References

[1] *Bara, M.:* Electrophysiological studies of the human amniotic epithelium in vitro. Satellite Symposia of XXVIII Int. Cong. Phys. Sc., Szeged (1980).

[2] *Bara, M.:* Perméabilité de la membrane amniotique humaine in vitro. Comparaison avec des membranes artificielles lipidiques. Thése de Doctorat d'Etat ès-Sciences, Université P. et M. Curie (1981).

[3] *Brame, R. G.:* Quantitation of transport of sodium across the amnio-chorion. Am. J. Obstet. Gynecol., **43** (1972) 1085—1089.

[4] *Brame, R. G., Harbert, M. D., Gaughey, H. S., Thornton, W. W.:* Fetomaternal exchange of sodium. A possible mechanism of the formation of hydramnios. Obst. Gynecol., **32** (1968) 241—268.

[5] *Brinley, F. J.:* Calcium and magnesium transport in single cells. Fed. Proc., **32** (1973) 1735—1739.

[6] *Guiet-Bara, A., Bara, M.:* Perméabilité de la membrane amniotique humaine isolée aux cations divalents. C. R. Acad. Sci. Paris, **286** (1978) 635—638.

[7] *Guiet-Bara, A., Bara, M.:* Transfert des cations monovalents à travers la membrane amniotique humaine isolée. C. R. Soc. Biol., **173** (1979) 875—880.

[8] *Wright, E. M., Diamond, J. M.:* Effects of pH and polyvalent cations on the selective permeability of gall-bladder epithelium to monovalent ions. Biochim. Biophys. Acta, **163** (1968) 57—74.

Reproduction, Université Pierre et Marie Curie, 7 Quai Saint-Bernard, 75230-Paris Cedex 05, France)

Behavioral and biological effects of oral magnesium, vitamin B6 and combined magnesium — vitamin B6 administration in autistic children*) **)

By *C. Barthelemy, B. Garreau, I. Leddet, D. Ernouf, J. P. Muh,* and *G. Lelord*

Service d'Explorations Fonctionnelles Psychopathologiques, Centre Hospitalier Universitaire Bretonneau, F-37044 Tours Cédex

Zusammenfassung

Es wird über Verhalten und biochemische Parameter von 52 autistischen Kindern berichtet, die unter Doppelblindbedingungen im Überkreuzversuch folgendermaßen behandelt wurden: A: Vit. B_6 + Mg; B: Magnesium; C: Vit. B_6. Die therapeutischen Effekte wurden einerseits mittels Rating-scales and andererseits anhand der Ausscheidung von Homovanillinmandelsäure (HVA) im Urin beurteilt.

Nach Gabe von Vitamin B_6 + Mg wurde eine Besserung des Verhaltens und eine Normalisierung der HVA-Ausscheidung beobachtet. Nach alleiniger Gabe von Vit. B_6 oder Mg wurden diese Wirkungen nicht gesehen.

Summary

This is a report of behavioral and biological effects of 3 therapeutic crossed sequential double-blind trials on 52 autistic children: Trial A (vitamin B6 + magnesium); Trial B (magnesium); Trial C (vitamin B6). Therapeutic effects were controled using behavior rating scales on one hand and urinary excretion of homovanillic acid (H.V.A.) on the other hand.

With vitamin B6 + Mg, a diminution of autistic symptoms is observed as well as a normalisation of urinary H.V.A. levels. These effects are not observed when vitamin B6 or Mg are administered alone.

Résumé

Les effets cliniques et biologiques du magnésium et de la vitamine B6 prescrits séparément ou associés sont étudiés. 52 enfants autistiques ont participé à 3 essais croisés doubleaveugle: Essai A (vitamine B6 + magnésium); essai B (magnésium); essai C (vitamine B6). L'appréciation des effets thérapeutiques est réalisée d'une part à l'aide d'échelles de comportement et d'autre part par le dosage de l'acide homovanilique urinaire (H.V.A.).

Lorsque Mg + B6 sont associés, on observe une amélioration clinique et une tendance à la normalisation des taux de l'H.V.A. urinaire. Ces effets ne sont par observés quand Mg et B6 sont prescrits séparément.

* * *

*) Results presented at the 3rd International Symposium on Magnesium, Baden-Baden, 22.—22. 8. 1981.

**) This study was supported by CNRS ERA (N-697) "Biological Methods in Neuropsychiatry", INSERM, ATP 78—97 and CL 79, Fondation pour la Recherche Médicale.

According to several studies [3, 17, 19], vitamin B6 has been found effective in the treatment of autistic children. The observed clinical improvement is associated with a decreased level of uri-

nary homovanillic acid (H.V.A.), one of the main dopamine metabolites [2, 14, 15]. In most of these studies, vitamin B6 was associated with magnesium. *Rimland* [18] had in effect pointed out that vitamin B6 prescribed alone would engender problems suggestive of a magnesium deficiency. In this study, we have attempted to clarify and differentiate the respective effects of magnesium (Mg), vitamin B6 (B6) and combined magnesium — vitamin B6 (Mg-B6).

Subjects and Methods

All 52 subjects were patients in the child psychiatry unit of Centre Hospitalier Régional Tours — St Benoit la Foret. The 34 boys and 18 girls varied in age from 3.2 to 16.0 years; mean age was 8.8 years. A diagnosis of infantile autism was made by two research child psychiatrists on the basis of DSM III [8] criteria. The children received a complete diagnostic work-up, including psychiatric, psychological, neurological and medical evaluation. Patients with severe seizure disorders, gross neurological deficits, endocrine or systemic disease were excluded from this study.

Three double blind therapeutic trials were performed. Trial A: Mg-B6 versus placebo (21 children, 17 boys, mean age 9.1 years); trial B: Mg versus placebo (35 children, 20 boys, mean age 8.5 years); trial C: B6 versus placebo (37 children, 21 boys, mean age 8.7 years). Only 7 children successively entered all three trials.

All three trials were carried out with identical formats. After a 2 weeks drug-free period (ANTE), children were randomly assigned to two groups. The first group received a placebo and the second group either Mg-B6, Mg or B6. After two weeks, the medication and placebo groups were switched for an additional two weeks. The two week treatment period (TREATMENT) was followed by a two week drug-free period (POST).

The children received oral medication daily at 8 a.m. and noon in the following doses: 30 mg/Kg/d B6 (pyridoxine chlorhydrate; daily doses up to 1 g/d), 10 to 15 mg/Kg/d Mg^{++} (magnesium lactate or pyrrolidone carboxylate) and a combination of Mg^{++} and B6 at these individual dose levels.

The clinical effects of the treatment were evaluated utilizing our own rating scale (Fig. 1) which quantifies the severity of various inappropriate aspects of the autistic child. This scale consists of 20 items grouped in 7 parameters. The first three parameters deal particularly with autistic features. The last four deal with accompanying symptoms. Children were rated twice a week during the whole trial period by two independent raters who had daily contact with each child, and who did not know which medication the child was receiving. The scores of the children were averaged for each 2 week period: ANTE, TREATMENT, PLACEBO, POST.

BEHAVIOR RATING SCALE
20 ITEMS SCORED FROM 0 TO 4

I LACK OF RESPONSIVENESS TO OTHER PEOPLE
1 AUTISTIC WITHDRAWAL
2 LACK OF INTEREST IN PEOPLE
3 AVERSION TO AFFECTION AND PHYSICAL CONTACT
4 LACK OF EYE CONTACT

II IMPAIRMENT IN VERBAL AND NON VERBAL COMMUNICATION
5 GROSS DEFICITS IN LANGUAGE
6 LACK OF APPROPRIATE FACIAL EXPRESSIONS AND GESTURES
7 UNEXPLAINED SCREAMING AND LAUGHING

III BIZARRE RESPONSES TO VARIOUS ASPECTS OF THE ENVIRONMENT
8 POOR ACTIVITY, LACK OF INITIATIVE
9 STEREOTYPED ACTIVITIES
10 PECULIAR INTEREST IN OR ATTACHMENT TO OBJECT (DOLL)

IV MOTILITY DISORDERS
11 AGITATION, RESTLESSNESS

V AFFECTIVE DISORDERS
12 AGGRESSIVENESS TOWARD SELF AND OTHERS
13 DISTRESS, ANXIETY
14 MOOD DIFFICULTIES

VI INSTINCTUAL DISTURBANCES
15 EATING PROBLEMS
16 BOWELS AND BLADDER CONTROL TROUBLES
17 DISTURBED SLEEP

VII DISTURBANCES OF ATTENTION AND PERCEPTION
18 EASILY DISTRACTED
19 DOESN'T SEEM TO LISTEN
20 DOESN'T SEEM TO UNDERSTAND

Fig. 1: The Autistic Status. Rating Scale consists of 20 items grouped in 7 parameters. The first three parameters deal particularly with autistic features. The last four deal with accompanying symptoms.

H.V.A. assays were carried out on a morning urine sample collected towards the end of each two-week period (day 10 or 11), between 9—10 a.m.. Urinary H.V.A. level was measured by a modified version of the techniques proposed by *Korf* et al. [12] and *Wadman* et al. [20] and was expressed as nmol/μmol creatinine.

Eleven healthy children (6 boys, ages 7 to 14 years; mean age of 10) were treated with combined Mg-B6 in an identical fashion and served as controls in the H.V.A. study.

Results

Clinical data analysis confirmed the effectiveness of combined Mg-B6 administration for autistic symptoms. By contrast, when given separately, Mg and B6 had no significant therapeutic effect.

The average parameter ratings (Sc.) recorded during the "ANTE" drug-free period and during treatment are compared on summary graphs combining all subjects for each of the three trials: Mg-B6, Mg and B6 (Bar graphs of Fig. 2). Four out of six parameters consistently responded to combined Mg-B6 treatment. The greatest differences between ANTE and Mg-B6 periods were seen in the autism parameters: AUT I Lack of responsiveness to other people ($t = 5.56$, $p < .001$); AUT II Impairment in communication ($t = 3.67$, $p < .01$); AUT III Bizarre responses to the environment ($t = 4.90$; $p < .001$, Student's t test). There was also a significant difference in parameter ACC VI Instinctual disturbances ($t = 3.63$; $p < .01$, Student's t test).

No significant change was observed in the mean behavior scores between the ANTE and TREATMENT periods when Mg and vitamin B6 were given separately.

Biological data analysis revealed that a significant decrease in urinary H.V.A. levels was associated with the clinical improvement observed during combined Mg-B6 administration. Comparisons between H.V.A. levels during ANTE and TREATMENT periods were performed for the three trials. (Bottom graph of Fig. 2). H.V.A. levels decreased during Mg-B6 administration (ANTE = 6.61 nmol/μmol creat; Mg-B6 = 4.43 nmol/μmol creat; $t = 3.23$, $p < .02$, Student's t test). No significant change was observed when Mg and vitamin B6 were given separately.

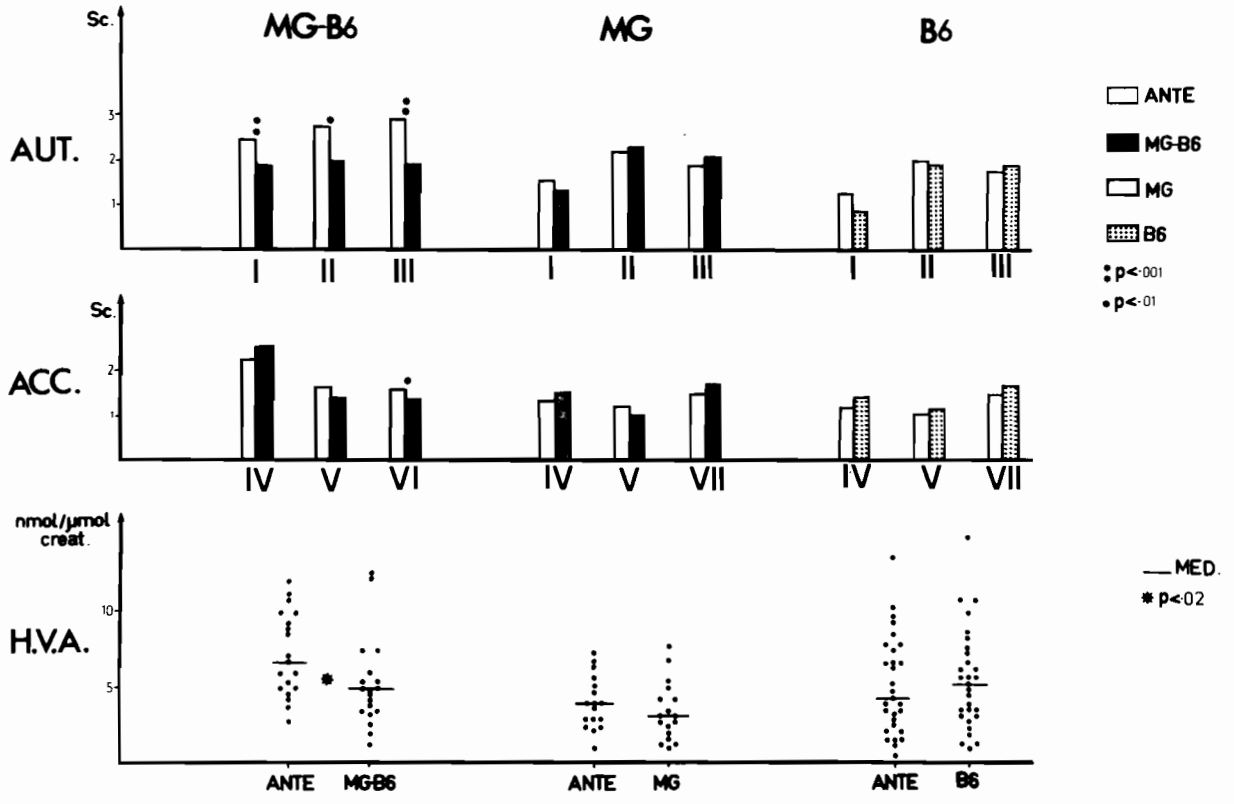

Fig. 2: Comparison of the vitamin B6 (B6), magnesium (Mg), and combined Mg-B6 trials.
Upper graphs: The average parameter ratings (Sc.) recorded during the ANTE drug-free period and during TREATMENT are compared on bar graphs combining all subjects for each of the three trials. (AUT: autistic symptoms; ACC: accompanying symptoms).
Bottom graph: Comparisons between H. V. A. levels (nmol/umol creatinine) during ANTE and TREATMENT periods of the three trials.
MED. = median value.

Urinary H.V.A. levels were initially higher in the autistic group than in the control group of children (AUT = 6.61 nmol/μmol creat; NOR = 2.08 nmol/μmol creat; $t = 6.16$, $p < .001$, Student's t test). Treatment with combined Mg-B6 decreased H.V.A. levels in the autistic group, but slightly increased H.V.A. levels in the control group (ANTE = 2.08 nmol/μmol creat; Mg-B6 = 2.52 nmol/μmol creat; $t = 3.07$, $p < .02$, Student's t test).

Discussion

This study supports previous observations [2, 14, 15] and confirms that Mg and B6 when administered in combination is therapeutic for autistic children, but have no beneficial effect when given separately.

The clinical effects were measured by a double blind method utilizing an objective rating scale. The greatest differences between ANTE and Mg-B6 treatment were seen in two parameters: Lack of responsiveness to other people and Bizarre responses to various aspects of the environment. In a recent work, *Lelord* et al. [15] reported Mg-B6 to be especially effective in reducing autistic symptoms.

In addition, the higher levels of urinary H.V.A. in autistic children (in contrast to control children) were decreased by combined Mg-B6 administration. No such effect was observed when Mg or B6 were given separately.

Thus, both clinical and biochemical findings indicate that Mg and vitamin B6 are effective only when combined. This synergic activity has already been described in a variety of cellular and metabolic mechanisms [9].

The presence of high urinary H.V.A. levels in autistic children can be interpreted as relating to a disturbance of dopamine metabolism. This hypothesis has already been suggested [4, 5, 11, 13].

Some researchers suggest that autism may result from a maturational impairment of certain CNS dopaminergic structures [7, 10, 16]. Both vitamin B6 and magnesium take part in the metabolism of this neurotransmitter [1, 6, 9, 21].

Although the effects of Mg-B6 cannot be attributed to any single mechanism, our results suggest that the clinical improvement observed in autistic children during Mg-B6 administration may be related to brain dopamine metabolism.

Acknowledgements

The authors wish to thank Dr. *Brouillot* (Specia Laboratories) and Dr. *Pechery* (Meram Laboratories) who helped make this work possible.

References

[1] *Barbeau, A., Rojo-Ortega, J. M., Brecht, H. M., Ganien, D., Minnich, J., Boucher, R., Geivest, J.*, In: *Durlach, J.*, (ed.): 1st International Symposium on magnesium deficit in Human Pathology. Vol. II. S.G.E.M.V., Vittel, (1973) 149—152.

[2] *Barthelemy, C., Garreau, B., Leddet, I., Sauvage, D., Domenech, J., Muh, J. P., Lelord, G.*: Therapie. **35** (1980) 627—632.

[3] *Callaway, E.*: Psychopharmacol. Bull. **13** (1977) 57—58.

[4] *Cohen, D. J.*: Arch. Gen. Psychiat. **31** (1974) 845—853.

[5] *Cohen, D. J., Caparulo, B. K., Shaywith, B. A., Bowers, M. B.*: Arch. Gen. Psychiat. **34** (1977) 545—575.

[6] *Coleman, M., Steinberg, G., Tippet, J., Bhagavan, H. N., Coursin, D. B., Gross, M., Lewis, C., Deveau, L.*: Biological Psychiat. **14** (1979) 741—751.

[7] *Damazio, A. R., Maurer, G.*: Arch. Neurol. **35** (1978) 777—786.

[8] D.S.M. III, In: *American Psychiatric Association* (ed.): Diagnostic and Statistical manual for Mental Disorders. Third edition. Washington, (1980) 87—90.

[9] *Durlach, J.*, In: *Vinken, P. J., Bruyn, G. W.*, (eds.): Handbook of Clinical Neurology. Vol. 28. North-Holland Publishing Company — Amsterdam — Oxford — New York, (1976) 545—579.

[10] *Garreau, B., Barthelemy, C., Domenech, J., Sauvage, D., Muh, J. P., Lelord, G., Callaway, E.*: Acta Psychiat. Belg. **80** (1980) 249—265.

[11] *Goldstein, M., Mahanand, D., Lee, J., Coleman, M.*, In: *Coleman, M.*, (ed.): The autistic syndromes. American Elsevier, New York, (1976) 57—63.

[12] *Korf, J., Ottema, S., Van Derveen, I.*: Anal. Biochem. **40** (1971) 187—191.

[13] *Lake, C. R., Ziegler, M. G., Murphy, D. L.*: Arch. Gen. Psychiat. **34** (1977) 553—556.

[14] *Lelord, G., Callaway, E., Muh, J. P., Arlot, J. C., Sauvage, D., Garreau, B., Domenech, J.*: Rev. Neurol. **134** (1978) 797—801.

[15] *Lelord, G., Muh, J. P., Barthelemy, C., Martineau, J., Garreau, B., Callaway, E.*: J. Autism Development. Dis. **11** (1981) 219—229.

[16] *Martineau, J., Garreau, B., Barthelemy, C., Callaway, E., Lelord, G.*: Biological Psychiat. 7 (1981) 625—639.

[17] *Rimland, B.*, In: *Hawkins, D., Pauling, L.*, (eds.): Orthomolecular psychiatry Freeman, W. H., New York, (1973) 513—539.

[18] *Rimland, B.*: Orthomolecular Psychiat. **3** (1974) 371—377.

[19] *Rimland, B., Callaway, E., Dreyfus, P.*: Am. J. Psychiatry. **135** (1978) 472—475.

[20] *Wadman, S. K., Ketting, D., Voute, P. A.*: Clin. Chim. Acta. **72** (1976) 49—68.

[21] *Yahr, M. D., Duvoisin, R. C., Cote, L., Cohen, G.*, In: *Ebadi, M., Costa, E.*, (eds.): Advances in Biochemical psychopharmacology. Vol. 4. Raven Press, New York, (1972) 185—194.

(For the authors: Dr. Catherine Barthelemy, C. H. U. Bratenneau Laboratoire D'Explorations fonctionelles Psychopathologiques, 2, Boulevard Tonelle, F-37044 Tours Cedex)