ISOHEMAGGLUTINATION AND BLOOD GROUPING. THEIR RELATION TO TRANSFUSION.

REPORT OF 5,135 BLOOD GROUPS IN PORTO RICO

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In 1892 Maragliano(1) stated that human cells were destroyed by alien serum. Ehrlich and Morgenroth(2), as early as 1900 were able to demonstrate isoantibodies (antibodies developed in the serum against cells of the same species), by injecting goats intravenously with blood of other goats. About the same time Shattuck(3) discovered the presence of isoagglutinins in blood serum. Subsequently Landsteiner(4) studied twenty-one persons and divided them into three groups or types in regard to isoagglutination, as follows:

Group A.—(Classified by Jansky(5) and Moss(6) as Group II). The serum of which agglutinates the corpuscles of Group B, but not those of Group C; the corpuscles of the latter being agglutinated by the serum of groups B and C.

Group B.—(Group II under Jansky's(5) and Moss'(6) classifications). The serum of which agglutinates corpuscles of Group A but not those of C; the corpuscles of Group B being agglutinated by the serum of Groups A and C.

Group C.—(Group I, Jansky(5); Group IV, Moss(6)). The serum of this group agglutinates the corpuscles of Groups A and B; the corpuscles of this group not being agglutinated by the serum of any of the persons examined. Landsteiner further found that no reaction took place upon mixing the serum and corpuscles of the same group. Von Decastello and Sturli(7), after examining 155 persons described a fourth group (Group IV, Jansky(5); Group I, Moss(6)), the serum of which did not agglutinate the corpuscles of any other group, but the corpuscles of which were agglutinated by the serum of all the three former groups described by Landsteiner.

In 1907 Jansky(5) described and classified these four groups as follows: Groups I, II, III, and IV. Later, in 1910, Moss(6) made a new arrangement and classification of these four blood groups in which Group I corresponds to Group IV of the Jansky classification, and vice-versa.

The adoption of the above two classifications has often led to confusion and misunderstanding in teaching, and even to a few accidents in blood transfusion, since different classifications are employed in the various laboratories and hospitals. In 1921 a joint committee([8]) representing the American Association of Immunologists, the Society of American Bacteriologists, and the Association of Pathologists and Bacteriologists, unanimously recommended that the Jansky classification be uniformly adopted on a priority basis.

Several years later Landsteiner([9]) presented and recommended for adoption a new classification based on the Landsteiner-Hektoen hypothesis of agglutinins and agglutinogens in the blood. The new Landsteiner classification gives the four groups as AB, A, B, and O. Group O represents the absence of both agglutinable substances A and B in the corpuscles. The Landsteiner-Hektoen theory assumes that two isoagglutinins exist in the serum: A and B. The first is present in Group II and the second in Group III; both being present in Group IV (Moss) and neither in Group I (Moss). Two receptors for these agglutinins, isoagglutinogens A and B are present among the corpuscles of the groups in such a way that an agglutinin and its corresponding receptor are never to be found simultaneously in the same blood. The presence of corresponding agglutinin and agglutinogen in a given mixture of blood results in agglutination of the corpuscles containing the receptors.

Notwithstanding Jansky's accepted priority in blood grouping and the recommendation of the American Committee in 1921 that the Jansky classification be adopted, Moss classification is the one most widely used in France, England, and particularly throughout the United States, where in 1929 Kennedy([10]), after a careful survey of 552 hospitals, arrived at the following conclusions:

1. The recommendation of the committees appointed to consider blood-grouping classification did not result in any considerable increase in the use of the Jansky classification which was the one they supported. (This recommendation rather than bringing order into chaos, has increased chaos.)

2. The marked opposition to the new Landsteiner classification bids fair to make matters worse, and this classification should be abandoned by institutions employing it.

3. Of 552 institutions which answered the questionnaire, about 75 per cent using the Moss classification and 70 per cent, using either the Moss or the Jansky classification, want no compromise in the new Landsteiner classification.

4. From 155 hospitals where blood grouping began after 1931, 81.3 per cent adopted the Moss classification in spite of the committees' advice to the contrary.

5. In view of these facts the Moss classification should be uniformly adopted.

In the light of Kennedy's survey and because of the fact that during his days as a medical student the writer was taught the use of the Moss classification and has ever since thought in terms of such, this classification will be adopted with reference to blood groups throughout this paper.

These isohemagglutinations and blood grouping phenomena are of vital importance in several respects:

1. Ashby[11] made use of these in determining the life span of the transfused erythrocytes. He transfused Group IV blood into Group II and made frequent counts of unagglutinable corpuscles in the recipient's circulation. Unagglutinable corpuscles were found 31 to 100 days after transfusion. It must be considered, however, that this period represents the life span for the transfused erythrocyte and is probably far from that of the red corpuscle in its normal medium.

2. It has been demonstrated by Von Dungern and Hirschfeld[12] that blood groups are inherited, according to the Mendelian law, and Ottenberg[13] has stated that this fixed inheritance of group characters can favorably be applied in forensic medicine in regard to the determination of paternity. The isoagglutination test has also been employed in medico-legal cases to determine the origin of blood stains. The simplest procedure is to test the agglutinin content in the stain by adding red cell suspensions II or III to the material or to an extract of it. A preliminary precipitin test is needed, however, to show that one is dealing with human blood.

3. Isoagglutination and blood grouping have quite a definite relation to anthropological studies. It has been found that the blood of anthropoid apes contains agglutinins and agglutinogens indistinguishable from those of human blood; thus the four blood groups are definitely established in anthropoids. Agglutinogens have also been found in the blood of lower animals but they have been shown not to be identical with human isoagglutinogens.

The above findings confirm the idea of a close biochemical relationship between ape and man, and it is probable that group agglutinogens appeared at a time before anthropoids and man arose from a common stock[9]. It has been found that the rate distribu-

tion of blood types varies in different races, and Hirschfeld and Hirschfeld(14) have developed a racial index which varies distinctly with different races. The racial index is obtained by dividing the percentage of agglutinogen *"b"* (Groups II and I) by that of agglutinogen *"a"* (Groups III and I). Thus, the racial index is a biochemical index.

4. Isohemagglutination and blood grouping have made blood transfusion a reasonably safe procedure. The following compatibility test and precautions should be taken before each transfusion:

(*a*) If possible, transfuse only among individuals belonging to the same blood group. Ottenberg(15) in 1911 advanced the idea that in ordinary transfusion we transfuse corpuscles; that is, the large amount of the recipient's serum dilutes the transfused serum so that usually nothing occurs *in vivo*, even in cases in which the donor's serum agglutinates the recipient's corpuscles *in vitro*. On the other hand, the transfused corpuscles come in contact with the recipient's serum which has been only slightly diluted by the transfused blood. This led to the vogue of using universal donors (Group IV, the corpuscles of which have no receptors and therefore are not agglutinable by serum of any other group), which, in my opinion, constitutes a practice justifiable only in emergencies or when no other suitable donor is at hand. Untoward effects due to isoagglutination frequently follow transfusions from universal donors, especially when large amounts of blood are transfused and the donor's serum has a high agglutinating titer for the recipient's corpuscles. In such cases the dilution of the transfused serum is not high enough to prevent agglutination of some of the recipient's corpuscles.

(*b*) Except in emergencies, always determine groups and proceed to direct matching of donor's and recipient's blood before each transfusion. By direct matching one is able to rule out exceptionally atypical agglutinin. Incompatibility may escape detection. Furthermore, it must be remembered that the group of an individual may change after one or more infusions. In this connection, it is interesting to note the observation of Vorschütz(16) and Harper and Byron(17). Vorschütz presents evidence to show that previous taking of certain drugs, general anaesthesia, or Roentgen irradiation may so modify the blood as to temporarily change it from one group to another. Harper and Byron are of the opinion that lack of sufficient green vegetables in the diet apparently affects agglutination of Group III corpuscles in Group II serum, and reduces the agglutinative power of Group III serum. These observers warn the

laboratory worker to give a diet rich in greens before extracting from known Group III individuals sera to be used for blood grouping.

(*c*) Blood counts and hemoglobin estimation, blood Wassermann and careful search for malaria in the donor's blood, are recommended.

(*d*) Inquiry as to allergic disease or tendency to same in the donor should be made.

By strict adherence to the above points the author has performed over sixty-five transfusions in Porto Rico (including six by the intraperitoneal route, in infants) with regrettable results in only two cases. One of these was a case in which some fifteen days after infusion the recipient developed a severe maculo-papular syphilitic eruption. The donor's blood gave a negative Wassermann reaction three days previous to transfusion and again three weeks after. A careful inquiry into the history, however, revealed a primary syphilitic lesion two years before. The other case[18] developed a positive Wassermann and symptoms of secondary syphilis a few months after several transfusions from donor who had given a negative Wassermann reaction. Since these occurrences, the writer has made it a habit to go into the donor's venereal history very carefully before transfusion.

In spite of all these precautions mild reactions such as chills and fever and various manifestations may follow transfusion. To account for these phenomena various observers have advanced different theories, among which are the following:

(*a*) Effect of sodium citrate when used as an anticoagulant. Drinker and Brittingham[19] have studied this problem thoroughly and find that citrated blood plasma is non-toxic but that the citrated corpuscles are toxic even if thoroughly washed and reinjected into the individual from whom they were taken.

(*b*) Individual variation of leucocytes. Doan[20], after careful investigation, has found a definite incompatibility between the blood and plasma of certain individuals and the white corpuscles of others. A definite classification of individuals on this basis is difficult, since he found at least 27 different group combinations possible. He recommends that the relative compatibility of plasma and W. B. C. be ascertained by direct matching prior to transfusion in cases in which extreme weakness of the patient makes an operation imperative.

(*c*) Extravascular blood changes.

(*d*) Effect of rubber tubing when used. It is a well known fact

that passage of blood through rubber tubing destroys the platelets to a great extent.

(*e*) Serum proteins injected.

(*f*) Difference in the red blood cells not detectable by the usual agglutination tests. In this connection, it will be well to mention the work of Guthrie and Huck([21]). These investigators have found two types of blood which do not conform exactly to any of the known types, and by various absorption experiments have demonstrated the existence of a third agglutinogen and a third agglutinin which they term "*c*" and C, respectively. According to these investigators, Groups II and III must be subdivided into two groups each. The sera of Group II contains agglutinin A and the red cells contain agglutinogens *b* and *bc*, respectively; the red cells of Group III contain the agglutinogen *a*, but the sera of this group contain agglutinins BC and C, respectively.

In the course of eight years of hospital practice the writer has only observed one very severe and untoward reaction following transfusion. This was a case where Type II blood was injected into a Type IV recipient due to an error in grouping and matching in the laboratory. The infusion was being made by the whole-blood syringe method and only 25 cc of blood were injected. In such cases the observer is struck by the suddenness and severity of the symptoms. In this particular instance the patient developed a distressing hacking cough, flushing of the face and severe lumbar and precordial pains as soon as the first syringe-full of blood was injected. The precordial pain, cough and erythema disappeared quite rapidly but the lumbar pain which was later accompanied by hemoglobinuria persisted for three days. All these symptoms were undoubtedly due to the combined effect of isoagglutination and isohemolysis of the transfused corpuscles. The relationship between isoagglutination and isohemolysis is indeed interesting and deserves a few words at this point. With the usual laboratory technic the latter occurs less frequently than the former, but by using large amounts of serum or by storing the red cells for several days it can be made to approach the incidence of the former([22]). Isohemolysis depends on the same constitutional group qualities as isoagglutination, and when it occurs it is liable to mask the latter.

The last part of this paper has been devoted to the presentation and brief comment of the data obtained by the writer on his studies of blood grouping in Porto Rico.

A total of 5,153 blood specimens from persons from thirty towns of the southern part of Porto Rico were grouped; over 75 per cent

of these specimens came from the cities of Ponce, Guayama and Mayagüez. There were eighteen cases of infants ranging from 3 months to 2 years of age, and in over 60 per cent of them no agglutination of either Type II or Type III corpuscles was obtained. Since it is an established fact that in young children isoagglutinogens are often missing, or present in very small amounts, and that the appearance of agglutinogens precedes that of agglutinins, it was decided not to include these specimens in our figures. Blood groups can be determined in the serum only after the first or second year of life. According to Happ([23]), who studied 131 individuals from birth to 10 years of age, the group characters are established first in the corpuscles, though this early acquisition of characters by the corpuscles is subject to change. Once the group characters are acquired by the serum those of the corpuscles undergo no further change.

Ottenberg([13]) gives the source of error in blood typing as:

1. Deteriorated sera.
2. Originally weak sera.
3. Hemolysis.
4. Incubation at 37° C.
5. Drying.
6. Setting of cells.
7. Use of the microscope.
8. Too thick a cell emulsion.
9. Undeveloped group characteristics.
10. Autoagglutination.

In order to obviate these sources of error, the writer used equal parts of unknown inactivated fresh serum and 3 per cent washed Groups II and III corpuscular suspensions in physiological NaCl solution. The macroscopic technic recommended by Ottenberg([13]) was followed and all readings taken 9 to 12 minutes after mixing the corpuscles and serum, letting the mixture stand at room temperature. As previously stated, sera from infants were not included in our data.

The sex and race distribution of the people studied was as follows:

Male	2,349
White *	3,988
Female	2,786
Colored **	1,147

* Under *"white"* are included dark-complexed individuals in some of whom there undoubtedly are traces of Indian or Negro blood, or both.

** The *"colored"* includes all frankly negroid types: mulattoes, negroes, etc. About 50 per cent of this group undoubtedly have traces of white or Indian blood, or both.

Table I shows blood group distribution in 5,135 Porto Ricans by age groups (In decades). Graph I shows curves in which the axis of obscissa represents the age groups in decades, and the axis of ordinate the percentage distribution of the blood groups. It is evident, from Table I and the graph, that blood group distribution does not vary much with age. This fact tends to prove definitely that group characteristics are constitutional in nature and ordinarily remain unchanged through life.

Tables II and III give blood group distribution by sex and race. The study of these tables reveals no difference in group distribution between the two sexes in either the black or the white race, the percentage distribution approaching one another very closely in both instances. However, some difference exists in the group distribution among the white and colored races. Groups IV and I show strikingly close figures for both races, whereas Group III is more common (12.96 per cent white, and 16.11 per cent colored) and Group II is less common among colored than among white. (27.63 per cent colored and 30.31 per cent white).

As stated by Lewis and Henderson[24], in European races Type II is two and a half to four and a half times as common as Type III, while among Asio-African races, including Melagasies, Negroes, Anameses and Hindus, Types II and III become more equal in number, Type III at times exceeding Type II.

In classifying 270 American negroes, Lewis and Henderson[24] found 26.9 per cent of Group II, 18.5 per cent of Group III and a racial index of 1.4. Among 1,147 Porto Rican negroes the writer found 27.6 per cent of Group II and 16.2 per cent of Group III, with a racial index of 1.41. The similarity in these figures is indeed striking. However, among 500 negroes in Senegal, Hirschfeld and Hirschfeld[25] obtained 22.6 per cent of Group II and 29 per cent of Group III, with a racial index of .8 (Table IV). As Lewis and Henderson[24] point out, this change in racial index in negroes in America, which shows a definite tendency to approach the racial index of the white races, is no doubt due to the inter-mixture of white and negro blood. (See footnote under Races in Porto Rico.)

In a study of over 8,000 individuals of the Asio-African races, including Japanese, Chinese, Koreans, Manchus, Hindus, Negroes in Senegal, Madagascans, Sumatrans, Javans and Anamese[26], the following average distribution of types was found: Group IV, 35.6 per cent; Group III, 28.6 per cent; Group II, 26.7 per cent; Group I, 8 per cent; Racial index: .99.

Europeans yield a much higher racial index than the Asio-African

races. In classifying a total of 8,325 individuals to include eleven European nationalities examined by several observers([26]), the following average distribution was found: Group IV, 39.4 per cent; Group III, 12.3 per cent; Group II, 43.5 per cent; Group I, 4.7 per cent; Racial index: 3.1 per cent. In white North Americans a similar distribution and racial index holds true. A so-called intermediate type of racial index has been found in Arabians, Turks, Russians and Spanish Jews([27]). The average index among 2,500 individuals of these nationalities, studied by various observers, yields 1.5.

In classifying 3,899 white Porto Ricans, the writer found 46.8 per cent of Group IV; 12.9 per cent of Group III; 30.3 per cent of Group II; and 9.85 per cent of Group I; and a racial index of 1.74. Racial lines in Porto Rico are sometimes difficult to draw. Slight mixtures often escape detection and people with a little negro blood may pass for white. Traces of Indian blood probably exist in many whites. These facts and what may remain of Moorish and Jewish strains from our Spanish ancestors may account for a lower racial index for the white group in Porto Rico as a whole.

SUMMARY AND CONCLUSIONS

1. The different classifications of blood grouping are herein discussed. The author gives preference to the Moss classification.

2. The importance of isohemagglutination and blood grouping in relation to the following topics, is discussed:

(*a*) Determination of the life span of erythrocytes.
(*b*) Determination of paternity.
(*c*) Anthropological studies.
(*d*) Blood transfusion.

3. The writer gives results of 65 transfusions in Porto Rico and points out the precautions to be taken before each transfusion.

4. Among 3,988 white Porto Ricans, the following group distribution was found: Group I, 9.85 per cent; Group II, 30.3 per cent; Group III, 12.9 per cent; Group IV, 46.8 per cent; Racial index, 1.74. These figures are compared with those obtained by other investigators for European races.

5. Among 1,147 colored Porto Ricans the following group distribution was found: Group I, 9.6 per cent; Group II, 27.6 per cent; Group III, 15.2 per cent; Group IV, 26.5 per cent; Racial index, 1.41. These figures are compared with those obtained from other observers of negroes in the United States and Senegal.

ACKNOWLEDGEMENTS

The writer is indebted to Dr. A. Fernós Isern, Commissioner of Health of Porto Rico, and to Dr. O. Costa Mandry, Director of the Biological Laboratory of the Department of Health of Porto Rico, for review and criticism of the manuscript.

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TABLE I

SHOWING BLOOD-GROUP DISTRIBUTION BY AGE-GROUPS (IN DECADES)

Age Groups	Group I (Moss)	Group II (Moss or Jansky)	Group III (Moss or Jansky)	Group IV (Moss)	Number of People
Under 10 years	8.0	30.0	13.7	48.3	138
10–20 years	9.0	30.0	13.0	48.0	1,357
20–30 years	9.4	30.8	12.7	47.0	1,710
30–40 years	10.4	29.8	15.5	44.1	1,015
40–50 years	10.1	28.4	15.5	46.0	489
Over 50 years	10.6	27.7	14.0	47.7	426

TABLE II

SHOWING GROUP DISTRIBUTION BY SEX AND RACE (SEPARATE SEXES IN EACH RACE)

Classification by sex and race	Group I (Moss)	Group II (Moss or Jansky)	Group III (Moss or Jansky)	Group IV (Moss)	Number of People
White males	10.40	30.50	12.80	46.30	1,836
White females	9.40	30.20	13.10	47.10	2,152
Colored males	8.38	26.20	15.60	49.70	513
Colored females	10.70	28.80	16.50	44.00	634

TABLE III

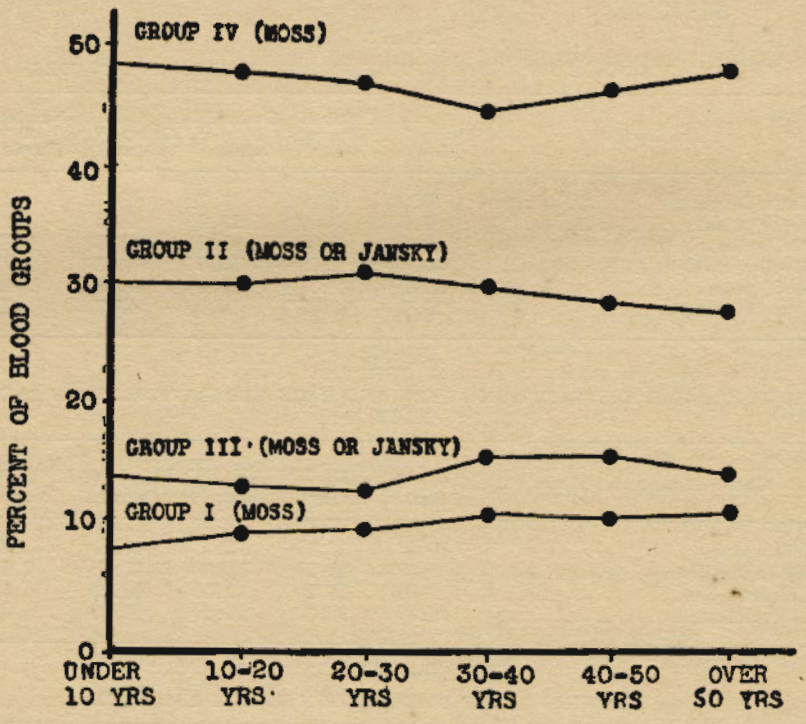
SHOWING GROUP DISTRIBUTION BY SEX AND RACE

Classification by sex and race	Group I (Moss)	Group II (Moss or Jansky)	Group III (Moss or Jansky)	Group IV (Moss)	Number of People
Male	9.8	29.7	13.1	46.8	2,349
Female	9.5	30.0	13.9	46.6	2,786
White	9.85	30.3	12.9	46.8	3,988
Colored	9.6	27.6	16.2	46.5	1,147

TABLE IV

COMPARING GROUP DISTRIBUTION OF NEGROES IN SENEGAL, UNITED STATES AND PORTO RICO

	Group I (Moss)	Group II (Moss or Jansky)	Group III (Moss or Jansky)	Group IV (Moss)	Race Index	Number of People
Negroes in Senegal....	5.0	22.6	29.0	43.2	.8	500
Americanized Negroes..	5.5	26.9	18.5	49.0	1.4	270
Porto Rican Negroes ..	9.6	27.6	16.2	46.5	1.41	1,147



GRAPH I. Showing Group Distribution by Age-Groups (in decades).