ABO Blood Groups and the Hardy-Weinberg Equilibrium

It is now 50 years since Bernstein's classic demonstration (*1*) that the two-locus interpretation of the genetic basis of the ABO blood groups made by von Dungern and Hirszfeld (*2*) was inconsistent with the population frequencies of the ABO phenotypes, and that the hypothesis of three alleles at a single locus fit those frequencies much better. The algebra involved in this analysis has been reproduced in all major genetics texts and has been taught to countless numbers of students as a convincing illustration of the power of the Hardy-Weinberg formulation in differentiating between alternative genetic systems. It may therefore not be inappropriate to point out that the first part of his analysis does not demand the use of the Hardy-Weinberg equilibrium expression for allele frequencies ($p^2 + 2pq + q^2$) at all, but is, in fact, independent of the allele frequencies at a specific locus.

We are concerned here only with that part of his argument which deals with the assumption of two independent loci, an A and a B locus, as the genetic basis for the ABO groups. The set of equations relating the frequencies of the A, B, AB, and O phenotypes with the corresponding genotypes and allele frequencies, as they appear in Bernstein's original paper and copied, with minor modifications, in standard textbooks on genetics, is as follows.

Genotype	Probability	
	Group O	
aabb	$(1-p)^2(1-q)^2$	$= \bar{p}^2 \cdot \bar{q}^2$
	Group B	
aaBB	$(1-p)^2q^2$	$= \bar{p}^2 \cdot (1-\bar{q}^2)$
aaBb	$2(1-p)^2q(1-q)$	
	Group A	
AAbb	$p^2(1-q)^2$	$= (1-\bar{p}^2)\bar{q}^2$
Aabb	$2p(1-p)(1-q)^2$	
	Group AB	
AABB	$p^2 \cdot q^2$	$= (1-\bar{p}^2)(1-\bar{q}^2)$
AaBB	$2 \cdot p(1-p)q^2$	
AABb	$2p^2q(1-q)$	
AaBb	$2p(1-p) \cdot 2q(1-q)$	

By inspection, $\bar{O} \times \overline{AB} = \bar{A} \times \bar{B}$. Also since $\bar{A} + \overline{AB} = 1 - \bar{p}^2$ and $\bar{B} + \overline{AB} = 1 - \bar{q}^2$, then $(\bar{A} + \overline{AB}) \cdot (\bar{B} + \overline{AB}) = \overline{AB}$. (A bar over a term indicates "the frequency of" that term.)

The preceding implies that the equation $(\bar{A} + \overline{AB})(\bar{B} + \overline{AB}) = \overline{AB}$ is a necessary relationship of the phenotypic frequencies revealed only after the application of the Hardy-Weinberg rule for the distribution of alleles in homozygotes and heterozygotes. However, a second glance at the equation suggests something quite different. What that equation states is that if there are two sets of alternative properties, A (and not-A) and B (and not-B), with the sets independent of each other, then the probability of A and B occurring simultaneously is the probability of A times the probability of B. This is obviously the statement of the elementary rule of probability and, as such, should not require the algebraic manipulation of the gene frequencies for its derivation. In fact, it does not. That this relationship is independent of allele frequencies may be easily verified by substituting nonequilibrium values for the homozygotes and heterozygotes at each locus. As long as the two loci are combined randomly, the simple relationship must hold.

Similarly, an alternative relationship, $\bar{O} \times \overline{AB} = \bar{A} \times \bar{B}$, suggested by the same method, is independent of gene frequencies and is simply obtained if not-A not-B is substituted for O, A not-B for A, and B not-A for B.

A little thought will convince one that not only must these relationships hold for this particular genetic theory, that of the two loci, but for any genetic hypothesis, no matter how simple or contrived, so long as A and B are independent. As a matter of fact, such tests for independence would be valid even if the characteristics were not genetically determined.

Bernstein was a mathematician with considerable facility in handling genetic formulations; this is well illustrated by his subsequent demonstration, in the same work, of the surprisingly good fit of the three-allele hypothesis with population data, as well as by his other contributions (*3, 4*). We might wonder, then, why he chose to present the argument against the two-locus hypothesis in this cumbersome way. It is possible, but not likely, that he overlooked the significance of his final equation for the two-locus case, that $p(A) \times p(B) = p(AB)$. Perhaps he recognized the predilection of geneticists for algebraic formulations and felt that they would be more convinced of the validity of his argument if he expressed it with detailed (and unnecessary) algebra. Or could it be that he possessed a rare sense of humor and was playing a practical joke on biologists? If so, it has worn well.

E. NOVITSKI

Department of Biology,
University of Oregon,
Eugene 97403

References and Notes

1. F. Bernstein, *Klin. Wochenschr.* **3**, 1495 (1925).
2. E. von Dungern and L. Hirszfeld, *Z. Immunitaetsforsch. Exp. Ther.* **8**, 526 (1911).
3. F. Bernstein, *Z. Induk. Abstamm. Vererbungsl.* **54**, 400 (1930).
4. ———, *ibid.* **57**, 113 (1931).
5. I thank Professor F. H. Sobels of the University of Leiden for making facilities available for relaxed contemplation during a sabbatical leave.

24 October 1975

"Serotonin Depression"—A Biochemical Subgroup Within the Affective Disorders?

Abstract. *The distribution of 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the cerebrospinal fluid of 68 depressed patients was bimodal. Twenty-nine percent of the patients were in the lower mode, with a concentration of 5-HIAA below 15 nanograms per milliliter. Although there were no differences in overall severity of depression between the two modes, there was a significant correlation between the concentration of 5-HIAA and severity of depression in the lower, but not in the upper, mode. The finding suggests the existence of a biochemical subgroup of depressive disorder, characterized by a disturbance of serotonin turnover.*

Evidence of a disturbance of serotonin turnover in the depressive disorders has accumulated over recent years, but the findings in the field are contradictory (*1*). Low (*2*) but also normal (*3*) concentrations of serotonin have been found in brains from suicide victims. Tryptophan, the precursor of serotonin, is claimed by some authors (*4*) to have antidepressant effects, while others (*5*) have found it to be of doubtful value. Low (*6*) but also normal (*7*) concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) from depressed patients have been reported.

We have developed a highly sensitive and specific mass fragmentographic method (*8*), which has been used to determine 5-HIAA in CSF from 43 depressed patients (*9*). The distribution of the metabolite appeared to be bimodal. This suggested the existence of two biochemically different types of depression, which might explain some of the divergences between earlier findings.

A further 25 patients have now been studied. In this second sample a more extensive rating of psychopathology was used; otherwise, the basic study design remained essentially the same. Only patients

with severe, primary depressive illness (*10*) were included. Sixteen patients had received tricyclic drugs before admission, almost all of them in inadequate dosages. There were 27 endogenous (*11*) and 16 nonendogenous depressions in sample 1. Sample 2 contained 17 endogenous and 8 nonendogenous depressions.

Severity of depression was assessed after a minimum observation period of 5 days, when no drugs were given except for barbiturates or nitrazepam for night sedation when needed, and occasional doses of diazepam or oxazepam to relieve severe anxiety. In sample 1, the modified Cronholm-Ottosson rating scale (CORS) for depression (*12*) was used. In sample 2, a recently developed, comprehensive psychopathological rating scale (CPRS) (*13*) was used. This scale covers a wider range of psychopathology. Severity of depression was assessed on a subscale. On the same day as the rating, a lumbar puncture was performed according to a standardized technique (*14*). The 5-HIAA concentration in the CSF was measured according to Bertilsson *et al.* (*8*).

The distribution of 5-HIAA in sample 2 was similar to that previously found in sample 1 (Fig. 1, A and B). When the two samples were combined (Fig. 1C), the deviation from the expected normal distribution was significant (χ^2 = 19.76, 9 d.f., P = .02). Twenty patients (29 percent) had 5-HIAA levels below 15 ng per milliliter of CSF and thus fell into the lower mode.

The proportion of men was somewhat higher in the low 5-HIAA mode (9/20 versus 10/48, χ^2 = 2.98, P = .08). The mean age was similar in the two modes [t = 1.12, not significant (N.S.)]. Neurotic depressions occurred in both modes, but were rarer in the low 5-HIAA mode (4/20 versus 20/48, χ^2 = 2.03, P = .15).

Tricyclic antidepressants are known to lower the concentration of 5-HIAA in the CSF (*9*). Even if previously given antidepressants were cleared from the patient's plasma after the washout period, there might still be residual effects on the turnover of serotonin. If this was the case, it was not reflected in the 5-HIAA concentrations, which did not differ between those who had and those who had not had such treatment before admission (t = 0.09, N.S.).

There were no differences in rated severity of depression between the modes (sample 1, Mann-Whitney z = 1.03; sample 2, z = 1.78; both N.S.). There was, however, a significant correlation in sample 1 between the severity of depression and the concentration of 5-HIAA within the lower mode (Spearman ρ = −0.55, N = 14, P = .04; Fig. 2A) but not in the upper mode (ρ = 0.36, N = 29, N.S.).

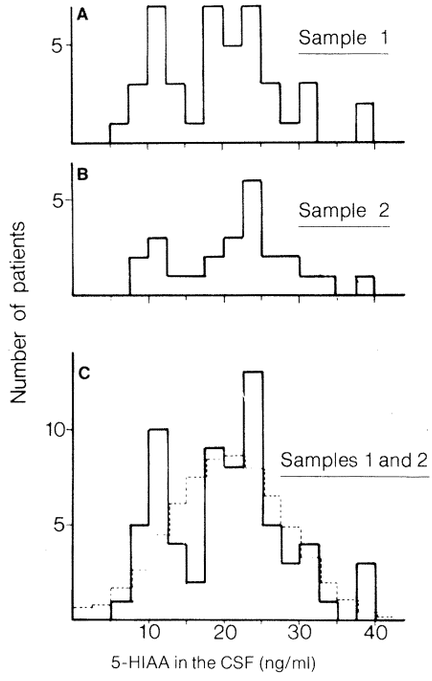

Fig. 1. Distribution of 5-HIAA in depressed patients: (A) sample 1, N = 43; (B) sample 2, N = 25; (C) samples 1 and 2 combined. The dashed line represents the expected normal distribution (mean ± standard deviation = 20.36 ± 7.77). The deviation from normality is significant (χ^2 = 19.76, 9 d.f., P = .02).

In sample 2, there was likewise a significant correlation between severity of illness and 5-HIAA concentration in the low mode (ρ = −0.94, N = 6, P = .005; Fig. 2B), but none in the high mode (ρ = −0.04). The magnitude of the correlation coefficient in sample 2 tells very little about the strength of the relationship because of the small number of observations. Its importance lies rather in the fact that the finding from sample 1 is replicated.

The results are consistent with the hypothesis (*15*) that there is a subgroup within the depressive disorders (particularly the so-called endogenous depressions) where concentrations of 5-HIAA are low, possibly reflecting a disturbance of serotonin turnover. Within this subgroup, there is a negative correlation between severity of depression and the concentration of the metabolite.

The existence of this subgroup may explain much of the apparent conflict in the data on serotonin turnover in depressed patients. "Serotonin depressions" may occur in different proportions in the patient samples previously studied and thus account for the varying mean levels of 5-HIAA. It is possible that treatment with agents that affect serotonin turnover (such as tryptophan) is most helpful in this subgroup. The finding of Van Praag and coworkers (*16*) of an antidepressant effect of 5-hydroxytryptophan in patients with a low probenecid-induced accumulation of 5-HIAA, and the lack of effect of nortriptyline (*9*) [which is predominantly a noradrenaline uptake inhibitor with very little effect on serotonin neurons (*17*)] in the low 5-HIAA group of sample 1, are both consistent with this hypothesis.

If serotonin depression were a specific disorder, one might expect differences between the two modes in other respects as well. One such difference, and an unexpected one, has emerged so far: depressive patients with low 5-HIAA concentrations are significantly more prone to attempt suicide than those with high concentrations, and they use more violent means (*18*).

The nature of the relationship between 5-HIAA concentrations and depression is far from clear. Coppen's observation (*1*) that concentrations remain low after recovery supports the idea that they reflect a constitutional deviation, which perhaps renders the individual more vulnerable to depressive reactions. Our finding of a negative correlation between 5-HIAA and severity of illness is more suggestive of a relationship to the depressive state, but the two hypotheses are not mutually exclusive. Another possibility [which has some sup-

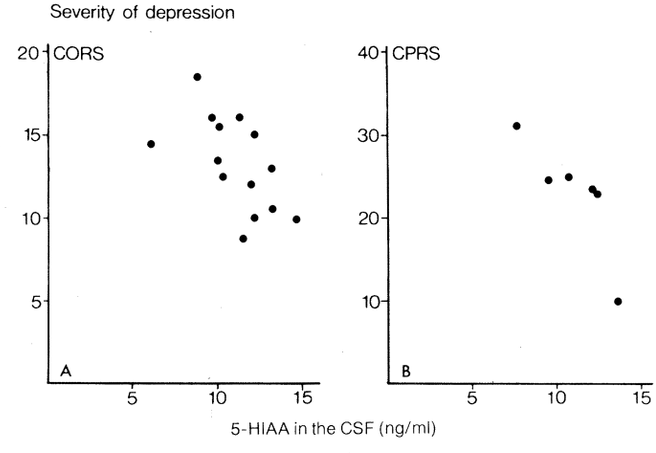

Fig. 2. Relationship between 5-HIAA in CSF and severity of depression in the low 5-HIAA mode (5-HIAA < 15 ng/ml): (A) sample 1, Spearman ρ = −0.55, N = 14, P = .04; (B) sample 2; ρ = −0.94, N = 6, P = .005.

port from animal data (*19*)] is that changes in serotonin turnover are part of a general stress reaction in certain individuals, and the depressed mood may be another facet thereof.

A most interesting question is how our findings are related to the well-known catecholamine hypothesis of affective disorder (*20*). According to this hypothesis, noradrenergic functions are disturbed in depression. Do disturbances of serotonergic and noradrenergic functions coexist, or is there another subgroup of disturbed noradrenaline turnover within the depressive spectrum? Further investigation may help to clarify this issue. Our preliminary findings indicate, however, that a negative correlation may indeed exist between concentrations of the noradrenaline metabolite, 4-hydroxy-3-methoxyphenyl glycol (HMPG) in CSF and severity of illness in endogenously depressed patients, but only in those who belong to the upper 5-HIAA mode.

MARIE ÅSBERG
PETER THORÉN
LIL TRÄSKMAN
Karolinska Institute, Department of Psychiatry, Karolinska Hospital, S-104 01 Stockholm 60, Sweden
LEIF BERTILSSON
VIVIANN RINGBERGER
Karolinska Institute, Department of Clinical Pharmacology, Huddinge Hospital, S-141 86 Huddinge, Sweden

References and Notes

1. For a review, see A. Coppen, *J. Psychiatr. Res.* **9**, 163 (1972).
2. D. M. Shaw, F. E. Camps, E. G. Eccleston, *Br. J. Psychiatry* **113**, 1407 (1967); C. M. B. Pare, D. P. H. Yeung, K. Price, R. S. Stacey, *Lancet* **1969-II**, 133 (1969); K. G. Lloyd, I. J. Farley, J. H. N. Deck, O. Hornykiewicz, *Adv. Biochem. Psychopharmacol.* **11**, 387 (1974).
3. H. R. Bourne, W. E. Bunney, Jr., R. W. Colburn, J. M. Davis, J. N. Davis, D. M. Shaw, A. J. Coppen, *Lancet* **1968-II**, 805 (1968).
4. A. Coppen, D. M. Shaw, B. Herzberg, R. Maggs, *ibid.* **1967-II**, 1178 (1967); A. Coppen, P. C. Whybrow, R. Noguera, R. Maggs, A. J. Prange, Jr., *Arch. Gen. Psychiatry* **26**, 234 (1972).
5. B. J. Carrol, *Clin. Pharmacol. Ther.* **12**, 743 (1971); W. E. Bunney, Jr., H. K. H. Brodie, D. L. Murphy, F. K. Goodwin, *Am. J. Psychiatry* **127**, 872 (1971).
6. G. W. Ashcroft, T. B. B. Crawford, D. Eccleston, D. F. Sharman, E. J. MacDougall, J. B. Stanton, J. K. Binns, *Lancet* **1966-II**, 1049 (1966); S. J. Dencker, U. Malm, B.-E. Roos, B. Werdinius, *J. Neurochem.* **13**, 1545 (1966); A. Coppen, A. J. Prange, Jr., P. C. Whybrow, R. Noguera, *Arch. Gen. Psychiatry* **26**, 474 (1972).
7. R. Papeschi and D. J. McClure, *Arch. Gen. Psychiatry* **25**, 354 (1971); R. Sjöström and B.-E. Roos, *Eur. J. Clin. Pharmacol.* **4**, 170 (1972); H. M. Van Praag, J. Korf, D. Schut, *Arch. Gen. Psychiatry* **28**, 827 (1973).
8. L. Bertilsson, A. J. Atkinson, Jr., J. R. Althaus, Å. Härfast, J.-E. Lindgren, B. Holmstedt, *Anal. Chem.* **44**, 1434 (1972). Dideuterium-labeled 5-HIAA is used as an internal standard. The standard deviation is about 7 percent below 20 ng of 5-HIAA per milliliter and less than 2 percent above this level.
9. M. Åsberg, L. Bertilsson, D. Tuck, B. Cronholm, F. Sjöqvist, *Clin. Pharmacol. Ther.* **14**, 277 (1973).
10. Exclusion criteria were signs or symptoms of schizophrenia, organic brain syndrome, and abuse of alcohol or drugs. A diagnostic inventory [C. Gurney, in *Proceedings of the 5th World Congress of Psychiatry* (Mexico City, 1971), p. 330] was used to ascertain the diagnosis of depression. The reliability of the inventory scale score was 0.93 ($P < .001$).
11. In sample 1, depression was classified as endogenous or nonendogenous according to the clinical criteria laid down by B. Cronholm and J.-O. Ottosson [*Acta Psychiatr. Scand.* **35**, (Suppl. 145), 69 (1960)]. In sample 2, the above-mentioned inventory was used.
12. M. Åsberg, P. Kragh-Sørensen, R. H. S. Mindham, J. R. Tuck, *Psychol. Med.* **3**, 458 (1973). The reliability for two cotrained raters was 0.97 ($P < .001$).
13. M. Åsberg, C. Perris, D. Schalling, G. Sedvall, in preparation. The reliability of the subscale used in this study was 0.92 ($P < .001$).
14. Samples (13 ml) of CSF were drawn at about 8 a.m., after a minimum 8 hours of fasting and bed rest. The samples were centrifuged immediately and stored frozen at −20°C in 2-ml portions.
15. F. Sjöqvist, *Int. Pharmacopsychiatry* **6**, 147 (1971).
16. H. M. van Praag and J. Korf, *Psychopharmacologia* **19**, 148 (1971); ———, L. C. W. Dols, T. Schut, *ibid.* **25**, 14 (1972).
17. L. Bertilsson, M. Åsberg, P. Thorén, *Eur. J. Clin. Pharmacol.* **7**, 365 (1974).
18. M. Åsberg, L. Träskman, P. Thorén, in preparation.
19. G. Curzon, *J. Psychiatr. Res.* **9**, 243 (1972)
20. J. J. Schildkraut, *Am. J. Psychiatry* **122**, 509 (1965).
21. We are grateful to Kerstin Isacson, Lennart Jörnestedt, and Lil Ericius, as well as to the staff of ward 7 of the Karolinska Hospital, for their assistance. The Ethical Committee of the Karolinska Hospital approved of the investigation. The study was supported by the Tercentenary Fund of the Bank of Sweden (68/90) and the Swedish Medical Research Council (61P-4567 and 21P-4676).

22 September 1975

Influence of Cadmium and Other Trace Metals on Human α_1-Antitrypsin: An in vitro Study

Abstract. *The effect of trace metals on plasma α_1-antitrypsin was studied in vitro by adding known concentrations of trace metals, either alone or in combination, to plasma. Cadmium was the only trace metal that reduced the concentration of α_1-antitrypsin and depressed the trypsin inhibitory capacity. No such effects were found with divalent lead, mercury, nickel, iron, and zinc ions. The present study appears to offer a plausible explanation for the emphysema that occurs in industrial workers exposed to cadmium.*

Considerable attention has been paid to the association between familial α_1-antitrypsin deficiency and pulmonary emphysema (*1, 2*). Although the role of intermediate α_1-antitrypsin deficiency in predisposing to pulmonary emphysema is still controversial (*2, 3*), the association with severe deficiency appears well established. Emphysema can also be caused by prolonged exposure to cadmium (*4–6*).

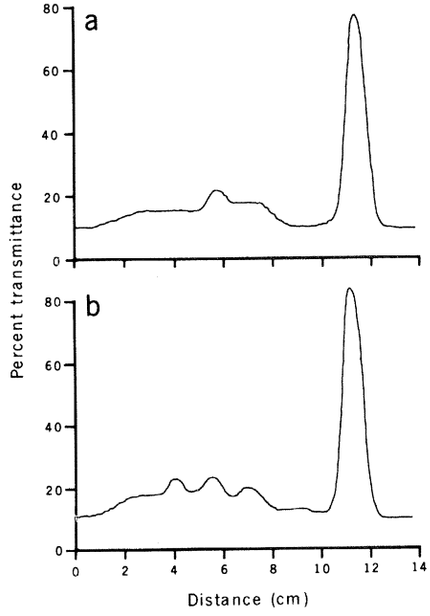

Fig. 1. Cellulose acetate electrophoretic patterns of (a) the plasma incubated with 50 μg of cadmium per milliliter and (b) normal human plasma (control). Percent transmittance (ordinate) and distance (abscissa) are in arbitrary units.

Since exposure to cadmium and severe α_1-antitrypsin deficiency are associated with emphysema, we wondered whether cadmium or other trace metals would alter human α_1-antitrypsin.

Experiments were carried out by adding a graded amount of trace metal in concentrations of 5 to 50 μg/ml to plasma and incubating the mixture at 37°C for 1 hour. Further experiments were conducted by combining two metals together, in each case one of the two being cadmium. Plasma blanks were incubated in triplicate for each set of experiments. After incubation the samples were centrifuged at 5000 rev/min and the following assays were performed in triplicate. α_1-Antitrypsin content was determined by a radial immunodiffusion method (*7*); trypsin inhibitory capacity (TIC) was measured according to the method of Dietz *et al.* (*8*); serum protein concentration was determined by cellulose acetate membrane electrophoresis with a Beckman Microzone electrophoresis apparatus (*9*); and total protein was determined by biuret reaction (*10*).

Figure 1 shows the electrophoretic pattern of normal human plasma and of plasma treated with cadmium solution, the concentration of cadmium being 50 μg/ml. The α_1-globulin peak is virtually absent in the plasma to which cadmium had been added. A decrease in the peak corresponding to the β-globulin fraction was also observed. Treatment of plasma with equivalent concentrations of Pb^{2+}, Hg^{2+}, Ni^{2+}, Fe^{2+}, and Zn^{2+} produced no such effects. The effects of various concentrations of