Blood Groups and Disease

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It is firmly established that erythroblastosis fetalis (hemolytic disease of the newborn) is a blood disease of the fetus and newborn resulting from isosensitization of the mother to an antigen on the red cells of the fetus which is lacking from her own blood, and the transplacental transfer of the maternal antibody into the fetal circulation, where it combines with the red cells of the fetus (Levine et al. 1941). Thus, there necessarily is a strong association between blood groups and this disease, so much so that Rh typing and Rh antibody testing have become a routine part of the prenatal care of expectant mothers, in order to predict *before* birth the occurrence of the disease in the fetus and newborn baby. While the purpose of this article is primarily to discuss the numerous investigations that have been carried out on the association of blood groups with diseases and conditions other than erythroblastosis fetalis, it will be necessary to compare these two kinds of investigations for the proper development of the argument.

The pathogenesis of erythroblastosis fetalis was elucidated when it was realized that the expectant mother could be isosensitized either by an injection of blood having the agglutinogen lacking from her red cells, or as a result of leakage of fetal blood into her circulation across the ordinarily impervious placenta. The presence in the mother's serum of the isoantibody produced in response to such stimulation and also coating the red cells of the newborn baby is pathognomonic of erythroblastosis. This is true both in the classic cases caused by $\mathbf{Rh_0}$ sensitization* and in atypical types due to sensitization to other Rh-Hr blood factors such as **rh″**, **hr′**, etc., or blood factors like **S**, **M**, etc., of other blood group systems. In the vast majority of cases of ABO hemolytic disease, the mother belongs to group O, and the baby, to subgroup A_1 or group B (Wiener 1961). Thus, the reason for the association between blood groups and hemolytic disease of the newborn is crystal clear and can be demonstrated in every individual case.

The rationale underlying the claimed associations between blood groups and other diseases, on the other hand, is not at all apparent. In such investigations (reviewed by Wiener 1943, pp. 361–379; Bourdel 1960; Race and Sanger 1962, pp. 385–400; Muschel 1966; Chakravartti 1967; Prokop and Uhlenbruck 1969, pp. 690–722), as large a series as possible is compiled of examples of a particular disease or a condition selected more or less at random (e.g., syphilis, malaria, feeblemindedness, skin

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* To avoid ambiguity, symbols for blood factors (serological specificities) and their corresponding antibodies are printed in **boldface** type, symbols for genes and genotypes are printed in *italics*, while symbols for agglutinogens and phenotypes are in regular type.

diseases, duration of life, dental caries, duodenal ulcer, carcinoma of the lung, fractured femur, cigarette-smoking habits, pernicious anemia, personality traits, hypertension, etc.) and the distribution of the ABO blood groups and subgroups, MN types, Rh-Hr types, etc., determined for the test series and compared with the distribution of the blood groups in a control population. The control population usually consists of blood donors or members of the armed forces. If a "statistically significant" difference is found in the frequencies of any particular blood group between the test and the control series, it is maintained that an association has been established between the blood groups and the condition in question. However, as has been mentioned, in none of the diseases for which an association has been claimed has a plausible rationale been established. In this paper, it is proposed to point out some of the fallacies underlying these investigations and to demonstrate that the claimed associations actually do not exist.

THE FALLACY OF STATISTICAL SIGNIFICANCE

The procedure usually used in the investigations on blood groups and disease is to determine the difference in the frequencies for every blood group between the test and control series, and then determine the ratio of that difference to its standard error. It has become conventional to consider any difference greater than twice its standard error as "statistically significant," on the basis that such a difference could occur by chance alone only once among 20 times; that is, $P = .05$. When the difference is much greater than twice its standard error, it is said to be "highly significant"; for example, $D/\sigma = 3$ implies that $P = .003$, approximately, so that such a difference could occur by chance only once in about 300 times. Thus, if it is found that $P < .05$, and certainly when $P < .005$, it is generally concluded that an association has been established between the blood group in question and the condition under investigation. However, it will be shown that, as a rule, the reasoning used is fallacious.

First, $P = .05$ means that deviation at this magnitude *will* occur by chance alone once in about 20 times. Therefore, if enough comparisons are carried out, a P value of .05 is bound to turn up sooner or later, even when no associations are present.Thus, if 20 comparisons are made, as have often been reported, and a P value of .005 is obtained for one of the comparisons, this should be multiplied by 20, thus reducing the P value to only .1, or about one chance in ten. If the difference found is still to be considered a significant one, then the study must be repeated on a *fresh* series of cases for the blood group in question. However, in almost all of the cases where this has been done, the original difference reported could not be confirmed, or when the same deviation was found, it could again be merely the result of chance. For example, a report (Cohen and Thomas 1962) that in white males there is a significant deficiency of group B individuals among heavy cigarette smokers and an excess of Rh-negative individuals in the white occasional-smoker class could not be confirmed by subsequent investigators (Higgins et al. 1963); the same was true for the claim that mongolism was associated with the Kell-positive type (Evans et al. 1968), etc.

A more serious flaw in the calculations of "statistical significance" is the failure to take into account the a priori probabilities (see Wiener 1960). When a sensitized Rh-negative mother has a baby with neonatal jaundice and anemia, the demonstration that the baby is Rh positive with red cells coated with Rh antibodies is generally

sufficient to establish the diagnosis of Rh hemolytic disease; if the baby is Rh negative, it becomes equally obvious that some other explanation must be sought for the baby's illness. Here the a priori probabilities of the occurrence of Rh hemolytic disease in the baby are quite high, so that it takes only a *single* case to establish the association of the diagnosis with the baby's Rh type. When diseases are selected at random and without clear rationale, however, the a priori probabilities of the existence of an association with blood groups are extremely remote. In such instances a P value of .05 or even of only .001 would hardly be enough to overcome the presumption that no association is present. It is revealing that in none or hardly any of the reports in which associations have been claimed to be established (as in studies on duodenal ulcer, pernicious anemia, carcinoma of the lung, etc.) have the a priori probabilities been taken into account or even mentioned.

A possible exception to this criticism is the investigations on the association between blood groups and particular infectious diseases (see Vogel 1965). It has been established, especially by the work of Springer (1966) and his associates, that many microorganisms have A-like, B-like, and H-like serological specificities. It has been reasoned that persons of blood groups A and AB should be more susceptible to infection with bacteria having A-like antigens because of the inability of persons having agglutinogen A to produce anti-**A** isoagglutinins. The same reasoning has been applied to infections with microorganisms having B-like and H-like antigenic specificities. However, attempts to establish associations between blood groups and such infectious diseases have so far given only inconclusive results. Some of the probable reasons for these disappointing results will be discussed.

The a priori probabilities by no means have a fixed value, but they change depending on the state of knowledge. For example, when the first studies were carried out on blood groups, secretor types, and alkaline phosphatase, the a priori probability that an association would be found appeared very small indeed. However, it has been found that an association actually exists. This discovery has therefore changed the situation and now makes it appear likely that further investigation may disclose additional associations between blood groups and isozymes, even though the basis for such associations has not yet been determined.

DILUTION OF SIGNIFICANT DATA WITH NONSIGNIFICANT DATA

Before the Rh factor was discovered and before adequate tests for Rh sensitization had been devised, it was obviously impossible to diagnose Rh hemolytic disease. For a long time it had been suspected that neonatal jaundice and anemia could be the result of ABO incompatibility between mother and baby. However, when blood grouping tests on babies with neonatal jaundice showed that a high percentage were group O, the concept of maternofetal ABO incompatibility was abandoned. Yet we now know that neonatal jaundice and anemia can and do result from maternofetal incompability with respect to the ABO blood groups. When the earlier studies were carried out, the Rh factor was still unknown and there was no way of distinguishing between instances of Rh hemolytic disease and ABO hemolytic disease; the two conditions were pooled together, and the mutual dilution cancelled out or obscured the presence of the association between the ABO blood groups and hemolytic disease.

It is firmly established that strong sensitization of the Rh-negative expectant

mother can cause her Rh-positive fetus to be stillborn, and, in fact, there is a strong correlation between maternal antibody titer and stillbirth rate (Wiener and Wexler 1963). Still, if one were to collect two series of women at random, one Rh positive and the other Rh negative, it would be extremely difficult if not impossible to demonstrate the association between Rh type and stillbirth in this way. The reason is that only a low percentage of Rh-negative women selected at random are sensitized, and among those sensitized most have low titers of antibodies. Therefore, any stillbirths encountered in a study of this nature would almost all be due to causes other than Rh sensitization and would so dilute the relatively few stillbirths actually due to erythroblastosis fetalis as to obscure the association between stillbirths and Rh type.

Similarly, in studies on infectious diseases, one often must deal with more than one strain of the same kind of microorganism, and the various strains may differ antigenically. Thus, some strains of *Escherichia coli* have B-like specificities, and other strains have A-like specificities, while still others have neither (Springer 1966). Therefore, studies on gastroenteritis in babies because of *E. coli* infections will generally include a heterogeneous series of cases so that any possible effect of the A-like and B-like antigens of the microorganisms on immunity could very well be diluted out. However, a greater flaw in the attempts to establish associations between blood groups and infectious diseases is that every microorganism has multiple antigenic specificities (theoretically infinite in number), among which the A-like and B-like specificities are not necessarily the most important (Springer and Wiener 1962). Thus the A-like specificity of a microorganism would not be an absolute bar to its antigenicity for individuals having the blood group substance A in their bodies. Finally, it must be mentioned that in a few cases where A-like specificity has been ascribed to a microorganism, this specificity could be shown to be a property not of the organism itself but of the medium on which it was grown for the purpose of the investigation.

THE FALLACY OF POOLING HETEROGENEOUS DATA

In an attempt to render the statistical significance greater for a claimed association of blood groups with a particular disease, many investigators have pooled together series of cases from various reports in the literature. The justification given for such pooling of data is a "test for heterogeneity," which, it is claimed, failed to show any evidence of heterogeneity among the results reported by different workers. However, there must be a fallacy in the way this test is being used because simple inspection demonstrates that many of the data that are being pooled are actually heterogeneous (Wiener 1962). Table 1 presents one striking example of this kind of fallacy.

In table 1 are compared the results of studies by two different investigators on the distribution of the ABO blood groups among patients with carcinoma of the lungs. In the series from Cremona, patients with carcinoma were reported to have a much lower frequency of group O and a much higher frequency of group A. The differences from the control series are about 14 times the standard error, so that the likelihood that the differences reported are a chance effect are infinitesimal. Thus, if the results were accepted at face value, the presence of an association could not be denied despite the very high a priori probabilities against the existence of such an association. In contrast, the considerably larger series of cases from Vienna shows no difference be-

tween the blood group distributions for the test and control series of cases. For Vienna there is no evidence at all of the existence of any association. The author (McConnell 1966) of the book from which these data are taken merely remarks: "In all, 71 separate series of carcinoma patients and their controls have been published. In no less than 55 of the series a significant increase in the frequency of group A was found. In 14 cases there was little difference from the control and in only 2 series there was a considerable deficiency of A." McConnell then concludes that the results

TABLE 1

ABO BLOOD GROUPS OF PATIENTS WITH CARCINOMA OF THE LUNGS

SOURCE	TOTAL NO. OF INDIVIDUALS	PERCENTAGE OF BLOOD GROUP			
		O	A	B	AB
Vienna, Austria:					
Carcinoma	1,146	36.2	44.1	12.5	7.2
Controls	10,000	36.3	44.2	13.4	8.0
Cremona, Italy:					
Carcinoma	300	10.3±1.7	84.7±2.1	3.3	1.7
Controls	1,762	38.1±1.1	46.9±1.2	10.4	4.7
Difference	.....	27.8±2.0	37.8±2.7	.....	.....

* SOURCE.—Abridged from McConnell (1966, table 25, p. 58).

leave no doubt that an association exists between blood groups and carcinoma of the lung. It is revealing that McConnell thus pools together heterogeneous data and findings, giving equal weight to all reports irrespective of the size of the series and the statistical significance of the differences reported, and failing to take into account the experience and reliability of the investigators, settles the entire matter simply by taking a vote. I prefer to give credence only to the results reported for Vienna (table 1) and find it impossible to believe that a very strong association between blood groups and carcinoma of the lung could exist in one city when there is none at all in another.

OTHER FALLACIES

One of the major flaws in the studies on blood groups and disease has been the presence of bias in the collection and analysis of the data. One of the more obvious sources of bias has been the use as "controls" of a population that differs in ethnic composition from the test series of patients. This gives rise to the error of so-called stratification, which may account, for example, for the seeming association between pernicious anemia and blood group A (McIntyre et al. 1959). In this regard, E. L. Conley (1960, personal communication) states: "The constitutional features which have been associated with pernicious anemia, including the increased frequency of blood group A, are really only the characteristics of the racial group in which pernicious anemia has its highest incidence." Hartmann and Stavem (1964) have suggested that this pitfall could be avoided by using as the control or reference distribution of blood groups the weighted average of the frequencies according to the place of birth of the patients. However, few, if any, investigators have taken the pains to follow this advice.

Another important source of bias is errors in blood grouping, which have occurred much more often than is realized. Graubard's (1954) claim of an association between Dupuytren's contracture and the Rh-Hr blood types, based on his finding that all his patients (more than 28 in succession) were type Rh_1Rh_2, obviously belongs here; other more reliable investigators (R. T. Simmons 1969, personal communication) have failed to confirm this claim. More subtle is the fallacy in the claim that type N persons are more prone to rheumatic fever (Buckwalter and Tweed 1962). As shown in table 2, however, among the 2,186 controls of this study, as many as 55.26% were reported to be type MN, even though the maximum frequency possible for this blood type is 50%. Thus, at least 115 individuals were incorrectly typed as MN among 1,208 tested, so that $\chi^2_{(1)} = 12.5$, alongside which the value of $\chi^2_{(1)} = 7.1$ claimed for the excess of type N in rheumatic fever pales into insignificance. Obviously a study containing so many errors in blood typing is worthless for drawing valid conclusions.

TABLE 2

MN TYPES IN RHEUMATIC FEVER

BLOOD TYPE	CONTROLS		RHEUMATIC FEVER		χ^2
	No.	%	No.	%	
M	587	26.85	128	23.7	1.4
N	391	17.89	128	23.7	7.10
MN	1,208	55.26	284	52.5	0.56
Totals	2,186	.....	540	.....	.....

SOURCE.—Modified from Buckwalter and Tweed (1962, p. 479).

A still more subtle source of bias derives from the fact that almost all the studies on blood groups and disease have been retrospective, in contrast with the studies on erythroblastosis. In other words, the reports have been based on data culled from hospital records. Because of ambiguities in diagnosis and classification of disease, a certain number of arbitrary decisions have to be made, so that unless the blind technique (Wiener 1954, pp. 726–733) is used when selecting the cases, as is never or hardly ever done, bias is almost unavoidable. For example, when Billington (1956*a*) was unable to confirm for his series of cases the claim of an association between ABO blood groups and carcinoma of the stomach, he then subdivided his series of cases according to the location of the lesions and concluded that the association actually did exist but only for lesions in the body of the stomach. This caused me (Wiener 1956) to inquire whether "in assigning the cancer to one or another area the investigator was subconsciously influenced in borderline cases by his prior knowledge of the patient's blood group." Billington (1956*b*) then forthrightly responded: "I admit I am unable to exclude the possibility that the figures in relation to the site of the gastric carcinoma might have been influenced in the borderline cases by my knowledge of the patient's blood groups. . . . An analysis of hospital records by a single interested observer (perforce myself) must always be regarded with suspicion."

Attention must also be called to serious errors in calculations in some of the reports

on blood groups and disease. For example, the claim of Kaklamanis et al. (1964) that their data proved the presence of an association between rheumatic fever and secretion types was shown to be based on a fallacious method of computing the χ^2 values, which, correctly calculated, showed no evidence of association (Wiener and Shapiro 1965).

SUMMARY AND DISCUSSION

Evidence has been presented that the reported claims of associations between blood groups and disease have almost all been fallacious except in the case of erythroblastosis fetalis. Among the errors made in such investigations are the failure to take into account the a priori probabilities and the number of comparisons being made, the use as controls of a series of individuals of different ethnic origin than the patients (i.e., stratification), pooling heterogeneous data, errors in blood grouping, and bias in the selection or classification of cases. "The proof of the pudding is in the eating." Thus, it is revealing that while the discovery of the role of maternofetal blood group incompatibility in the pathogenesis of erythroblastosis fetalis opened up a new field of medical science—immunohematology—the studies on association of blood groups and other diseases are at exactly the same point today as 50 years ago. While routine Rh testing is an essential part of prenatal care, no one has yet suggested the use of blood grouping tests in the differential diagnosis of duodenal ulcer, pernicious anemia, carcinoma of the lungs, or the like, despite the published claims of associations.

An exception to these remarks is the recent discovery of the association between plasma alkaline phosphatase isozymes and the ABO blood groups and secretor types (Arfors et al. 1963; Schreffler 1965; Beckman 1968). Significantly, similar associations of blood types with alkaline phosphatase have been found in cattle. Moreover, in pigs, an association has been established (Andresen 1966) between the red-cell antigen I_b and the serum amylase enzymes, while in sheep there is a close association between M red-cell antigen and serum potassium concentration (Rasmusen and Hall 1966; Kauf and Tosteson 1969). So far no satisfactory explanation for these associations has been demonstrated. They are, however, reminiscent of the negative association in man between the ABH secretor types and the Lewis blood type (Grubb 1948), which has been explained by competition between the *Se* and *Le* genes for a common substrate. A similar plausible explanation for the associations between the blood types and isozymes could be that the products of the blood group genes are somehow involved in the biosynthesis of the isozymes. At any rate, the association between blood groups and isozymes constitutes a new field of research which promises to yield important results once the biochemical basis for the associations has been discovered.

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