THE RELATIONSHIP BETWEEN RH FACTOR INCOMPATIBILITY AND MENTAL DEFICIENCY

By

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A THESIS

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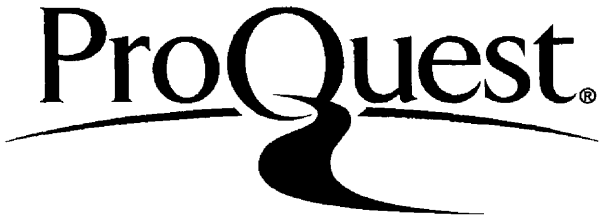

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ABSTRACT

Blood samples from 278 mentally deficient patients at three Michigan state institutions were examined for the ABO and Rh antigens. Samples from the mothers of these patients were also obtained and typed. The patients were divided into three groups, based on the etiology of their condition; namely, "cause known," "cause unknown," and mongoloid. Those patients whose case histories include a diagnosis of Rh factor incompatibility were placed in the "cause unknown" group since the etiology of the mental deficiency in these cases was not clearly established. The "cause unknown" category was designated as the experimental group; the "cause known" category as the control group. Those designated as mongoloids were treated separately since they do not fit clearly into either of the other groups because of the manifold character of their syndrome and the heterogeneous nature of its etiology.

Statistical analyses of the antigen frequency data, the genotype frequency data, and the incidence of simple and multiple mother-child antigen incompatibilities were made using the chi-square test for good-

ness of fit. Each of the three groups studied was compared with the general population to determine whether they may be considered as discreet populations or as a part of the general population. These statistical analyses show a high degree of conformity of the groups studied with the general population. In each case, where the antigen frequency data or the genotype frequency data indicate a difference between the groups studied and the general population, the analysis of the mother-child incompatibility data shows that this difference is not sufficient to permit relating the mental deficiency to the atypical antigen or genotype frequency. The mother-child simple and multiple incompatibility data for all three groups examined fit very closely those expected in the general population.

These results suggest that mother-child incompatibility with regard to any of the antigens examined in this investigation does not play a significant role in the etiology of mental deficiency. Multiple incompatibilities, i. e., mother-child pairs incompatible for more than one antigen of the Rh series and/or the ABO series, were also shown not to be statistically significant as causative agents. While this does not

preclude the possibility that maternal isoimmunization with any of the Rh factors may be responsible for some cases of mental deficiency, the evidence gathered in this study indicates that no general pattern of isoimmunization may be inferred. It appears, therefore, that antigen incompatibility in a mother-child pair does not play a significant role in the etiology of mental deficiency.

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CHAPTER I

INTRODUCTION

The role of blood group incompatibility between mother and child in the etiology of mental deficiency has been the subject of speculation for many years. Even before the discovery of the Rh group of antigens, various hypotheses were advanced regarding ABO group incompatibility as a causative agent of mentally deficient progeny. Hemolytic disease of the newborn (or of the fetus) has been known for many years. Orth in 1875 and later Schmorl in 1904 related hemolytic disease to prenatal damage to the brain. Later workers showed a higher incidence of mental deficiency among children who had suffered severe jaundice at birth than is found in the general population. This severe jaundice indicates affliction with hemolytic disease, which in turn suggests blood group incompatibility in the mother-child pair.

The discovery of the Rh series of antigens was instrumental in explaining the phenomenon of hemolytic disease in ABO compatible mother-child pairs. Furthermore, it is well known that hemolytic disease may, through damage to the brain tissue, cause mental deficiency.

The role of blood group incompatibility, in the absence of any known hemolytic disease, as an etiological agent for mental deficiency, has been the subject of many interesting speculations. This is the problem with which the present investigation is concerned. It is conceivable, that due to mother-child incompatibility, and subsequent isoimmunization of the mother, the fetus is afflicted with a very mild case of hemolytic disease. So mild is this affliction, that none of the external symptoms are observable at birth. However, the reasoning continues, perhaps the undiscovered disease, mild as it was, has already damaged the brain in utero sufficiently to cause the child to be mentally deficient.

There have been several investigations in this area, but there is no universal agreement among the investigators regarding their findings. These are reviewed in Chapter III. In recent years, advances in blood-grouping techniques have been very rapid. Thus it is now possible to examine blood samples of individuals and determine their genotypes with much greater accuracy than ever before.

The subjects for the present investigation are mentally deficient patients at three of Michigan's

state institutions and their parents. The officials at these institutions were eager to have this study done and cooperated in every way to make possible the gathering of the necessary data. The procedure followed in this phase of the investigation is presented in Chapter IV. The data and statistical analyses permit various conclusions to be drawn. The results of the experimentation and statistical analyses appear in Chapter V, while a discussion of these results is presented in Chapter VI. The summary of the investigation is found in Chapter VII.

While this investigation is an attempt to shed further light on the problem of the cause of mental deficiency, it does not purport to be the definitive word on the matter. In the field of blood-group genetics, as, indeed, in every branch of Science, advances are constantly being made by investigators the world over. Future discoveries will, undoubtedly, lead to revision of hypotheses which seem quite tenable today. This investigation was undertaken with these facts in mind and was conducted within these limitations. It is in this spirit that the results are presented.

CHAPTER II

THE HISTORY OF THE RH BLOOD FACTORS

The study, in 1939, by Levine and Stetson, of isoimmunization in man due to blood group antigens other than A or B, was the beginning of a new era in blood group studies. Isoimmunization had been studied earlier, by Dienst in 1905 and by Ottenberg in 1923, but their work was largely ignored. It was in 1939, that the antigen later to be called Rh was first described. When Landsteiner and Wiener in 1940 published their findings of a new agglutinable factor in humans which is identified by use of serums from rabbits and guinea pigs immunized with Rhesus monkey blood, they named the factor "Rh" and did not associate it with the work of Levine and Stetson of the previous year.

At that time, Levine and Stetson (1939) reported that they had found an atypical immune agglutinin in the serum of a woman who had recently given birth to a dead fetus (second pregnancy.) This agglutinin reacted with an antigen in the erythrocytes of the father, but absent in the mother herself. Upon further testing, this agglutinin reacted with the erythrocytes of 83 out of 104 persons of blood group O. The authors suggested

that the antigen was inherited by the fetus from the father, and that the mother had become immunized by trans-placental transfer of fetal red blood cells containing the antigen.

In their original work on the subject, Landsteiner and Wiener (1940) showed that this antibody not only agglutinated Rhesus red blood cells, but also the erythrocytes of 85% of the white population in New York City. The following year, Wiener and Peters (1940) recognized that this anti-Rh antibody was the cause of hemolytic reactions in repeated transfusions of patients with blood which was compatible in the ABO blood groups.

Levine and his associates (1941a, 1941b, 1941c) in a series of papers announced several important discoveries. They showed that the formation of atypical immune agglutinins by a woman during pregnancy frequently caused miscarriage or stillbirth. In the case of infants surviving to full term, one or more of the syndromes grouped together as "erythroblastosis fetalis" was usually present. The authors proposed that the antibody which had been found in the mother's circulation penetrated the placenta and by action on the fetal erythrocytes induced the disease. (It is pref-

erable to use the terms "hemolytic disease of the newborn" or "hemolytic disease of the fetus" rather than "erythroblastosis fetalis" since these terms describe the main phenomenon of the disease. [Mollison, Mourant, and Race (1948)].) Further, they found that over 90% of the mothers in their study were Rh negative (as compared with 15% in the general population) who were married to men who were Rh positive. This led to the discovery that the agglutinins involved in hemolytic disease of the newborn were the same as those involved in the transfusion reaction studies by Wiener and Peters (1940) and the same therefore, as those described by Landsteiner and Wiener (1940) in their original paper.

Landsteiner and Wiener in 1941 published a complete account of their work with the new antigen and its antibody. They tested 448 white persons with guinea pig anti-Rh serum and with human anti-Rh serum with the following results:

Rh positive	379	84.6%
Rh negative	69	15.4%
TOTAL	448	100.0%

The Rh groups of sixty families with 237 children were tested and are given in Table 2-1.

TABLE 2-1

SUMMARY OF FAMILY MATERIAL

(After Landsteiner and Wiener, 1941)

Mating	Number of Families	Number of Children		
		Rh pos.	Rh neg.	Total
Rh pos. x Rh pos.	42	151	7	158
Rh pos. x Rh neg.	12	37	11	48
Rh neg. x Rh neg.	6	0	31	31
TOTAL	60	188	49	237

Assuming that the Rh factor was a simple Mendelian dominant, they calculated the following gene frequencies:

$$rh = \sqrt{0.154} = 0.392$$

$$Rh = 1 - 0.392 = 0.608$$

The genotype frequencies, based on these are:

$$Rh\ Rh = (0.608)^2 = 37.0\%$$

$$Rh\ rh = 2\ (0.608)(0.392) = 47.6\%$$

Rh pos.

$$rh\ rh = (0.392)^2 = 15.4\%$$

Rh neg.

Landsteiner and Wiener (1941) next computed the expected frequency of Rh positive and Rh negative children from each of the three types of matings above and obtained the results shown in Table 2-2.

TABLE 2-2

EXPECTED RESULTS OF RH MATINGS

(Landsteiner and Wiener, 1941)

Mating	Calculated Number of each type	
	Rh positive	Rh negative
Rh pos. x Rh pos.	145.5	12.5
Rh pos. x Rh neg.	34.5	13.5
Rh neg. x Rh neg.	0.0	31.0

From this they concluded that the observed results fitted well with the expected values based on the assumption that a simple Mendelian dominant character was involved. The fit was good enough to give strong support to their hypothesis.

At this point, the presence of the Rh antigen in an individual was believed to be due to a single dominant gene. Those individuals whe were Rh negative were presumed to be homozygous for the recessive allele. The dominant gene was designated Rh and the recessive one rh.

This was, however, an oversimplification. It was earlier recognized that there were differences in the anti-Rh serums (Levine et al, 1941c; and Landsteiner and Wiener, 1941.) Those differences were

thought to be due to antigenic differences. Levine et al (1941c) also found a serum, which they designated anti-Hr, which gave strong reactions on bloods which were Rh negative with one of their antiserums. They recognized that this latter serum was composed of one component of their original anti-Rh serum. Levine (1942) found that the original Rh serum could be split into two components by absorption, and that one of these reacted positively with all the bloods which were negative with the anti-Hr serum. Further mention of the anti-Hr serum was made in the same paper:

> Another blood factor genetically related to Rh was recently demonstrated with the aid of an atypical agglutinin observed in an Rh positive woman, who recently delivered an erythroblastotic infant. This agglutinin acted by preference on bloods which were Rh negative with the 70% anti-Rh serum. This new agglutinable factor, probably allelomorphic with an antigenic component of the Rh factor . . . (Levine, 1942)

It was now clear that the anti-Rh serum was composed of at least two components, and that the "Rh factor" or antigen must likewise be composed of two or more antigens. Further, the allele to one of these was also known. The two components which were identified in the anti-Rh serum are the anti-D and anti-C of the British workers or the anti-Rh_o and anti-rh' of the Americans. The anti-Hr serum of Levine was

allelic to the anti-C (anti-rh'.) Wiener and Landsteiner (1943) demonstrated that these antigenic differences which were defined by the anti-D and anti-C serums were inherited, and postulated the existence of three genes, R_1, R_2, and r. Race and Taylor (1943) at this same time, working with anti-D and anti-c serum had also identified three alleles.

Table 2-3 summarizes the different Rh types recognizable at the end of 1942.

TABLE 2-3

REACTIONS OF RH TYPES WITH THREE ANTISERUMS KNOWN AT THE END OF 1942

Serum	Rh Allelic Type		
	R_1	R_2	r
Anti-D (Anti-Rh_o)	+	+	-
Anti-C (Anti-rh')	+	-	-
Anti-c (Anti-hr')	-	+	+

In 1943, Race et al described a new serum, anti-E (anti-rh") obtained from an Rh positive mother. At the same time, Wiener and Sonn (1943) described a mixed serum - anti-D E, and were later able to separate it into its anti-D and anti-E components. With all the

serums that were now available, Race and his colleagues (1944) were able to define four additional allelic types, for a total of seven. Table 2-4 summarizes these findings, giving the names of each of the seven types. Wiener (1943) using only three serums (anti-D, anti-C, and anti-E) was able to identify six of the alleles, and his work was in complete agreement with that of Race et al (1944) where the two schemes overlapped.

At the end of 1943, Fisher (cited by Race, 1944) presented a theory regarding the nature and inheritance of the Rh blood groups. He assumed, since the reactions of anti-C and anti-c were antithetical, that the genes and antigens which are identified by these two antibodies were alleles. The other two serums which were in use in British laboratories at the time, anti-D and anti-E, were not antithetically related to each other or to any other serum. Fisher proposed that the antigens (and genes) D and E had alleles, which he designated d and e respectively which were not yet discovered. He further suggested that both these antigens would be capable of producing their own antibodies, anti-d and anti-e, under proper circumstances.

TABLE 2-4

SUMMARY OF RH ANTIGENS AND ANTIBODIES AT END OF 1943**

Anti-serum	Rh Allelic Types						
	R_1	R_2	r	R_o	R"	R'	R_z
Race (1944)							
Anti-D (Anti-Rh_o)	+	+	−	+	−	−	?
Anti-C (Anti-rh')	+	−	−	−	−	+	?
Anti-E (Anti-rh")	−	+	−	−	+	−	+
Anti-c (Anti-hr')	−	+	+	+	+	−	−
Wiener (1943)							
Anti-D (Anti-Rh_o)	+	+	−	+	−	−	
Anti-C (Anti-rh')	+	−	−	−	−	+	
Anti-E (Anti-rh")	−	+	−	−	+	−	

** After Race and Sanger - Blood Groups in Man.

At this point, a conflict arose over systems of nomenclature and the mode of inheritance. Fisher (cited by Race, 1944) proposed that each of the Rh antigens (four known, two hypothesized) was produced by a single gene and that these occurred in three allelic pairs, i. e., D-d, C-c, and E-e. Furthermore, he stated that the three genes involved must be very closely linked. Wiener had originally proposed (Wiener and Landsteiner, 1943) that a single gene was involved in the production of these antigens. As more antigens were discovered, Wiener postulated more genes, and frequently changed designations of his earlier genes (Wiener, 1944; Wiener et al, 1944; Wiener, 1946a; Wiener, 1946b; Wiener, 1949.) In 1946, Wiener (Wiener and Sonn) denied the linkage theory of Fisher, and stated further that in accordance with his (Wiener's) views, the two additional factors, d and e, hypothesized by Fisher, could not exist.

With regard to nomenclature of genes and antigens, both are designated by the same letter (as are the allelic pairs) in the Fisher-Race system. The Wiener system has undergone many changes and modifications and is, by far, more complex. Tables 2-5 and 2-6 show a comparison of the two systems.

TABLE 2-5

RH ANTIGENS

Fisher-Race	Wiener	Fisher-Race	Wiener
D	Rh_o	d	Hr_o
C	rh'	c	hr'
E	rh"	e	hr"

TABLE 2-6

RH NOMENCLATURE

Fisher-Race*	Wiener**	Much used notation***
DCe	Rh_1	R_1
DcE	Rh_2	R_2
Dce	Rh_o	R_o
DCE	Rh_1Rh_2	R_z
dce	rh	r
dCe	rh'	R'
dcE	rh"	R"
dCE	rh'rh"	R_y

* Fisher cited by Race (1944)

** Wiener (1949)

*** Race and Sanger (1950) - Blood Groups in Man

Within a few years after its synthesis, the Fisher theory was confirmed by the results of various investigators. The two unknown reactions of the R_z group (see Table 2-4) were observed by Murray et al (1945) to be exactly as predicted by Fisher. Furthermore, Mourant (1945) found the anti-e serum and here again all reactions were as predicted. Additional confirmation came when Diamond (1946) reported the first anti-d serum. Hill and Haberman (1948) and Haberman, Hill, et al (1948) confirmed Diamond's findings and this was the final confirmation of the factors hypothesized by Fisher in 1944, and denied by Wiener in 1946.

This, however, did not end the disagreement. Wiener and his associates still insist that multiple alleles are involved, and have not only attacked Fisher's theory of inheritance, but his system of nomenclature as well. In the words of Race and Sanger in Blood Groups in Man (1950):

> Numerous attacks by Wiener on the theory (Fisher's) have in fact been attacks on the highly academic and interesting point whether the three allelomorphic sites of Fisher are to be placed within or without the boundary of one gene. . . . From these attacks the impression may unfortunately be received that Fisher's great contribution, the recognition of the three allelomorphic antigens, is wrong and has been disproved. The only real disagreement between the two schools, of Fisher and Wiener, is over the academic point referred to, and over the notation.

Regardless of the mode of inheritance, the existence of six antigens is now well established, and each can be detected clearly by means of separate specific typing serums. With regard to nomenclature of antigens, both systems are used in the United States, with many workers in the field favoring the Fisher-Race system.

In addition to the three pairs of well established antigens and genes, a number of sub-groups have been found, due perhaps to gene mutations. The first of these was the C^w antigen reported by Callendar and Race in 1946. Since then several examples of the specific anti-serum, anti-C^w, have been found. Another member of the D-d series has also been found. This, the D^u factor was first reported by Stratton in 1946. Also, two other alleles at the C-c-C^w locus are known. The c^v and C^u antigens were found by Race, Sanger, and Lawler (1948a, 1948b.) At the E-e locus, a third allele is also known; the E^u antigen, which was first reported by Ceppellini et al in 1950.

Many studies have been done to determine the Rh chromosome and gene frequencies in recent years. Fisher and Race in 1946 published the results of their investigations. In 1948, Race, Mourant, Lawler, and Sanger published the most comprehensive study to date,

based on 2,000 blood samples. Tables 2-7, 2-8, and 2-9 are based on their work.

The dCE or R_y chromosome which was not found by Race et al (1948) (see Table 2-9) was assumed to be very rare. Since that time, it has been reported by several workers (Van den Bosch, 1948; Wiener, 1948; and Johnstone, 1950) who agree that its occurrence is indeed rare.

It will be observed that the subgroups mentioned earlier are considered to be variations of their alleles. For example, the C^W antigen, which gives a positive reaction not only with anti-C^W serum, but also with anti-C serum, is considered with the C antigen.

TABLE 2-7

THE GENE FREQUENCIES OF THE RH GENES

C	0.4327500	D	0.5896698	E	0.1554045
c	0.5672500	d	0.4103302	e	0.8445955

TABLE 2-8

THE RH GENOTYPE FREQUENCIES

CC	0.1873	DD	0.3477	EE	0.0242
Cc	0.4909	Dd	0.4839	Ee	0.2625
cc	0.3218	dd	0.1684	ee	0.7133

TABLE 2-9

THE RH CHROMOSOME FREQUENCIES

DCe	R_1	42.04795 %
DcE	R_2	14.10870
Dce	R_o	2.56677
DCE	R_z	0.24356
dce	r	38.86134
dCe	R'	0.98349
dcE	R"	1.18819
dCE	R_y	very low

CHAPTER III

THE HISTORY OF RH INCOMPATIBILITY AND MENTAL DEFICIENCY

Hemolytic disease of the newborn had been known for many years before Levine and Stetson (1939) first suggested that blood groups other than the ABO system could be responsible for immunization of the mother during pregnancy. Levine, Katzin, and Burnham in 1941 reported that from a group of women in whose obstetrical histories there was a high incidence of abortions, miscarriages, and stillbirths, there was also a relatively high incidence of infants afflicted with hemolytic disease. Later in that same year, a statistical study by Levine, Burnham, Katzin, and Vogel (1941) of 153 mothers of erythroblastotic infants gave strong evidence that this disease was due to isoimmunization of the mother by the Rh factor. Since that time many other workers, among them Potter (1943), Race, Taylor, Cappell, and McFarlane (1943), Boorman, Dodd, and Mollison (1944), Plaut, Barrow, and Abbott (1945), and Broman (1944), have confirmed Levine's hypothesis. The serological evidence based on the findings cited above is now used as the final proof in the diagnosis of the disease.

The three main symptoms of hemolytic disease, any or all of which may be present, are (1) edema of the fetus (hydrops fetalis), (2) anemia of the newborn, and (3) icterus gravis neonatorum (jaundice of the newborn.) The disease was usually limited to the neonatal period, during which time the infant either recovered or died. It was reported as early as 1875 by Orth and later by Schmorl (1904) that in many cases of infants with icterus gravis who died shortly after birth, there were discolorations of certain nuclei in the brain. Schmorl described two types of lesions: in one type, small pin point areas of degeneration were noted in the white matter, while in the other type the basal ganglia and medulla were affected. To the latter type, he gave the name "kernicterus." Out of 120 post-mortem examinations in cases of icterus gravis neonatorum, he found six cases of kernicterus. Fitzgerald, Greenfield, and Kuonine (1939) reported a clinical and pathological study of children who had suffered severe jaundice shortly after birth. Although they had recovered from the acute disease, the children still showed signs of various kinds of disorders of the central nervous system and a marked degree of mental retardation. They mention the possibility that

this involvement of the central nervous system might be related to the kernicterus observed in the post-mortem examinations of infants dying from icterus gravis neonatorum.

This mental retardation and involvement of the central nervous system as sequelae of hemolytic disease of the newborn led to the consideration that perhaps various forms of neurological disease and mental deficiency were the result of Rh factor incompatibility. Yannett and Lieberman (1944) were the first to report a statistical study in which a significant increase in Rh negative mothers of mentally deficient children occurred. They tested about 100 mothers and divided them into two groups. Those mothers in whose children a diagnosis of a specific etiological category was made, form the control group, while the remainder, mothers of children in the "undifferentiated" category (i. e., no specific etiological diagnosis having been made), are the experimental group. They found that in the control group, the occurrence of the Rh negative mother-Rh positive child combination was approximately the same as in the population at large. However, in the experimental group, they reported a statistically significant increase in the occurrence of this combination.

Snyder, Schonfeld, and Offerman (1945a) reported findings substantially in agreement with Yannett and Lieberman (1944.) Among individuals (and their mothers) whose cases were diagnosed as a specific type of mental deficiency, the distribution of the Rh factor was normal (i. e., the same as in the general population.) In those cases diagnosed as "undifferentiated mental deficiency" an abnormal distribution of the Rh factor was found. The frequency of the Rh negative mother-Rh positive child combination was greater than would be expected on the basis of chance. In a second report during the same year, Snyder, Schonfeld, and Offerman (1945b) stated findings which essentially agreed with their earlier work. Statistically, however, their second paper reported the deviation of Rh factor distribution from normal in undifferentiated mental deficiency cases to be only "significant" instead of "highly significant" as they reported earlier.

In 1946, another paper by Yannett and Lieberman reported results similar to those obtained in their study in 1944. Working with a different group of patients, they further substantiated their earlier findings.

Docter (1945), in a clinical study of kernicterus,

stated that erythroblastosis fetalis always precedes kernicterus. The degeneration of nerve cells, particularly the basal ganglia, which occurs in kernicterus is traced to icterus gravis neonatorum in every case.

With regard to the etiology of kernicterus, Vaughn (1946) wrote:

> Kernicterus is not yet, however, clearly accounted for in terms of an antigen-antibody reaction. We do not know whether it represents a primary nerve cell injury, with secondary pigmentation and later morphologic change, or whether it is primarily a vascular disease with secondary pigment deposition and nerve cell injury. . . .

Vaughn further restated Docter's findings that kernicterus was one of the consequences of erythroblastosis fetalis. These were some of the considerations which led to the original work of Yannett and Lieberman. In the cases of those erythroblastotic infants who do not die, might there nevertheless be damage to the nervous system (as in kernicterus, milder perhaps) which causes mental deficiency? This seemed well established on the basis of Yannett and Lieberman's work and the later corroboration by Snyder et al. It must be mentioned, however, that Cook in 1944 suggested that perhaps in their original work, Yannett and Lieberman did not have a truly random sample of undifferentiated mentally

deficient individuals.

In 1947, Cappell reported his disagreement with the previous findings. He stated that cases diagnosed as kernicterus or icterus gravis should not be included in the sample. He agreed that these abnormalities were caused by Rh factor incompatibility, but nonetheless insisted that they be removed from the sample. If this were done, the previous work would coincide with his (Cappell's) and there would be no difference in Rh factor frequencies between the general population and one selected on the basis of undifferentiated mental deficiency in the offspring.

Book et al (1949) agreed with Cappell. He stated:

> Obvious cases of nuclear jaundice in combination with mental deficiency should not be classified as undifferentiated mental deficiency of unknown etiology. . . .

Furthermore, he added that if such individuals were excluded from the samples of Yannett and Lieberman (1944, 1946) and Snyder et al (1945a, 1945b), their results would agree with his as well as with Cappell's (1947). Book further criticized the work of Snyder et al (1945a, 1945b) in that they included first-born children in their sample. Hemolytic disease and its sequelae never affect first-born, unless the mother has been previously immunized by transfusion. It would seem, therefore,

that first-born children should be eliminated from the sample unless the mother had been previously transfused, which could have the same effect as an earlier pregnancy. Book concluded that except for cases where it causes hemolytic disease and kernicterus, Rh factor incompatibility plays no important part in the etiology of undifferentiated mental deficiency.

This same view was reported by Gilmour (1950) and by Richards (1951).

Scholl, Wheeler, and Snyder (1947) conducted an immunological study of the mentally deficient patients and their mothers who had been studied in 1945 by Snyder, Schonfeld, and Offerman. They examined blood samples from the mothers in an attempt to establish Rh sensitization. Their results, however, offer no support for the earlier work since only one case of Rh sensitization was found. It should be mentioned, however, that these tests were not performed soon enough following delivery to afford conclusive evidence.

In 1951, Zwerling, Gold, Jervis, and Ginsberg reported the results of the most recent investigation. In all the studies prior to this one, only one antigen of the Rh series, the D antigen, was investigated. In this study, Zwerling et al tested their blood samples

with anti-D, anti-C, anti-E, and anti-c typing serums. They reported the occurrence of incompatibility in each group, and concluded that their results confirm those of Cappell (1947), Gilmour (1950), and Book et al (1949) rather than those of Yannett and Lieberman (1944, 1946) or of Snyder et al (1945a, 1945b). They stated further, that their results should not be regarded as conclusive, since "the data suggest, but do not establish statistically, that isoimmunization with the D antigen may be responsible for a very small number of cases of mental deficiency, even when evidence of hemolytic disease is lacking. . . ."

Thus it seems that while the early investigations into the problem showed a positive correlation between mother-child Rh incompatibility and mental deficiency, this is refuted in the more recent papers, though by no means conclusively. A total of approximately 2,000 defective persons have been examined by the various researchers, showing no overall statistical evidence to support the hypothesis that maternal-fetal Rh factor incompatibility is involved in the etiology of mental deficiency. This sample is statistically too small to afford conclusiveness to these findings. In addition, there is disagreement among the various investigators

as to whether those mentally defective patients whose condition is diagnosed as due to Rh factor incompatibility (i. e., kernicterus as a sequel to hemolytic disease) should properly be included in such a study. It appears that if they are included, there is a significant correlation between mother-child Rh factor incompatibility and mental deficiency, but if they are omitted, there is none. Apparently, the situation requires further investigation before more valid conclusions can be drawn.

CHAPTER IV

OUTLINE OF PROBLEM AND METHOD OF INVESTIGATION

This investigation was undertaken not only to ascertain whether any relationship exists between Rh factor incompatibility and mental deficiency, but if so, which of the Rh factors is involved. The experimental population was comprised of mentally deficient patients at the State Home and Training Schools at Lapeer, Coldwater, and Mount Pleasant, Michigan, and their parents.

At the Lapeer institution, blood samples for this study were taken from 1,000 patients and typed. These patients were selected on the basis of whether the hospital records showed the mother to be available. It would have been desirable to type the mothers first and then type only those patients from whose mothers data had been obtained. However, the hospital authorities requested that the patients be typed first, even at the expense of some efficiency. Since it did ease the administrative burden, this was done. Unfortunately, samples were not available from all the mothers, and so the number of families investigated is somewhat smaller than the number of patients originally typed.

At the Coldwater and Mount Pleasant institutions it was possible to obtain samples and type the mothers first. The parents were contacted through officers of the Mount Pleasant and the Coldwater Parents Associations and samples obtained at chapter meetings in Detroit, Kalamazoo, Grand Rapids, Lansing, and Ann Arbor. Samples from the patients were taken only after a sample from the mother had been obtained.

The patients were divided into three groups: (1) those for whom a diagnosis of a specific etiology, other than Rh incompatibility, existed; (2) those for whom a diagnosis of a specific etiology was lacking, or for whom a diagnosis of Rh incompatibility was made; and (3) those cases diagnosed as mongoloids. Group #1 is the control group; group #2, the experimental group. The cases diagnosed as mongoloids were placed in a third category since this diagnosis does not fit clearly into either of the other groups. In each case, the diagnosis was obtained from the official records at the institution in which the patient resided. Table 4-1, based on the official list of diagnoses of the Michigan Department of Mental Health, shows the group in which each diagnosis was placed.

TABLE 4-1

DIAGNOSES INCLUDED IN EACH CATEGORY

Category	Dept. of Mental Health Code Designator	Diagnosis
1	011	Familial
(Known etiology)	021	Due to epidemic encephalitis
	022	Due to syphilis
	023	Other infections
	031	Natal trauma
	032	Post-natal trauma
	041	Idiopathic epilepsy
	042	Symptomatic epilepsy
	050	With endocrine disorder
	060	With familial amaurosis
	070	With tuberous sclerosis
	080	With other organic nervous diseases
2	013	With developmental cranial anomalies
(Unknown etiology)	014	With congenital spastic paralysis
	091	Undifferentiated

TABLE 4-1 (continued)

DIAGNOSES INCLUDED IN EACH CATEGORY

Category	Dept. of Mental Health Code Designator	Diagnosis
3	012	Mongoloid
Omitted from study	092	Undetermined
	101	Not mentally defective (with psychoses)
	102	Not mentally defective (no psychoses)
	110	Mentally defective with psychoses

Blood samples of patients and mothers were typed and compared. Any factor present in the patient, but absent in the mother was considered to be incompatible. The results obtained in the three groups were compared with each other and with the general population. These results appear in Chapter V.

Samples of three to five ml. of whole blood were taken from the subjects by venipuncture. Each sample was identified by a number and a list of names with corresponding sample numbers was compiled. These samples, usually about 50 at one time, were sent or taken to the laboratory at Michigan State College. Those shipped, were sent via Special Delivery and so were received in the laboratory on the day after they were taken.

At the beginning of the study, a group of samples were taken by auto, immediately after being removed from the subjects, to the laboratory and were typed within a few hours. The results were recorded and an experiment was set up to determine the maximum allowable time between taking the blood sample from the subject and typing in the laboratory. Samples kept at room temperature gave accurate tests for seven days after removal from the subjects. Those which were

refrigerated (5°-8° C) within 24 hours after removal gave accurate tests for 16 days. Samples 17 or more days old gave variable results and were therefore considered unreliable.

Laboratory tests of the blood samples were carried out within two days after removal from the subjects. Samples, when not in use, were kept under refrigeration, and were available for re-testing in case of error or questionable reaction.

For typing, 2% red blood cell suspensions were made in 0.9% saline solution. The cells were washed twice in saline and resuspended. (Washing consists of suspending the cells in saline, centrifuging at approximately 2,000 r. p. m. for two minutes, and decanting the supernatent liquid, while the cells remain packed at the bottom of the tube.) These washed cells were used in all subsequent tests.

TESTS FOR THE ABO ANTIGENS

The procedure for determining the ABO group of an individual was as follows: Two drops of the 2% blood cell suspensions in saline were placed in each of two 10 x 75 mm. tubes. To one, one drop of anti-A serum was added; to the other, one drop of anti-B

serum. The tubes were shaken thoroughly, and allowed to stand at room temperature for one hour. After one-half hour, they were shaken again. At the end of one hour, the results were read, using a hand lens. Readings were made by gently shaking and dislodging the sedimented cells. The presence of clumps of agglutinated cells indicated a positive reaction. Table 4-2 summarizes the reactions of the different groups.

TABLE 4-2

REACTIONS WITH ANTI-A AND ANTI-B SERUMS

Group	Agglutination with	
	Anti-A	Anti-B
O	−	−
A	+	−
B	−	+
AB	+	+

TESTS FOR THE D, C, AND E ANTIGENS

For the Rh groups, the procedure varied depending on the group involved. For the D (Rh_o), C (rh'), and E (rh") antigens, the following procedure was used. Two drops of the 2% blood cell suspensions in saline were placed in each of three 10 x 75 mm. tubes. One drop of typing serum, anti-D, anti-C, and anti-E was added to the respective tubes. The tubes were shaken thoroughly and were then incubated in a water bath at 37.5° C. After half an hour they were again shaken and returned to the water bath. At the end of a second one-half hour period, the tubes were removed and centrifuged for one to two minutes at low speed (1,000 r. p. m.) after which they were read with the aid of a hand lens. A round, smooth, and firm sediment even after gentle agitation indicates the absence of agglutination, and hence a negative reaction. The presence of agglutination was noted by irregular clumps and loose sediment observed after gently agitating the tube. These were read as positive.

The serums used in the tests for the D, C, and E factors contained a pool of saline agglutinins and were designed for use with saline suspended cells. The

use of saline suspended cells is more advantageous than serum or albumin suspended cells since the former give clear-cut specific reactions without confusing effects due to rouleaux or autoagglutination (Levine, 1950.)

TESTS FOR THE c AND e ANTIGENS

The serums available for this study for detection of the c (hr') and e (hr") antigens were of the albumin agglutinin or blocking antibody type, and designed to cause agglutination with specific cells suspended in serum or plasma. The reactions with serum suspended cells are subject to the difficulties mentioned above and so these serums were used on saline suspended cells in conjunction with the Direct Coombs Test (Coombs, Mourant, and Race, 1945a, 1945b, 1946.)

Two drops of the 2% blood cell suspension in saline were mixed with one drop of the typing serum (anti-c or anti-e) containing the albumin agglutinins in a 10 x 75 mm. tube. The tubes were thoroughly shaken and incubated in a water bath at 37.5° C for one hour. They were removed for shaking after 30 minutes but were promptly returned to the water bath for the remainder of the incubation period. After the incubation period, the cells were washed three times with

tubefuls of fresh saline. After each washing the tubes were centrifuged at high speed (3,000 r. p. m.) for two to three minutes, and the supernatent poured off. After the last washing, the supernatent was decanted completely leaving the cells packed at the bottom. Two drops of anti-human serum were added to each tube. The tubes were then shaken and allowed to stand at room temperature for 15 minutes, after which they were centrifuged lightly (500 r. p. m.) for one minute. The packed cells were resuspended by gentle agitation and examined with the aid of a hand lens for agglutination. Those tubes which contained agglutinated red blood cells were read as positive, those which did not were negative.

The agglutinins in the typing serums are albumin agglutinins. As such they cannot agglutinate cells containing the antigens for which they are specific when these cells are in a saline suspension. The action of these agglutinins in saline is to coat the outside of any erythrocytes which contain antigens for which they are specific. In the Direct Coombs Test, these coated cells are agglutinated by the anti-human serum after the excess typing serum has been removed by the saline washings. If there has been no coating of the cells by

the antibodies in the typing serum, these antibodies and the serum are removed by the saline washings and the anti-human serum will cause no agglutination. Hence, a positive reaction in the Direct Coombs Test, i. e., agglutination, indicates the presence of the c or e antigen, whichever is involved in the test.

TESTS FOR THE D^u ANTIGEN

Stratton (1946) and Stratton and Renton (1949) showed that there are variations of the D factor, known as D^u, which are not detected by the usual methods of typing. Neither saline nor albumin agglutinins, of anti-D specificity, will agglutinate D^u cells. However, the anti-D albumin agglutinins do coat these cells, and this is detected by use of the Direct Coombs Test. Therefore, all samples of D negative blood were subjected to the D^u test. Several D positive samples were included in each run to provide positive controls.

Two drops of the 2% suspension of blood cells in saline were placed in each of two 10 x 75 mm. tubes. To one tube, one drop of anti-D typing serum, containing albumin agglutinins, was added, while one drop of bovine albumin was added to the other. The latter tube serves as a control to show autoagglutination and non-

specific coating of the cells. The tubes were shaken and incubated for one hour in a water bath at 37.5°C. They were removed for shaking after one-half hour but returned at once to the incubator. After the incubation period, the cells were washed three times with tubefuls of fresh saline. After each washing, the tubes were centrifuged at high speed (3,000 r. p. m.) for two to three minutes, and the supernatent poured off. After the last washing, the supernatents were completely decanted, leaving the cells packed at the bottom. Two drops of anti-human serum were added to each tube. The tubes were thoroughly shaken and allowed to stand at room temperature for 15 minutes, at the end of which time they were centrifuged lightly (500 r. p. m.) for one minute. The packed cells were resuspended by gentle agitation and read with the aid of a hand lens. Those showing agglutination were positive, the others negative.

If there was no agglutination in either of the tubes containing a given blood sample, this sample is D^u negative. If agglutination appears only in the tube containing the anti-D serum, then the sample is D^u positive. Such an individual is classified as D positive, even though the original test for the D antigen

was negative. If agglutination appears in both tubes, this indicates autoagglutination or non-specific coating of the cells. In this last case, the test should be repeated and if the same results occur, the sample cannot be accurately tested for the D^u antigen. No such samples were found in this investigation.

Table 4-3, which follows, gives a list of the common Rh genotypes, the percentage of their incidence in England, and their reactions with the various typing serums. No such figures are available for the population as a whole in the United States nor for the region from which the sample population for this study was drawn (southern Michigan.)

The mother-patient pairs were classified with regard to the compatibility of the blood grouping antigens, i. e. , a factor present in the patient, but lacking in the mother was considered incompatible. This examination was made for all the antigens in the Rh blood group series as well as for those in the ABO series. The results appear in Chapter V.

TABLE 4-3

INCIDENCE AND REACTIONS OF RH GENOTYPES

Genotype	Incidence*	Agglutination with				
		Anti-D	Anti-C	Anti-E	Anti-c	Anti-e
DCe/dce	32.6808 %	+	+	−	+	+
DCe/DCe	17.6803	+	+	−	−	+
dce/dce	15.1020	−	−	−	+	+
DCe/DcE	11.8648	+	+	+	+	+
DcE/dce	10.9657	+	−	+	+	+
DCe/Dce	2.1586	+	+	−	+	+
Dce/dce	1.9950	+	−	−	+	+
DcE/DcE	1.9906	+	−	+	+	−
DCe/dcE	0.9992	+	+	+	+	+
dcE/dce	0.9235	−	−	+	+	+
DCe/dCe	0.8270	+	+	−	−	+
dCe/dce	0.7644	−	+	−	+	+
DcE/Dce	0.7243	+	−	+	+	+
TOTAL	98.6762 %					

*After Race, Mourant, Lawler, and Sanger (1948)

The incidence of each of the remaining genotypes is exceedingly low, none being greater than 0.34%.

CHAPTER V

RESULTS OF EXPERIMENTATION AND STATISTICAL ANALYSIS

Although patients from three institutions were studied in this investigation, they were by no means equally distributed among the three. Table 5-1 shows the number of patients examined from each of the three institutions.

The patients were placed in groups based on their diagnoses according to Table 4-1. Table 5-2 shows the sex distribution of patients in the three groups. Those patients for whom the etiology of mental deficiency is unknown comprise the experimental group. Those whose diagnoses show an etiology for their condition were placed in the control group. Those cases diagnosed as mongoloids could not clearly be placed in either group and were therefore treated separately.

The ABO blood group distribution is given in Table 5-3. The percentage distribution for each group and for the general population is given in Table 5-4. The figures for the general population are based on the work of Dobson and Ikin (1946) who examined the blood groups of 190,177 people in the United Kingdom. No studies on such large numbers of individuals are available for the population of the United States.

TABLE 5-1

DISTRIBUTION OF PATIENTS FROM INSTITUTIONS

Institution	Number of patients examined
Lapeer	237
Coldwater	35
Mt. Pleasant	6
TOTAL	278

TABLE 5-2

SEX DISTRIBUTION OF PATIENTS IN EACH CLASSIFICATION

Group	Male	Female	Total
Experimental	69	55	124
Control	63	32	95
Mongoloid	31	28	59
TOTAL	163	115	278

TABLE 5-3

DISTRIBUTION OF ABO BLOOD GROUPS
AMONG PATIENTS

Group	Type A	Type B	Type AB	Type O	Total
Experimental	36	22	5	61	124
Control	39	10	3	43	95
Mongoloid	24	7	4	24	59
TOTAL	99	39	12	128	278

TABLE 5-4

PERCENTAGE DISTRIBUTION OF ABO BLOOD GROUPS AMONG PATIENTS AND GENERAL POPULATION

Group	Type A	Type B	Type AB	Type O
Experimental	29.0	17.7	4.0	49.2
Control	41.1	10.5	3.2	45.3
Mongoloid	40.7	11.9	6.8	40.7
General Population*	41.7	8.6	3.0	46.7

A chi-square analysis of the ABO blood group distribution of each of the three groups was performed, with the results shown in Table 5-5.

It will be seen by inspecting Sub-table A of Table 5-5 that the experimental population cannot be considered, on the basis of these data, as a part of the general population. The P value obtained from the chi-square analysis shows that the deviation in the ABO blood group frequencies between the experimental group and the general population is probably not due to chance. (Fisher, 1948.) The high P value obtained for the control group (Sub-table B of Table 5-5) shows that the ABO blood group frequencies of this group conform with those of the general population very closely and further serves to accentuate the divergence of the experimental group. On the basis of the P value obtained for the mongoloid group in Sub-table C of Table 5-5, the difference between it and the general population is probably due to chance. (In the tables which follow, the symbol "X" is used for the Greek letter "Chi." The symbol $|o-e|$ denotes the absolute difference between the observed and the expected values.)

TABLE 5-5

CHI-SQUARE ANALYSIS OF THE ABO BLOOD GROUP FREQUENCIES

Sub-table A Experimental Group

Blood Group	Observed	Expected	$\lvert o-e \rvert$	$(o-e)^2/e$
A	36	51.7	15.7	4.77
B	22	10.7	11.3	11.93
AB	5	3.7	1.3	0.46
O	61	57.9	3.1	0.17
Total	124	124.0		17.33

$X^2=17.33$; D. F.=3; $P<.01$

Sub-table B Control Group

Blood Group	Observed	Expected	$\lvert o-e \rvert$	$(o-e)^2/e$
A	39	39.6	0.6	0.009
B	10	8.2	1.8	0.395
AB	3	2.8	0.2	0.014
O	43	44.4	1.4	0.044
Total	95	95.0		0.462

$X^2=0.462$; D. F.=3; $P=.93$

TABLE 5-5 (continued)

CHI-SQUARE ANALYSIS OF THE ABO BLOOD GROUP FREQUENCIES

Sub-table C Mongoloid Group

Blood Group	Observed	Expected	|o-e|	$(o-e)^2/e$
A	24	24.6	0.6	0.015
B	7	5.1	1.9	0.708
AB	4	1.8	2.2	2.689
O	24	27.5	3.5	0.445
Total	59	59.0		3.857

$X^2=3.857$; D. F.=3; P=.28

To compare the incidence of A and B antigen incompatibilities in the selected populations with those expected in the general population, the work of Dobson and Ikin (1946) was again used. The antigen (and gene) frequencies for the general population are as follows:

A: .25690 = p

B: .06000 = q

O: .68310 = r

Table 5-6 shows the probability of obtaining a given mother-child incompatible pair along with the percentage incidence of these pairs in the general population.

The distribution of ABO group incompatible pairs found in the three groups examined is shown in Table 5-7. A chi-square analysis of the distribution of these data was made with the results shown in Table 5-8.

Inspection of the P values obtained in Table 5-8 shows no significant difference between the experimental group and the general population. The data for the control group, too, fit well those of the general population. The mongoloid group conforms with the general

population except in two cases; the mother B - child A combination and the mother B - child AB combination. These occur more frequently in the mongoloid group than in the general population. While the probability of such an occurrence is indeed small, it may be assumed that their greater frequency here is due to chance.

TABLE 5-6

PROBABILITY OF MOTHER-CHILD ABO GROUP INCOMPATIBLE PAIRS AND INCIDENCE IN GENERAL POPULATION

Mother	Child	Probability	Incidence
O	A	pr^2	11.99 %
O	B	qr^2	2.80
A	B	pqr	1.05
A	AB	pq(p+r)	1.45
B	A	pqr	1.05
B	AB	pq(q+r)	1.15

TABLE 5-7

DISTRIBUTION OF ABO GROUP INCOMPATIBLE MOTHER-CHILD PAIRS

Group	Experimental		Control		Mongoloid	
	No.	%	No.	%	No.	%
Mother O - Child A	13	10.5	10	10.5	5	8.5
Mother O - Child B	5	4.0	5	5.3	1	1.7
Mother A - Child B	4	3.2	1	1.1	0	0.0
Mother A - Child AB	2	1.6	2	2.1	1	1.7
Mother B - Child A	0	0.0	0	0.0	3	5.1
Mother B - Child AB	2	1.6	1	1.1	3	5.1
Total incompatible pairs	26	21.0	19	20.0	13	22.0

TABLE 5-8

CHI-SQUARE ANALYSIS OF ABO INCOMPATIBLE MOTHER-CHILD PAIR DATA

Sub-table A Experimental Group

Incompatibility		Observed	Expected	|o-e|	X^2	P
Mother	Child					
O	A	13	14.9	1.9	0.24	.63
O	B	5	3.5	1.5	0.64	.43
A	B	4	1.3	2.7	5.60	.02
A	AB	2	1.8	0.2	0.02	.88
B	A	0	1.3	1.3	1.3	.25
B	AB	2	1.4	0.6	0.26	.62

Sub-table B Control Group

Incompatibility		Observed	Expected	|o-e|	X^2	P
Mother	Child					
O	A	10	11.4	1.4	0.17	.68
O	B	5	2.7	2.3	1.96	.16
A	B	1	1.0	0.0	0	1.
A	AB	2	1.4	0.6	0.26	.62
B	A	0	1.0	1.0	1.00	.31
B	AB	1	1.1	0.1	0.01	.90

TABLE 5-8 (continued)

CHI-SQUARE ANALYSIS OF ABO INCOMPATIBLE MOTHER-CHILD PAIR DATA

Sub-table C Mongoloid Group

Incompatibility		Observed	Expected	|o-e|	X^2	P
Mother	Child					
O	A	5	7.1	2.1	0.62	.44
O	B	1	1.7	0.7	0.29	.59
A	B	0	0.6	0.6	0.62	.44
A	AB	1	0.9	0.1	0.02	.88
B	A	3	0.6	2.4	9.60	<.01
B	AB	3	0.7	2.3	7.90	<.01

The distribution of the Rh antigens in each of the three groups is shown in Table 5-9. The percentage incidence is also shown in each case as well as percentage frequencies for the antigens in the general population.

The chi-square analysis of the Rh antigen frequency data in Table 5-10 shows no significant difference between any of the groups and the general population.

Table 5-11 shows the distribution of the Rh genotypes in the three groups. The frequency in the general population of each of the genotypes is also given. It will be noted that all of the genotypes are not represented in the table since some of the rarer combinations were not found in this investigation. The combined frequency of these rare genotypes is approximately three per-cent.

TABLE 5-9

DISTRIBUTION OF RH ANTIGENS AMONG PATIENTS AND GENERAL POPULATION

Antigen	Experimental		Control		Mongoloid		General Population*
	No.	%	No.	%	No.	%	
D	101	81.5	77	81.1	40	67.8	83.2
C	78	62.9	59	62.1	31	52.5	67.8
E	39	31.5	23	24.2	18	30.5	28.7
c	110	88.7	91	95.8	54	91.5	81.3
e	119	96.0	91	95.8	59	100.0	97.6

* Percentages only, after Race, Mourant, Lawler, and Sanger (1948)

TABLE 5-10

CHI-SQUARE ANALYSIS OF RH ANTIGEN DISTRIBUTION

Sub-table A Experimental Group

Antigen	Observed	Expected	|o-e|	X^2	P
D	101	103.2	2.2	0.047	.84
C	78	84.1	6.1	0.442	.50
E	39	35.6	3.4	0.325	.59
c	110	100.8	9.2	0.840	.36
e	119	121.0	2.0	0.033	.87

Sub-table B Control Group

Antigen	Observed	Expected	|o-e|	X^2	P
D	77	79.0	2.0	0.051	.83
C	59	64.4	5.4	0.453	.50
E	23	27.3	4.3	0.677	.44
c	91	77.2	13.8	2.467	.11
e	91	92.7	1.7	0.005	.94

TABLE 5-10 (continued)

CHI-SQUARE ANALYSIS OF RH ANTIGEN DISTRIBUTION

Sub-table C Mongoloid Group

Antigen	Observed	Expected	|o-e|	X^2	P
D	40	49.1	9.1	1.687	.20
C	31	40.0	9.0	2.025	.16
E	18	16.9	1.1	0.072	.79
c	54	48.0	6.0	0.750	.40
e	59	57.6	1.4	0.034	.87

TABLE 5-11

DISTRIBUTION OF RH GENOTYPES AMONG PATIENTS AND GENERAL POPULATION

Genotype	Experimental		Control		Mongoloid		General Population*
	No.	%	No.	%	No.	%	
DCe/DCe	13	10.5	4	4.2	5	8.5	17.7
DCe/DcE	17	13.7	10	10.5	8	13.6	11.9
DCe/dce	44	35.5	44	46.3	18	30.5	32.7
DCe/dCe	1	0.8	0	0.0	0	0.0	0.8
DCe/dcE	1	0.8	0	0.0	0	0.0	1.0
DcE/DcE	4	3.2	2	2.1	0	0.0	2.0
DcE/DCE	1	0.8	0	0.0	0	0.0	0.01
DcE/dce	15	12.1	9	9.5	9	15.3	11.0
DcE/dcE	0	0.0	1	1.1	0	0.0	0.3
Dce/dce	6	4.8	6	6.3	0	0.0	2.0
DCE/dce	0	0.0	1	1.1	0	0.0	0.2
dce/dce	20	16.1	18	18.9	18	30.5	15.1
dce/dCe	1	0.8	0	0.0	0	0.0	0.8
dce/dcE	1	0.8	0	0.0	1	1.7	0.9

* Percentages only, after Race, Mourant, Lawler, and Sanger (1948)

Table 5-12 shows the results of the chi-square analysis of the genotype frequency data. In each of the three sub-tables, there is a slight discrepancy between the total number of observed cases and the total number of expected cases. This is due to the fact that certain rare genotypes, comprising approximately three per-cent of the general population were not found in this investigation. Some genotypes of very low incidence were found. However, the complete absence of others causes the total expected number to be approximately three per-cent less than the total observed number.

The data for all three groups conform closely with those for the general population. P values of less than 0.01 occur in a few cases, but these may be attributed to chance.

TABLE 5-12

CHI-SQUARE ANALYSIS OF RH GENOTYPE FREQUENCIES OF PATIENTS

Sub-table A Experimental Group

Genotype	Observed	Expected	|o-e|	X^2	P
DCe/DCe	13	21.9	8.9	3.617	.06
DCe/DcE	17	14.8	2.2	0.327	.58
DCe/dce	44	40.5	3.5	0.302	.60
DCe/dCe	1	1.0	0.0	0.000	1.00
DCe/dcE	1	1.2	0.2	0.033	.87
DcE/DcE	4	2.5	1.5	0.900	.35
DcE/DCE	1	0.01	0.99	98.000	<.01
DcE/dce	15	13.6	1.4	0.144	.70
DcE/dcE	0	0.4	0.4	0.400	.52
Dce/dce	6	2.5	3.5	4.900	.03
DCE/dce	0	0.3	0.3	0.300	.60
dce/dce	20	18.7	1.3	0.090	.77
dce/dCe	1	1.0	0.0	0.000	1.00
dce/dcE	1	1.1	0.1	0.009	.93
Total	124	119.51			

TABLE 5-12 (continued)

CHI-SQUARE ANALYSIS OF RH GENOTYPE FREQUENCIES OF PATIENTS

Sub-table B Control Group

Genotype	Observed	Expected	\o-e\	X^2	P
DCe/DCe	4	16.8	12.8	9.752	<.01
DCe/DcE	10	11.3	1.3	0.150	.70
DCe/dce	44	31.1	12.9	5.350	.02
DCe/dCe	0	0.8	0.8	0.800	.38
DCe/dcE	0	1.0	1.0	1.000	.31
DcE/DcE	2	1.9	0.1	0.005	.94
DcE/DCE	0	0.01	0.01	0.010	.93
DcE/dce	9	10.5	0.5	0.024	.88
DcE/dcE	1	0.3	0.7	1.633	.20
Dce/dce	6	1.9	4.1	8.847	<.01
DCE/dce	1	0.2	0.8	3.200	.08
dce/dce	18	14.3	3.7	0.957	.34
dce/dCe	0	0.8	0.8	0.800	.38
dce/dcE	0	0.9	0.9	0.900	.34
Total	95	91.81			

TABLE 5-12 (continued)

CHI-SQUARE ANALYSIS OF RH GENOTYPE FREQUENCIES OF PATIENTS

Sub-table C Mongoloid Group

Genotype	Observed	Expected	|o-e|	X^2	P
DCe/DCe	5	10.4	5.4	2.804	.10
DCe/DcE	8	7.0	1.0	0.143	.70
DCe/dce	18	19.3	1.3	0.088	.77
DCe/dCe	0	0.5	0.5	0.500	.48
DCe/dcE	0	0.6	0.6	0.600	.45
DcE/DcE	0	1.2	1.2	1.200	.28
DcE/DCE	0	0.0006	0.0006	0.0006	.98
DcE/dce	9	6.5	2.5	0.962	.34
DcE/dcE	0	0.2	0.2	0.200	.66
Dce/dce	0	1.2	1.2	1.200	.28
DCE/dce	0	0.1	0.1	0.100	.75
dce/dce	18	8.9	9.1	9.304	<.01
dce/dCe	0	0.5	0.5	0.500	.48
dce/dcE	1	0.5	0.5	0.500	.48
Total	59	56.9			

The distribution of Rh factor incompatibilities is given in Table 5-13. The table is divided into five sub-tables, one for each of the antigens investigated. The incidence of incompatibility expected in the general population has been computed and included in each sub-table. The computations were based on the gene frequencies for a population assumed to be mating at random. A chi-square analysis of these data was performed, and appears in Table 5-14. The expected values in each case are based on the frequency of incompatibility in the general population.

TABLE 5-13

DISTRIBUTION OF RH ANTIGEN INCOMPATIBILITIES

Sub-table A Mother D-, Child D+

Group	No.	Incompatible	
		No.	%
Experimental	124	7	5.65
Control	95	9	9.47
Mongoloid	59	3	5.08
General Population			9.92

Sub-table B Mother C-, Child C+

Group	No.	Incompatible	
		No.	%
Experimental	124	15	12.10
Control	95	15	15.79
Mongoloid	59	4	6.78
General Population			13.92

TABLE 5-13 (continued)

DISTRIBUTION OF RH ANTIGEN INCOMPATIBILITIES

Sub-table C Mother E-, Child E+

Group	No.	Incompatible	
		No.	%
Experimental	124	12	9.68
Control	95	7	7.37
Mongoloid	59	6	10.17
General Population			11.08

Sub-table D Mother c-, Child c+

Group	No.	Incompatible	
		No.	%
Experimental	124	6	4.84
Control	95	5	5.26
Mongoloid	59	2	3.39
General Population			10.62

TABLE 5-13 (continued)

DISTRIBUTION OF RH ANTIGEN INCOMPATIBILITIES

Sub-table E Mother e-, Child e+

Group	No.	Incompatible	
		No.	%
Experimental	124	6	4.84
Control	95	0	0.00
Mongoloid	59	2	3.39
General Population			2.04

TABLE 5-14

CHI-SQUARE ANALYSIS OF RH ANTIGEN INCOMPATIBILITY DATA

Sub-table A Mother D-, Child D+

Group	Observed	Expected	|o-e|	X^2	P
Experimental	7	12.3	5.3	2.251	.13
Control	9	9.4	0.4	0.017	.89
Mongoloid	3	5.9	2.9	1.425	.23

Sub-table B Mother C-, Child C+

Group	Observed	Expected	|o-e|	X^2	P
Experimental	15	17.3	2.3	0.306	.60
Control	15	13.2	1.8	0.245	.64
Mongoloid	4	8.2	4.2	2.151	.15

Sub-table C Mother E-, Child E+

Group	Observed	Expected	|o-e|	X^2	P
Experimental	12	13.7	1.7	0.211	.66
Control	7	10.5	3.5	1.167	.29
Mongoloid	6	6.5	0.5	0.038	.85

TABLE 5-14 (continued)

CHI-SQUARE ANALYSIS OF RH ANTIGEN INCOMPATIBILITY DATA

Sub-table D Mother c-, Child c+

Group	Observed	Expected	|o-e|	X^2	P
Experimental	6	13.2	7.2	3.927	.05
Control	5	10.1	5.1	2.575	.12
Mongoloid	2	6.2	4.2	2.845	.09

Sub-table E Mother e-, Child e+

Group	Observed	Expected	|o-e|	X^2	P
Experimental	6	2.5	3.5	4.900	.03
Control	0	1.9	1.9	1.900	.17
Mongoloid	2	1.2	0.8	0.533	.47

In addition to single Rh factor incompatibilities, each family studied was examined for multiple incompatibilities. The results of this examination are shown in Table 5-15. Only incompatibilities which were found in this investigation are included in the table. In each the expected percentage incidence in the general population of the incompatibility is included. A chi-square analysis of the multiple Rh factor incompatibility data is given in Table 5-16.

The incidence of ABO blood group incompatibility in addition to one or more Rh factor incompatibilities was computed for the general population. Computations were made only for Rh incompatibilities found in this investigation. Table 5-17 summarizes these. The frequencies of combined ABO and Rh factor incompatibilities were determined for each of the three groups under investigation. These frequencies appear in Table 5-18.

Table 5-19 shows the results of a chi-square analysis of the combined ABO and Rh factor incompatibilities for each of the three groups. A chi-square analysis of the incidence in each group of subjects who did not show combined ABO and Rh factor incompatibilities is given in Table 5-20.

TABLE 5-15

FREQUENCIES OF MULTIPLE RH FACTOR INCOMPATIBILITIES

Incompatible Factors	Group	Number	Percentage
DC	Experimental	1	0.8
	Control	6	6.3
	Mongoloid	2	3.4
	General Population		6.8
DE	Experimental	0	0.0
	Control	2	2.1
	Mongoloid	1	1.7
	General Population		2.3
DCE	Experimental	0	0.0
	Control	1	1.1
	Mongoloid	0	0.0
	General Population		0.036

TABLE 5-15 (continued)

FREQUENCIES OF MULTIPLE RH FACTOR INCOMPATIBILITIES

Incompatible Factors	Group	Number	Percentage
CE	Experimental	0	0.0
	Control	1	1.1
	Mongoloid	0	0.0
	General Population		0.041
Ce	Experimental	1	0.8
	Control	0	0.0
	Mongoloid	2	3.4
	General Population		1.007
cE	Experimental	3	2.4
	Control	2	2.1
	Mongoloid	0	0.0
	General Population		2.8

TABLE 5-16

CHI-SQUARE ANALYSIS OF MULTIPLE RH FACTOR INCOMPATIBILITY DATA

Sub-table A Experimental Group

Factors	Observed	Expected	|o-e|	X^2	P
DC	1	8.4	7.4	6.50	.01
DE	0	2.8	2.8	2.80	.10
DCE	0	0.04	0.04	0.04	.85
CE	0	0.05	0.05	0.05	.83
Ce	1	1.25	0.25	0.05	.83
cE	3	3.5	0.5	0.07	.79

Sub-table B Control Group

Factors	Observed	Expected	|o-e|	X^2	P
DC	6	6.4	0.4	0.03	.88
DE	2	2.2	0.2	0.02	.89
DCE	1	0.03	0.97	31.30	<.01
CE	1	0.04	0.96	23.00	<.01
Ce	0	1.0	1.0	1.00	.31
cE	2	2.7	0.7	0.18	.69

TABLE 5-16 (continued)

CHI-SQUARE ANALYSIS OF MULTIPLE RH FACTOR INCOMPATIBILITY DATA

Sub-table C Mongoloid Group

Factors	Observed	Expected	|o-e|	X^2	P
DC	2	4.0	2.0	1.00	.31
DE	1	1.3	0.3	0.07	.79
DCE	0	0.02	0.02	0.02	.89
CE	0	0.02	0.02	0.02	.89
Ce	2	0.6	1.4	3.27	.08
cE	0	1.7	1.7	1.70	.19

TABLE 5-17

PERCENTAGES OF MULTIPLE RH AND ABO GROUP INCOMPATIBILITIES EXPECTED IN THE GENERAL POPULATION

Incompatible Rh Antigens	Mother O		Mother A	
	Child A	Child B	Child B	Child AB
D	1.19	0.28	0.10	0.14
C	1.67	0.39	0.15	0.20
E	1.33	0.31	0.12	0.16
c	1.27	0.30	0.11	0.15
e	0.24	0.06	0.02	0.03
DC	0.81	0.19	0.07	0.10
DE	0.27	0.06	0.02	0.03
DCE	0.004	0.001	0.0004	0.0005
CE	0.005	0.001	0.0004	0.0006
Ce	0.12	0.03	0.01	0.01
cE	0.34	0.08	0.03	0.04

TABLE 5-17 (continued)

PERCENTAGES OF MULTIPLE RH AND ABO GROUP INCOMPATIBILITIES EXPECTED IN THE GENERAL POPULATION

Incompatible Rh Antigens	Mother B	
	Child A	Child AB
D	0.10	0.11
C	0.15	0.16
E	0.12	0.13
c	0.11	0.12
e	0.02	0.02
DC	0.07	0.08
DE	0.02	0.03
DCE	0.0004	0.0004
CE	0.0004	0.0005
Ce	0.01	0.01
cE	0.03	0.03

TABLE 5-18

FREQUENCY OF MOTHER-CHILD PAIRS SHOWING MULTIPLE RH AND ABO GROUP INCOMPATIBILITIES

Sub-table A Experimental Group

ABO Groups		Rh Groups	No.	%	% in General Population
Mother	Child				
O	A	D	1	0.81	1.19
O	B	C	1	0.81	0.39
O	B	E	1	0.81	0.31
A	B	C	1	0.81	0.15
A	B	c	1	0.81	0.11
B	AB	Ce	1	0.81	0.01

Sub-table B Control Group

ABO Groups		Rh Groups	No.	%	% in General Population
Mother	Child				
O	A	D	1	1.05	1.19
O	A	C	2	2.11	1.67
O	A	DC	1	1.05	0.81
O	B	c	1	1.05	0.30

TABLE 5-18 (continued)

FREQUENCY OF MOTHER-CHILD PAIRS
SHOWING MULTIPLE RH AND ABO
GROUP INCOMPATIBILITIES

Sub-table C Mongoloid Group

ABO Groups		Rh Groups	No.	%	% in General Population
Mother	Child				
B	A	E	1	1.69	0.12
B	A	c	1	1.69	0.11
B	AB	E	2	3.39	0.13

TABLE 5-19

CHI-SQUARE ANALYSIS OF MULTIPLE RH AND ABO GROUP INCOMPATIBILITY DATA

Sub-table A Experimental Group

ABO Groups		Rh Groups	Observed	Expected	|o-e|	X^2	P
Mother	Child						
O	A	D	1	1.5	0.5	0.17	.68
O	B	C	1	0.5	0.5	0.50	.48
O	B	E	1	0.4	0.6	0.90	.35
A	B	C	1	0.2	0.8	3.20	.08
A	B	c	1	0.1	0.9	8.10	<.01
B	AB	Ce	1	0.01	0.99	98.0	<.01

Sub-table B Control Group

ABO Groups		Rh Groups	Observed	Expected	|o-e|	X^2	P
Mother	Child						
O	A	D	1	1.1	0.1	0.01	.92
O	A	C	2	1.6	0.4	0.10	.75
O	A	DC	1	0.8	0.2	0.05	.84
O	B	c	1	0.3	0.7	1.63	.20

TABLE 5-19 (continued)

CHI-SQUARE ANALYSIS OF MULTIPLE RH AND ABO GROUP INCOMPATIBILITY DATA

Sub-table C Mongoloid Group

ABO Groups		Rh Groups	Observed	Expected	|o-e|	X^2	P
Mother	Child						
B	A	E	1	0.07	0.93	12.3	<.01
B	A	c	1	0.06	0.94	14.7	<.01
B	AB	E	2	0.08	1.92	46.1	<.01

TABLE 5-20

CHI-SQUARE ANALYSIS OF INCIDENCE OF SUBJECTS NOT SHOWING MULTIPLE ABO AND RH GROUP INCOMPATIBILITIES

Group	Observed	Expected	|o-e|	X^2	P
Experimental	118	112.5	5.5	0.269	.62
Control	90	86.2	3.8	0.168	.68
Mongoloid	55	53.5	1.5	0.042	.85

CHAPTER VI

DISCUSSION

This study was undertaken in an attempt to determine the role of the Rh factors in the etiology of mental deficiency. In the course of the investigation, data on the ABO groups were also collected and examined. The serological procedure was designed to identify particularly which factor or factors, if any, were involved. The previous work, as reviewed in Chapter III, does not afford any conclusive findings since there are disagreements among the findings of the various investigators.

It appears from the data presented in Chapter V that no clear relationship between blood group incompatibility and mental deficiency exists. The close agreement between the data for the control group and those for the general population indicates that the size of the sample is adequate.

In general, the results here agree with those of Zwerling, Gold, Jervis, and Ginsberg (1951.) The samples were subjected to more tests in this investigation than in the one by Zwerling *et al*, but the overall results are very similar. Book, Grubb, Engleson, and

Larson reported a similar investigation in 1949. While their study involved only one of the Rh factors, the D factor, their results agree with those obtained in this investigation.

The distribution of the ABO blood types in the experimental group is quite different from that expected. An excessive number of Type B individuals was found, whereas the number of Type A patients was considerably below that expected. This suggests that perhaps the ABO types are somehow involved in the etiology of mental deficiency. However, when the mother-child pairs in this group are examined for frequency of incompatibility, the statistical analyses show a good fit of these data with those expected in the general population. This refutes the hypothesis that immunization of the mother to the A or B antigens may have a role in causing mental deficiency. Furthermore, this atypical distribution of the ABO blood types of the experimental group was not found by any of the previous investigators. It would seem, therefore, that this distribution may be due to chance.

The distribution of the Rh antigens, genotypes, and incompatible mother-child pairs among the groups studied conforms closely with that of the general

population. In nearly all cases, the statistical analysis indicates that the selected population of mentally deficient individuals may be considered as a part of the general population. Among the exceptional cases which did not conform with the data for the general population, most are due to genotypes of very low frequency. The order of magnitude of these is such that in a sample ten times the size of the present one, no additional examples of these genotypes would be expected. However, in a few cases, frequencies of genotypes of common occurrence were found to be quite different from the expected values. The DCe/DCe genotype frequency was below that expected in all three groups. In the mongoloid group, the dce/dce genotype was found approximately twice as often as expected.

These deviations from the expected frequencies of the general population would, in themselves, give rise to some speculation regarding the role of the Rh factors in the etiology of mental deficiency. However, when the mother-child pairs are examined for incompatibility, no statistically significant difference from the general population may be found.

When the mother-child pairs are examined for multiple factor incompatibilities (i. e., each pair is

incompatible for more than one antigen of the Rh series and/or the ABO series) as suggested by Cotterman (1951, 1952), no clear pattern is apparent. Limiting the examination to antigens in the Rh series, only six types of multiple factor incompatibilities were found. These included four which although rare, are not uncommon in the general population, i. e., their expected frequencies vary from one to seven cases per hundred. However, the remaining two types are very rare; their expected frequencies in the general population being approximately four cases in ten thousand. Inspection of these data shows that both of these extremely rare incompatibility types are due to the same mother-child pair. The CE incompatible type (see Table 5-15) and the DCE incompatible type which were found are of an overlapping nature. The poor fit of these types is explained by the extreme rarity of the genotype combinations which could cause this incompatibility. The frequencies of the remaining incompatible types are in good agreement with the frequencies expected in the general population.

Further examination of the sample populations for ABO group incompatibilities accompanying Rh factor incompatibilities, yielded generally the same pattern

as had previously been obtained. The data obtained for the experimental group and for the control group fit quite closely the data for the general population. The only exceptions were due to incompatibilities caused by genotype combinations of extremely low frequency. The mongoloid group, however, appears at first not to fit at all with the general population. Closer examination reveals that the incompatibilities found are due to very rare genotype combinations. When the number of subjects in each of the three groups not showing ABO incompatibilities accompanied by Rh incompatibilities is compared with the expected number in the general population there is a high degree of conformity. Thus it appears that the selected populations studied in this investigation do not differ from the general population with respect to multiple factor incompatibilities.

An overall evaluation of all the data obtained in this investigation would be in agreement, essentially, with the work of Zwerling et al (1951), Book et al (1949), Cappell (1947), and Gilmour (1950). Although the last three of these studies were concerned only with the D antigen of the Rh series, no further relationships were discovered by investigating the remaining antigens.

Zwerling et al (1951) investigated some of these, but also found no evidence to justify the conclusion that certain of the Rh antigens, besides the D antigen, are involved in the etiology of mental deficiency. Yannett and Lieberman (1944), and Snyder et al (1945a, 1945b) reported a higher incidence of D factor incompatibility in a selected population of mentally deficient individuals and their mothers than was expected in the general population. The results of this investigation do not confirm their findings.

CHAPTER VII

SUMMARY

Blood samples from 278 mentally deficient patients at three Michigan state institutions were examined for the ABO and Rh antigens. Samples from the mothers of these patients were also obtained and typed. The patients were divided into three groups, based on the etiology of their condition; namely, "cause known," "cause unknown," and mongoloid. Those patients whose case histories include a diagnosis of Rh factor incompatibility were placed in the "cause unknown" group since the etiology of the mental deficiency in these cases was not clearly established. The "cause unknown" category was designated as the experimental group; the "cause known" category as the control group. Those designated as mongoloids were treated separately since they do not fit clearly into either of the other groups because of the manifold character of their syndrome and the heterogeneous nature of its etiology.

Statistical analyses of the antigen frequency data, the genotype frequency data, and the incidence of simple and multiple mother-child antigen incompat-

ibilities were made using the chi-square test for goodness of fit. Each of the three groups studied was compared with the general population to determine whether they may be considered as discreet populations or as a part of the general population. These statistical analyses show a high degree of conformity of the groups studied with the general population. In each case, where the antigen frequency data or the genotype frequency data indicate a difference between the groups studied and the general population, the analysis of the mother-child incompatibility data shows that this difference is not sufficient to permit relating the mental deficiency to the atypical antigen or genotype frequency. The mother-child simple and multiple incompatibility data for all three groups examined fit very closely those expected in the general population.

These results suggest thst mother-child incompatibility with regard to any of the antigens examined in this investigation does not play a significant role in the etiology of mental deficiency. Multiple incompatibilities, i. e., mother-child pairs incompatible for more than one antigen of the Rh series and/or the ABO series, were also shown not to be statistically significant as causative agents. While this does not

preclude the possibility that maternal isoimmunization with any of the Rh factors may be responsible for some cases of mental deficiency, the evidence gathered in this study indicates that no general pattern of isoimmunization may be inferred. It appears, therefore, that antigen incompatibility in a mother-child pair does not play a significant role in the etiology of mental deficiency. It should be stated that the results of this and previous investigations do not exclude further research in this area. While statistically significant samples have been examined, the number of cases investigated is a very small fraction of the total number of individuals afflicted with mental deficiency of unknown etiology. It would seem, therefore, that further research in this area on new samples of mentally deficient patients and their mothers is desirable.

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