpine depletion of catecholamines. Through their action on the supply of amino-acids, nucleic acids, and energy sources, as well as their hormonal influence on enzymatic activities vital for protein synthesis, the phenothiazines are capable of affecting the synthesis of antibodies in man (Saunders *et al.*, 1961).

## Summary

The phenothiazines, perphenazine and chlorpromazine, when administered for 28 days prior to oral poliovirus vaccine, produce a significant immunological inhibition as indicated by antibody titres. If the phenothiazines and oral poliovirus vaccine are administered simultaneously, there is no significant inhibition of immunization. We also observed that a 100 mg. t.i.d. maintenance dose of chlorpromazine is less likely to inhibit antibody synthesis than 4 mg. t.i.d. of perphenazine when given for 28 days prior to the induction of immunization.

Theories of antibody formation in the past have some precedent in biological data, but have been greatly influenced by the mechanisms of inducible enzyme synthesis in bacteria. This experiment in man with phrenotropic drugs provides another tool to aid in the elucidation of this mechanism.

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John C. Saunders, M.D., *Principal Research Scientist (Pharmacology), Research Facility, Rockland State Hospital, Orangeburg, New York*

Elizabeth Muchmore, M.D., *Clinical Research Associate, Research Facility, Rockland State Hospital, Orangeburg, New York*