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Naturopathic Hypothesis

Does ABO "bias" in Natural Immunity imply an innate difference in T-cell response?

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The philosophy of naturopathic medicine asserts that special attention be given to responses arising from each individual's reaction to their disease. This has been paraphrased as "treating both the patient and the disease". We have examined response variation in several hypersensitivity states due to the effects of ABO polymorphism. Sera from sixteen group O subjects reporting either urticaria, history of anaphylaxis, endometriosis, eczema or asthma were evaluated for the presence of isohemagglutinin-A (anti-A) by saline titration and human-specific murine antiglobulins IgG_{1-4}. Especially high saline titers and strong antiglobulin reactions were noted with urticaria, endometriosis and anaphylaxis when plotted against controls. Urticaria and anaphylaxis were also found to correlate with the broadest spectrum of IgG subclasses, with IgG_2 and IgG_3 appearing most consistently. Polymorphic factors may factor in pathogenesis, as differences in "natural immunity" observed with group O individuals appear to result in a less restricted, poorly behaved response (when T-cell dependent) to substances possessing A-like antigenicity.

INTRODUCTION

FROM THE AGE OF 6 MONTHS normal subjects show "naturally occurring" antibody to non-self ABO antigens. These antibodies are largely IgM, but under certain circumstances, particularly in group O, large amounts of "immune" IgG antibody may be produced. Almost thirty years ago, Rawson and Abelson*(1)* qualified physicochemical differences between isoanti-A,B and isoanti-A or B in attempting to explain the preponderance of group O women among mothers of infants with ABO hemolytic disease of the newborn. This observance, coupled with evidence that patients with aquired hemolytic anemias are more likely to belong to group O than to groups A or B, stimulated interest in further qualifying the differences between isohemagglutinin subclasses and bloodgroup. By a combination of chromatography and ultracentrifugation, they separated the isohemagglutinin and isohemolysins into molecular types *(i)* gamma 1 and 2-globulins with S7 sedimentation coefficients (IgG) and *(ii)* gamma 1-globulins with S19 sedimentation coefficients (IgM). In general, isohemagglutinins of S7 class showed maximal activity (titration endpoint) in the presence of antiglobulin serum, whereas S19 isohemagglutinins are maximally active in sodium chloride solution. IgM class antibodies, although having relatively low affinity for single antigenic determinants, bind with great affinity to antigens with multiple epitopes and are efficient agglutinating and cytolytic agents. Membrane bound monomeric IgM is the major antibody receptor used by B-lymphocytes to recognize antigen, a very potent initiator of classical complement

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ABBREVIATIONS: DTT: dithiothreitol; AHG: antihuman-globulin; BGD: blood group degrading enzymes

Strength of reaction	Grade	Score Value	Appearance
4+	"Complete"	12	A single agglutinate. No free cells detected.
3- 1/2+	4+w or 3+s	11	
3+	3+	10	Strong reaction. A number of large agglutinates.
2-1/2+	3+w or 2+s	9	
2+	2+	8	Large agglutinates in a sea of smaller clumps, no free cells.
2+w	2+w	7	
1-1/2+	1+s	6	Many medium and small agglutinates and a background of free cells
1+	1+	5	Many small agglutinates and a backround of free cells
1+w	1+w	4	Many very small agglutinates with many free cells
1/2+ or -	+/- macro	3	Weak granularity in cell suspension. A few macroscopic agglutinates but numerous agglutinates microscopically
trace or micro	(+) micro	2	Appears negative macroscopically. A few agglutinates of 6-8 cells in most fields.
questionable	(0r) rough	1	Rare agglutinates observed microscopically
0	0	0	An even cell suspension. No agglutinates observed microscopically.

TABLE 1. Interpretation of agglutination reactions. *S=strong, W=weak. (source: AABB Technical Manual, 10th edition. American Association of Blood Banks, 1117 North 19th St. Ste 600 Arlington VA, 22209)*

fixation, and an effective first line of defense against bacteremia. The major class of isohemagglutinins (anti-A, anti-B) and many of the "natural" antibodies to micro-organisms are usually IgM. These have been postulated to arrive out of inapparent microbial immunization,*(2)* or are acquired characteristics resulting from oral immunization with animal foods containing A and B antigens.*(3)* Moreover, under some circumstances, particularly in subjects of group O, large amounts of "immune" IgG class anti-A and anti-B can also be produced.

This heterogenicity of isohemagglutinin subclass, characteristic of group O, coupled with the wide spectrum of naturally occuring substances in the environment that possess blood group antigenicity, stimulated interest in investigating the activities of isohemagglutinins found in subjects of this blood group presenting with clinical symptoms of hypersensitivity, a correlation first advanced by Mourant,*(4)* who speculated that the presence of both anti-A and anti-B antibodies (in addition to a third hemolysin, anti-A,B) might render group O individuals more susceptible to allergic reactions.

METHODS

In order to test this hypothesis, saline titration endpoints of group O individuals with a past or current history of allergic or inflammatory disorders were measured and compared to controls. When elevated saline titers were encountered, thiol neutralization and antiglobulin titration were performed via standard technique.*(5)* Strong antiglobulin reactions were additionally subjected to further analysis via specific murine derived human-specific antiglobulin directed at subset groups IgG_1,IgG_2, IgG_3 and IgG_4 in a technique previously utilized by Kay and De Locke.*(6)*

Serum from sixteen subjects, all group O (3 men, 13 women) reporting clinical symptoms suggestive of immune hypersensitivity were examined for elevated titers of anti-A via saline agglutination endpoint technique. None of these subjects had ever been transfused, nor had any of the women reported heterospecific pregnancies.

As this method is semi-quantitative, titration results were assigned a number based on observed strength of reaction *(Table1)*. The sum of these values represent the "score" - a measurement of antibody reactivity. A difference of 10 or more between different test samples has arbitrarily been deemed significant.*(5)*

Saline titration

Saline titration is considered a good indicator of IgM isohemagglutinin activity. Test tubes were labeled according to serum dilution (1 in 1, 1 in 2 etc.). Serum samples were number coded to eliminate observer bias. Serum (1 ml) was delivered to the first tube, and .5 ml of saline delivered to all tubes except the first. An equal volume (.5 ml) of serum was added to the second tube to make the 1 in 2 dilution. The tubes were agitated and one-half the contents of tube 2 transferred to tube 3 to make the 1 in 4 dilution. This was continued until the final dilution of serum (1 in 5096).

Reagent group A1 cells were washed three times and a suspension of 4% cells in saline produced. Reagent cells were type Rh- and grouped in 3 banks according to MN bloodgroups. This was done to eliminate the only other major hemolysins (anti-Rh, anti-M and anti-N) which might have compromised the results. Reagent A1 cells (30ul) were

then added via calibrated pipette. The samples were mixed and incubated at 37°C for 15 minutes. Centrifugation at 1000 rpm for 45 seconds produced a cell button at the bottom of each tube which was then dislodged and graded by two observers. No effort was made to distinguish reactions which would require microscopic analysis (reaction less than 1/w; score less than 4).

Thiol neutralization

Serum sample with strong (>1+) reactions, and high (>128) titration endpoints were subjected to further analysis for IgG subset activity. Thiol reagents possess the ability to cleave the intersubunit bonds characteristic of IgM, while leaving intact the disulphide bonds also characteristic of IgG and IgA monomers. Treating sera with thiol reagents abolishes both the agglutinating and complement-activating activity of serum IgM. Thiol neutralization is useful in determining immunoglobulin classes within the serum by allowing detection of co-existing IgG class antibodies otherwise masked by the higher avidity of IgM class antibodies.

In one tube .5 ml of serum was mixed with an equal volume of 0.01 M dissolved dithiothreitol (DTT) in phosphate buffered saline (PBS) and incubated at 37°C for two hours. Thus all indirect subclass determinations were performed at a 1:2 dilution. No effort was made to determine titration endpoint on the individual samples.

IgG subclass determination

IgG subclass specific antihuman-globulin (AHG) were obtained from Zymed Immunochemicals Inc. Each sample was tested for the presence of incomplete IgG anti-A subclasses using each of the four subclass (anti $IgG_{1,4}$) specific AHG reagents. The treated serum was thoroughly mixed with .1 ml group A1 cells and incubated for 15 minutes at 37°C, after which the cells were washed using the *Simwash*® system (Ortho Diagnostics). The appropriate IgG subclass specific AHG (.1 ml) was then added, mixed and spun for 30 seconds at 1000 rpm. Each indirect subclass specific AHG test was inspected macroscopically and microscopically. Reaction strength was determined by the assessment score system previously described.

RESULTS

As summarized in *Table 2*, we found that individuals of group O blood reporting a previous urticaria or anaphylaxis showed high residual titers of anti-A isohemagglutinins. When a score value was assigned to each agglutination reaction to allow for characterization, it was found that individuals with these disorders showed remarkably high titration scores when measured against controls of the same bloodgroup *(Figures 1a and b)*. In several cases their scores showed almost threefold increases when measured against controls. A mild increase was noted for group O subjects with severe eczema or asthma but total scores were only marginally greater than controls and in both cases the numbers studied were quite small. However, a striking association was shown for group O women suffering from endometriosis.

Analysis of IgG subset characteristics showed consistent strongly positive reactions in the anaphylaxis and urticaria group when measured with murine derived human-specific antiglobulin sera directed against individual IgG fractions. While almost all subjects had some IgG subset activity, the anaphylaxis and urticaria group had the most diverse and generally stronger (3 or 4+) reactions. What is particularly interesting in these groups was the unexpectedly high strength reactions consistently seen against both anti-A IgG_2 and anti-A IgG_3. This unique characteristic was also noted in both endometriosis subjects.

DISCUSSION

It might be expected that since allergy is an immunological disorder, allergic diseases would tend to show disturbances of blood group frequencies. It is therefore rather disappointing to find that apart from asthma (in 1684 cases the A/O relative incidence was 1·13 - just below significance; the B/O ratio of 1·47 - highly significant) and eczema (no significant departure from controls), cases examined for blood groups have been rather few and for allergy as a whole rather inconclusive.*(4)* Although the goal of our study was to examine possible response variations and not blood group frequencies, the relative blood group frequency of previous anaphylaxis and group O should be further examined. Of patients presenting at our clinic in the last two years reporting urticaria, or previous anaphylaxis *(n=11)*, two urticaria patients were not group O. Several subjects reported more than one concurrent disorder *(Table 2)*. Reports of previous anaphylaxis were noted to be exclusively from group O patients. Eczema and asthma were reported in relatively equal numbers regardless of ABO group.

The occurence of particular subclasses cannot be accounted for by expected percentage as IgG_2 and IgG_3 account for less than 30% of the total IgG found in healthy serum.*(6)* IgG_3 is the most potent of all the subsets at fixing complement via the classic pathway, in addition to possessing a high avidity for monocyte binding.

Bloodgroup associations are fraught with difficulty.*(7)* Concurrent factors such as race and geography can be great obstacles to analysis. This study should not be interpreted as implying direct causality exists between isohemagglutinin

	Anti-A Saline Endpoint (Reciprocal of serum dilution)													Anti-A Subsets			
	2	4	8	16	32	64	128	256	512	1M	2M	5M	Scr	IgG_1	IgG_2	IgG_3	IgG_4
Subject A	4	4	4	4	4	4	3	2	2	1.5	1/w		108	1	1	3	
Urticaria	12	12	12	12	12	12	10	8	8	6	4						
Subject B	4	4	4	4	4	4	3	3	2.5	2	2/w	1/w	120	2		4	1
Urticaria	12	12	12	12	12	12	10	10	9	8	7	4					
Subject D	4	4	4	4	4	3	3	3	2/w	1/w			101		3	4	1
Urticaria[3,5]	12	12	12	12	12	10	10	10	7	4							
Subject G	4	4	4	4	4	3.5	3	2/w	1/w				92	1	1	3	2
Urticaria[4]	12	12	12	12	12	11	10	7	4								
Subject J	4	4	4	4	4	4	3	3	2	2/w	1/w		111	1	1	4	
Urticaria	12	12	12	12	12	12	10	10	8	7	4						
Subject C	4	4	4	4	4	3	3	2	2	1.5	1/w		106	2	3	4	2
Anaphyl.[2]	12	12	12	12	12	10	10	8	8	6	4						
Subject F	4	4	4	4	3	3	3	3	2	2/w	1/w		107	1	1	4	2
Anaphyl.[1]	12	12	12	12	10	10	10	10	8	7	4						
Subject M	4	4	4	4	4	3	3	2/w	1/w				81		1	3	1
Anaphyl.	12	12	12	12	12	10	10	7	4								
Subject O	4	4	4	3.5	3	3	2/w	2/w	1/w				85	1	2	4	1
Anaphyl.	12	12	12	11	10	10	7	7	4								
Subject E	4	4	4	3	1w								50	1			
Asthma	12	12	12	10	4												
Subject H	4	4	4	3	2	1/w							58	1			1
Asthma	12	12	12	10	8	4											
Subject L	4	4	2	1/w									36				
Asthma	12	12	8	4													
Subject I	4	4	4	3	1/w								40	1	1		
Eczema	12	12	12	10	4												
Subject K	4	4	3	2/w	1/w								45			1	
Eczema	12	12	10	7	4												
Subject N	4	4	4	4	4	3	3	3	2	1/w			102	1	2	4	1
Endomet.[5]	12	12	12	12	12	10	10	10	8	4							
Subject P	4	4	4	3	3	2	2/w	2/w	1/w				82	1	3	3	
Endomet.[2]	12	12	12	10	10	8	7	7	4								

TABLE 2. Summary of saline titer agglutination endpoints for 16 group O individuals reporting a variety of hypersensitivity conditions. Each reaction is rated in terms of macroscopic agglutination pattern (4-1/w; upper number, *see table 1*) and individual score value (12-4; lower number). Cummulative score values (Scr) represent the total of individual score numbers. **Summary of anti-A IgG subsets as measured by indirect antiglobulin reaction.** When noted, reactions were graded from 4 (strong) to 1 (very weak). **NOTES.** [1] Also reported asthmatic symptoms. [2] Reported previous urticaria. [3] Reported previous anaphylaxis. [4] Also reported food and airborne allergies. [5] Also reported positive rheumatoid factor. **CONTROLS:** (not shown, *n=16*, group O) were randomly selected from a bank of lysophilized, frozen sera and tested for saline titer scores. Observer bias was controlled by assigning random identification numbers to both the hypersensitivity subjects and the controls.

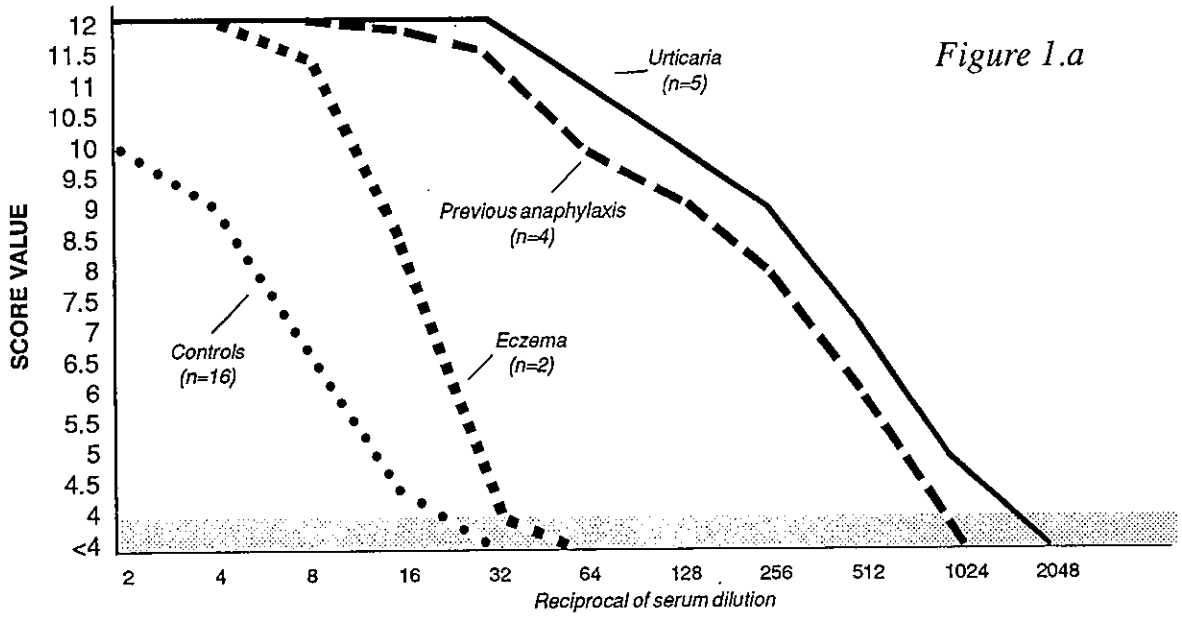

Figure 1.a

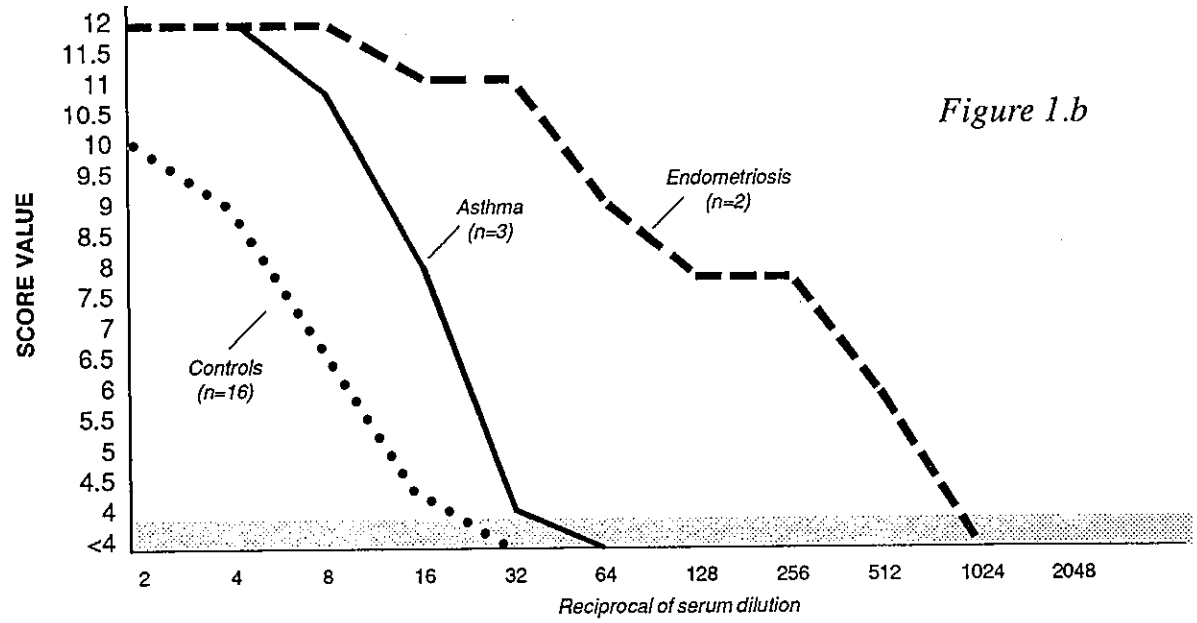

Figure 1.b

FIGURES 1 (a,b) Saline anti-A agglutination score averages in group O individuals for controls and a variety of hypersensitivity conditions plotted against saline titration endpoints. The shaded areas represent titration endpoints plotted for score values under 4.

titer and the pathogenesis of any of the conditions reported. Rather, it would indicate that in group O subjects these mechanisms are interactive if not interdependent. A "paleo-serologic" theory of bloodgroup phylogenetics would help explain some of these variants, and it has been hypothesized that group O preceded groups A and B,*(9)* which are teleologically closer to each other than either is to O.*(10)* Isohemagglutinins and other "natural" antibodies of early phylogenetic appearance may be of greater significance in the host response of group O when directed against microbiological and environmental challenges possessing opposing bloodgroup antigenicity.

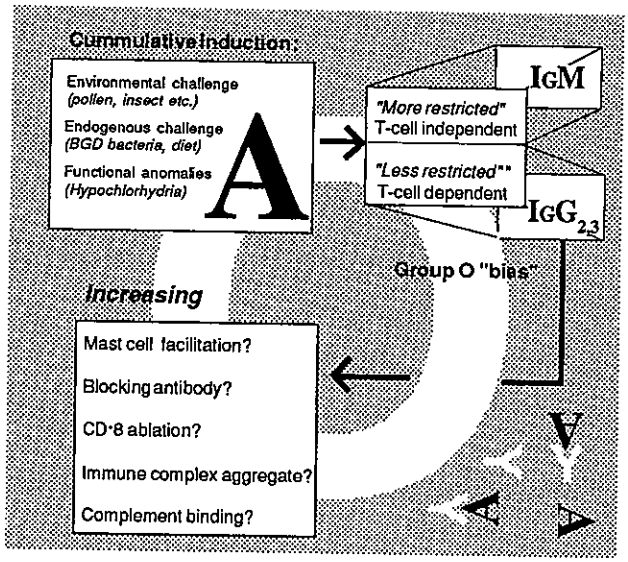

FIGURE 2. Hypothetical mode of hypersensitivity induction in group O individuals via isohemagglutinin A stimulation from exogenous and endogenous sources.

In general, isohemagglutinin studies on groups A and B are unrewarding. These antibodies are almost entirely IgM class and rarely show dramatic elevations in titer score. McDuffie and Kabat*(11)* studied the Coombs test behavior of anti-A and anti-B and reported that of 18 group O individuals the sera of 15 showed enhancement of anti-A and anti-B by the Coombs test as compared to only 2 of 10 sera from non-group O sources. Contreras *et al* showed that the IgG response is greater in group O subjects than those of groups A or B after deliberate immunization with appropriate antigen.*(12)* It was also noted that even before immunization the presence of some IgG antibody could be shown in all blood groups, although in a lower titer in those of groups A and B. In 1986 Kay and Locke*(13)* concurred with this finding and characterized particular IgG_{1-4} subsets in those groups with elevated IgG titers. Their findings showed that group O subjects produced a wider spectrum of response and a greater strength of antibody within each subclass. Although only a few subjects in their study produced strong reactions within IgG_1 and IgG_3 subclasses, with the exception of one subject, all were group O.

In a previous report, Kay had speculated that group O subjects could produce IgG antibodies to non-self ABO system antigens more readily than those of groups A or B because there is a greater antigenic disparity between group O and groups A/B than between group A and group B. This was postulated to allow a greater ability to provide T cell help during the response to T-dependent (glycoprotein) forms of ABO system antigens in subjects of group O.*(14)* ABO glycoprotein is the predominant environmental form observed in substances possessing A-like antigenicity. T cell help is known to allow responding B cells to change from IgM production to IgG, giving a less restricted response. Responses to T independent antigens are known to be more restricted, often to IgM alone.*(15)*

Glycoproteins with A antigenicity are ubiquitous in nature, occuring not only in humans, but in the blood and tissue of lower animals,*(16)* a variety of natural products,*(17)* and a wide variety of microbia.*(18)* One study showed almost 50% of 282 aerobic Gram negative bacteria were found to be blood group active.*(19)* They are extensively degraded in the human colon ecosystem, mostly from heat sensitive obligate anaerobes.*(20)* Human feces contains many blood group degrading enzymes (BGD); most if not all glycosidases. BGD are absent from meconium and appear in infant stool after the first few months of life, perhaps indicating increasing induction from developing microflora. Interestingly, they have been found in achlorhydric gastric secretions but not in normal acidic gastric juice.*(21)* The specificity of BGD is affected by ABO group and secretor status.*(20)* These observations should be viewed as interdependent: the large enteric population of fecal anaerobes are an important source of A antigen substrate for A-degrading bacteria. Exogenous and enterically developed A substance and its degradation by-products present a non-A host with a constant, dynamic antigen burden, which appears to be of special significance to individuals of group O who possess the inherent ability to produce anti-A in both IgM and IgG classes, resulting in a less restricted, "poorly behaved" response to challenge by substances with A-antigenicity.

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FROM THE ARCHIVES

"Watch out for personality-crises occurring mainly at growth periods. Schizophrenia (dementia of the young) is usually found in the 15th to 25th years' group (80,000 cases per year). At puberty personality-crisis may affect the boy or girl who shrinks from leaving the dependence of childhood behind -the adolescent who fears the assumption of adult responsibilities. Those in the "early twenties" have to conquer their fears of the burdens of marital and family responsibilities. The menopausal woman may have a stormy time with physical changes, and poignant regrets at leaving her attractive youth behind. Therapy is one of encouragement in the belief that innate intelligence takes charge of both physical changes in maturation, and also of mental and emotional development. The psychotherapist can often in one or two explanatory talks remove anticipatory anxiety which, if not dealt with, leads later on to grave personality crises."

"The Practice of Naturopathic Psychotherapy"
Milton Powell, N.D., M.B.N.O.A. From the
British Naturopathic Journal & Osteopathic Review
Vol. 9 No. 7, Winter 1974-5