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Scope of Report: Facts concerning blood tests, recent findings in this branch of biological science, and their many different applications has not been and is not now common knowledge to the average person. Many teachers of biology and general science suffer a deficit of knowledge in this technical area of science. Since many recent discoveries have been made in blood research, most biology text books that have not been published in the last few years are outdated in this respect. The purpose of this report is to present an overall picture of the background in this area of science, the knowledge available at this time, and the many new applications of the various tests to crime, identity establishment and disputed paternity. Also included in this report is a procedure for typing blood that could be used in a high school biclogy laboratory with a minimum amount of equipment. This report is not intended to be a technical medical report but rather a general overall report that can be easily understood by any person with a reasonable amount of sciontific knowledge.

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A REPORT ON THE BLOOD GROUPING SYSTEMS, NEW BLOOD TESTS, AND THEIR APPLICATION

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INTRODUCTION

Mankind has long marveled at the various functionings of the body. Possibly none are more fascinating than that of the circulation of the blood. In fact it has not been known for more than several centuries that the blood did circulate in the veins of the body.

When man was sure that the body had a complicated circulatory system he began to wonder about the purpose of blood. People until recent times felt that a person could get "bad blood" from time to time and that it was necessary to drain some of it from the body in order to purify the blood. Boils were thought to have been caused by "bad blood".

All this speculation about the purpose, content, and value of the blood to the human body caused man to begin to wonder if it would not be possible to give a human some blood from an animal and thus save his life after an extensive loss of blood. In the 15th century one man in England tried giving another man a transfusion of sheep's blood. Since the man lived over this it was assumed that the transfer was a success. However, on the next transfusion the patient died.

Man learns from mistakes and it was then realized that there was a lot more to this business of passing

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blood from one organism into another than met the eye.

Since that time great advancements have been made in the science of blood transfusion, methods of typing and the handling of blood and blood plasma. Possibly World War II did as much to accelerate the growth of knowledge in technical areas of blood transfusion and blood typing as any other event. This came as a result of dire necessity.

This is a very broad area of science in itself. I shall try in this report to bring out some of the recent developments and findings concerning blood types, blood grouping systems and the application of this information to cases of law, identity establishment, and possible blood donors.

BLOOD GROUPS

The systems of blood grouping are comparatively new in comparison even to the history of medical practice. In fact they are recent enough taht few people know much about them and the scientists are making more and more discoveries and are finding more groups and classifications into which they fit different individual's blood.

The most widely known systems are the International or A, B, O and AB type; the Jansky; the Moss; and the MN. Of course these are just the major systems under which fits many series and different sub-divisions that include the Rh factor, the hemophilic factor, the factor for night blindness, the factor for a scaly skin condition in adult life, and the factor for color blindness (which is sexlinked as are many of them).

Dr. Weiner, the discoverer of the Rh factor estimates that at this time there are 500 divisions if all the finest points of division and grouping are considered.¹ Such a large number of divisions is somewhat complicated but at the same time offers much value in crime detection as will be brought out later in this report. Many new factors are being discovered all the time which to the layman would

1. L. J. Lawler and S. D. Lawler, <u>Human Blood Groups and</u> Inheritance, Harvard University Press, 1957, pp. 58-65.

appear to only put things in a state of confusion. It works on the contrary, however, in that it helps the doctors and clinicians to prevent mistakes that would cost lives.

So much progress has been made in the last few years that it is now a recognized fact that to prevent loss of lives needlessly, blood grouping must be done by laboratory technicians that are skilled in this area rather than internes and less specialized persons.

It is established by now that universally about 85% of the population is Rh positive and the other 15% Rh negative. Many persons become frightened at the mention of Rh but this is because they do not understand what it implies and are not well enough informed. A marriage between persons with different Rh factors does not definitely spell disaster, rather it gives rise to a possibility for future trouble.

Some other minor blood group systems are the Kell system, the Lewis system, the Lutheran system, the Duffy system and the Kidd system. These systems however, are not as universally understood and commonly known as are the other four listed earlier in this chapter.

When Mrs. K. gave a pint of blood for Britian's National Blood Transfusion Service in Sheffield, the doctor and nurses who checked her saw nothing unusual.

But when technicians typed the blood, they did a double take, and with good reason: Mrs. K. was the first human being in medical history with a double set of blood groups. Her cells were 61% type 0 and 39% type A.

Puzzled, the researchers asked Mrs. K. whether she was a twin. Mrs. K. replied that she had had a twin brother who died at birth. That explained it, they figured: in the womb there had been a connection between the arteries of the fraternal twins, and Mrs. K. had picked up some of her brothers blood making cells.²

While the discovery of the various blood groups was made possible by the existence of antibodies to various factors in human and animal sera, by the deliberate immunization of animals, and by isoimmunization resulting from transfusion or pregnancy, the rational interpretation and organization of the data was made possible only on the basis of the firmly established principle of Mendelian genetics. The first suggestion that the blood group of an individual was genetically determined was made by Epstein and Attenberg in 1908 on the basis of studies in two families, but it was not until 1924 that Bernstein formulated the exact manner of inheritance in the A, B, O blood group system.³

- 2. "Double Blood", <u>Time</u>, July 20, 1953, p. 70.
- 3. Elvin Abraham Kabat, <u>Blood Group Substances</u>, Academic Press Inc., 1956, p. 26.

BLOOD GROUPING TECHNIQUE

Today blood transfusion is used extensively in the treatment of the sick and injured. Many countries have set up large organizations to ensure that adequate supplies of blood of the correct type are readily available whenever and whereever they are required. In Great Britain this organization is called the National Blood Transfusion Service.

The actual process of determining the blood groups of a donor involves the use of several relatively simple techniques. These techniques can be quickly learned, but the workers must have a great deal of practice as well as the experience of interpreting the results before their work becomes reliable. Since the techniques used in the various blood group systems are often the same, a general blood grouping laboratory may help in the understanding of the accounts of the individual blood group systems.

Whenever a sample of blood is taken from a new donor it must be "grouped" so that the blood donations can be used with safety. Usually only the A B O and Rh blood groups of the donor are determined. This is because the antibodies reacting with the A and B antigens occur naturally, and therefore incompatibility of the A B O blood groups is always dangerous in transfusion and Rhesus group incompatibility may be so, especially in women.

Antigens other than those of the A B O and Rh systems are not normally important in transfusion and are determined only in special cases. The main investigations of these other groups are carried out by research workers to find, for example, how the blood groups are inherited and distributed to different populations.

To "group" an individual fully it is necessary to have a number of samples of human sera containing antibodies corresponding to the antigens of the various systems. These samples of serum are called antisera, and for some systems, the amount available is extremely small, and therefore the antiserum is very precious. There may be only one small tube of such antiserum available at one time. These rare types contain antibodies formed sometimes by mothers whose blood was different from that of their children or by people who have had incompatible transfusions. Because the amounts of these antisera are sometimes very small, the techniques involved must allow accurate grouping to be done with small volumes.

If a sample of blood, the cells of which contain a certain antigen, is mixed outside the body with a sample of serum containing the corresponding antibody, agglutination results and the cells come together in clumps. These clumps of cells are sometimes big enough to be seen with the naked eye. The reaction is affected by external conditions such as temperature, length of time

following the mixing, and the alkalinity of the solution. Usually the red cells are separated from the plasma and then suspended in a saline solution of the same strength as normal plasma. This solution is made up of nine grams of common salt in a liter of distilled water and is called isotonic saline solution. Any alteration in the salt concentration may cause a distortion, or bursting, of the red cells: isotonic saline solution allows them to keep their natural shape.

The sample of blood to be grouped is received in a small bottle either clotted or, if mixed with an anticoagulant, unclotted. The groups are determined by antigens which are found in association with the red cells, and therefore these are separated from the serum or plasma and used in a saline suspension. This serum is tested to see what antibodies are present.

When preparing a red cell suspension from clotted blood a small piece of clot is broken up with a pair of forceps in a tube containing isotonic saline solution. As the clot is broken up, the separated cells, being microscopic, become suspended in the saline solution, and this suspension is removed with a fine pipette into another tube which is then placed in a centrifuge. This machine, by spinning very rapidly causes the red cells to settle to the bottom of the tube, leaving the supernatant saline above. This saline can now be pipetted

off and replaced by fresh solution. By shaking the tube, the packed cells are resuspended and said to be "washed". The suspension is adjusted to the required strength by judging the color. With experience this can be done very accurately. When citrated blood is used, it must first be spun in the centrifuge, the citrated plasma pipetted off, and then washing and resuspension of the cells in isotonic saline can be done as with the cacerated clot.

The cells containing the antigens are now ready to be tested against the stock antisera, whose antibodies are known. If the cells possess a certain antigen, then they will be agglutinated by serum containing the corresponding antibody, and from the results obtained the correct group of the donor of the cells can be estimated.

The actual test is performed on a white glazed tile or in a small percipitin tube. This tube is rimless and measures 7 mm. in diameter by 50 mm. in length. Transference of cell suspension or serum is done with a Pasteur pipette, which has a long nozzle with a bore of capillary fineness. Suction is provided by a rubber teat and the nozzle is ringed with a grease pencil so that a very small, but equal volume can be measured. After each operation the pipette is washed out in isotonic saline solution.

The tile test is extremely simple; a drop of antiserum is placed on the white glazed surface with the Pasteur pipette, the pipette is washed out, and then a

drop of the test cell suspension is added. The two drops are thoroughly mixed with the corner of the microscope slide and the tile is rocked gently. Any agglutination can usually be seen with the naked eye, but occasionally a hand lens of about 10 X magnification has to be used.

The precipitin tubes are used when only small quantities of sera are available or when the conditions for optimum reaction have to be controlled. A measured volume of the antiserum is pipetted into the bottom of the tube, the pipette is rinsed out and an equal volume of the red cell suspension is added. The two tiny drops are mixed by flicking the tube with a fingernail. Now, normally many bloods are tested against several antisera at the same time. Each reaction requires a separate precipitin tube and each must be identifiable, so a wooden block, containing fifty holes of 12 mm. diameter in five rows of ten holes, is used to hold the tubes. The positions of the tubes are recorded on a work sheet, on which the results are subsequently recorded. Each precipitin tube is covered by a glass The tests are left for the optimum time at the cap. optimum temperature which has been discovered by previous experiment. The maximum reaction between an antigen and a naturally occuring antibody usually takes place at room temperature, about 20 degrees Centigrade, while

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the reactions of immune antibodies are best at body temperature, about 37 degrees Centigrade.

When the reaction time is completed, the cap of the precipitin tube is removed and the drop is carefully pipetted out from the tube and spread evenly and gently on a microscope slide with the end of the pipette. Agglutination may be visible to the naked eye but if it is not, several parts of the drop are inspected under the low-power of a microscope. The result is recorded on the record sheet.

When all the necessary tests have been carried out the blood groups of the individual donor concerned are recorded and the bottle of blood is labelled. The donor is given a card upon which his blood groups are shown. This is not only useful for the people at the Transfusion Center but might also be useful to the donor if he became involved in an accident himself and required a transfusion.

To obtain the antisera used in these experiments the appropriate blood is collected without anticoagulant, left to stand, and then spun in a centrifuge until the clot is packed at the bottom of the tube. The clear straw-colored serum is pipetted off and stored, frozen solid, at -200 Centigrade, in a deep freezing machine similar to that used commercially for ice cream. In this condition it will keep for years.

Each antiserum must contain one particular antibody in sufficient strength to insure a clear agglutinating reaction when it is mixed with cells carrying the appropriate antigen. There must be no other antibody present which might interfere with this reaction. The technique by which pure antisera are prepared is called "adsorption" and by its use an undesirable antibody is removed from a serum containing more than one. Suppose a serum contains a mixture of "anti-X" and "anti-Y" and pure "anti-X" is required, then "anti-Y" can be removed by mixing the serum with red cells which contain the "Y" antigen but which lack the "X" antigen.

The red cells used for such an adsorption are collected in 3.8 per cent sodium citrate solution and then are washed in isotonic solution. They are spun down in a centrifuge and the saline is pipetted off, leaving the packed cells; thus when the serum is added it is not appreciably diluted. The serum and cells are thoroughly mixed and allowed to stand at the optimum temperature. After a suitable length of time the mixture is centrifuged and the serum, now lacking or partly lacking the "anti-Y", is removed. The adsorbed serum is tested to make sure that it no longer agglutinates red cells carrying the "Y" antigen, and also to see that "anti-X" is still present in sufficient strength. To make sure that all the "anti-Y" is removed, this adsorption process may

have to be performed several times--each time, of course, with fresh red cells of the correct type.

The strength of an antibody is measured by the dilution at which it still remains active. Since it is difficult to concentrate an antiserum, it is important that the strength of the antibody should be known. The technique used for this estimation is called titration, and the antibody strength is spoken of as the titer.

The titer is estimated by diluting the serum with saline solution and seeing at which dilution it will still agglutinate cells carrying the corresponding antigen. The small precipitin tubes are placed in rows in a block and a small measured volume of saline is put in every tube except the first. This measure is made accurately by using the pipette marked with a grease pencil. An equal volume of undiluted serum is put in the empty first tube. To the saline solution in the second tube is added an equal volume of undiluted serum and the two volumes are thoroughly mixed by running the fluid up and down the pipette. One volume--that is, half the fluid in the second tube--is drawn and put into the third tube, where the dilution is repeated. Again half this dilution is removed and placed in the fourth tube, and so on until the last tube is reached. When this final dilution has been made, the last volume of fluid is thrown away. Now

the series of tubes contain a serial dilution of strengths 1 in 1, 1 in 2, 1 in 4, 1 in 8, and so on.

One volume of red cell suspension is put in each tube in the series. These cells are chosen because they contain the antigen which reacts with the antibody being tested. The serum dilution and the cell suspension are mixed and kept at the optimum temperature until the reaction has had time to take place.

When the results of the titration are read, a system of scoring is used so that the amount of agglutination can be judged accurately. As the antibody becomes diluted so the reaction becomes weaker until finally, there is no agglutination at all. For an antiserum to be of practical value, the amount of agglutination should be sufficient for the clumps of red cells to be visible to the naked eye when the mixture is looked at on the microscope slide. If this does not happen readily, even when the serum has not been diluted, the serum then is too weak and cannot be used for routine blood typing. It will be realized that, in the case of rare antisera, the more they can be diluted the greater the number of tests which can be done. It is therefore of some importance to know at which dilution such sera can be used with confidence.⁴

A classroom experience in blood grouping can serve

^{4.} Sylvia D. Lawler and L. J. Lawler, <u>Human Blood Groups</u> and <u>Inheritance</u>, Harvard University Press, 1957, pp. 10-18.

several purposes: (1) to allow students to observe blood cells in the suspended and in the clumped state. (2) To learn the blood types of several students and their parents, thereby stimulating interest in the physiology of the human body. (3) To evoke parental interest in school activities. (4) To show that blood type is inherited according to certain well founded principles. This objective can be emphasized in the unit on inheritance. (5) To show that blood transfusions cannot be performed indiscriminately. (6) To teach a lesson in racial tolerance, i. e., that all races of mankind have the same blood types and the same blood chemistry. (7) To suggest to college students the principle that underlies the performance of blood tests in suits involving disputed paternity.

With the realization of these objectives the project becomes a success. Certainly, the objective dealing with racial tolerance should be brought to the attention of the students. As Dunn and Dabzhansky of Columbia University have pointed out, the fact that the four blood groups occur in mankind in the same approximate proportion among all races indicates that human beings regardless of race, color or nationality, have been "drawn from a common pool". All races of men apparently, have sprung from the same common ancestors. Since the blood Chemistry of the several human races seemingly is identical

then, contrary to popular prejudice, "blood" certainly does not determine "race". And a white person who refuses transfused blood from a member of one of the colored races on the ground that his own blood will be "tainted" has no scientific basis whatever for his thinking.⁵

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5. School Science and Mathmetics, November, 1947, p. 724.

THE Rh FACTOR

The Rh factor was so named because it was found first in the red blood corpuscles of the Rhesus monkey. It has no other name. The factor occurs in human blood but not in all persons. There are Rh positive and Rh negative persons. The marriage of an Rh negative woman to an Rh positive man may lead to harmful effects on the blood and health of their offspring. This untoward result is explained as follows: The father being Rh positive, some of his children will be Rh positive and from an Rh positive child in the uterus the Rh factor may pass through the placenta into the blood of the Rh negative The mother in response may produce a new submother. stance which, passing through the placenta into the blood of the child, causes destruction of its red corpuscles, general anemia and jaundice. The danger lies not in the marriage of an Rh positive man and an Rh negative woman, but in the marriage of an Rh positive man and an Rh negative woman. Whether a person is Rh positive or negative can be determined by properly made blood tests, but it is not possible to determine by blood tests that the marriage of a given Rh positive man to a given Rh negative woman is bound inevitably to result in disaster to offspring.⁶

6. "The Rh Blood Factor", Hygeia, June 1944, p. 460.

Attending an expectant mother in Solihull, near Birmingham, the doctors had good reason to believe that, like 85 per cent of humanity, their patient's baby would have blood containing the mysterious factor Rh in positive form. Such infants carried in the womb of a mother whose Rh factor is negative, occasionally develop afatal anemia known as erythroblastosis fetalis. The Solihull mother had already lost three babies for that reason.

This time the doctors removed her baby by Caeserean section three weeks before its time, set to work giving it a complete new supply of blood. With infinite delicacy they inserted a transfusion needle in the baby's tiny vein, while at another spot they drained off the child's own toxic blood. Three weeks later they handed the mother a healthy rosy baby, purring like a well-lubricated car.⁷

A warning of Rh danger in blood transfusions to women and even to little girls who some day may be mothers has been given by doctors of the Johns Hopkins Medical School.

The danger is that Rh negative women and little girls may be sensitized through transfusions with Rh positive blood. Then if they marry Rh positive men their babies will be born with the severest form of erythroblastosis

7. Time, May 28, 1945, p. 70.

and usually will not survive.

Rh negative women who have babies by Rh positive fathers become sensitized by the Rh positive blood of their own babies. But this sensitization proceeds rather slowly and the first and often the second child born under such circumstances will be spared.

Transfusions with Rh positive blood are much more powerful in sensitizing the Rh negative woman than repeated bearing of Rh positive children the Hopkins doctors say. Even a small amount of blood at an early age may be dangerous. As an example of this they cite the case of a 22 year old woman whose first baby was jaundiced five hours after birth because of anti-Rh positive substances in his mother's blood. The anti-Rh substances developed as a result of transfusion of about five ounces of blood when the young mother was herself a two months old baby with dysentery.

The danger can be averted by testing for Rh factor everytime a woman of childbearing age or a female child is given a blood transfusion. A high degree of negligence may be charged if such a test is not carried out. It is necessary in cases of great emergency when there is not time to test the patient's blood, only Rh negative blood should be given.⁸

8. Science News Letter, January 31, 1948, p. 76.

It had been suspected for a long time that the cause of many transfusion reactions was due to specific differences in blood other than the four main blood groups originally described by Landsteiner. Strong evidence of this view was furnished in 1939 when Levine and Stetson offered an explanation for the origin of an atypical agglutinin held to be the cause of a severe reaction in a recently delivered woman at her very first transfusion. The serum of this Group O patient, who had just delivered a macerated fetus, agglutinated the cells of about 80 per cent of Group O individuals. It was suggested that the fetus inherited a dominant agglutinable factor from the father, which was not present in the mother's blood. Isoimmunization could then have resulted from the transplacental passage of minute quantities of fetal red blood cells into the maternal circulation. It was this concept of placental isoimmunization which paved the way for the subsequent findings on the pathogenesis of erythroblastosis. At the same time a new blood factor was described to which no name was assigned.

Landsteiner and Wiener at that time were investigating a factor in the red blood cells of Rhesus monkeys related to, but not identical with, the human M factor. In the course of their subsequent studies of the reactions of human blood with an anti-Rhesus serum produced in rabbits, another factor was differentiated which was

called Rh. As in the case of the M factor, it indicated a property in Rhesus blood related to, but not identical with the factor in human blood. This factor was subsequently found to be identical with the human blood factor previously described by Levine and Stetson.

The Clinical significance of the Rh factor, known more specifically as D or Rho, became apparent as Levine, Wiener, and other investigators working independently demonstrated the antigenicity of this new blood factor. Levine and his co-workers investigated the blood of a number of patients similar to the one described with Stetson. In each case there was a severe or fatal reaction, at the very first transfusion, in a woman who had recently delivered. The obstetrical histories of these women were striking because of the high incidence of fetal and neonatal morbidity. It was suggested that the phenomenon of isoimmunization with fetal blood, responsible for intragroup transfusion reactions, was directly correlated with the fetal and neonatal morbidity. When the histories revealed that these infants suffered from one or another form of erythroblastosis fetalis it was a simple matter to suggest that the intrauterine blood destruction was brought about by the action of the maternal antibodies which found their way into the fetal circulation to react with and destroy the infant's Rh positive blood.

In the vast majority of cases of erythroblastosis fetalis the mother is Rh negative and the father Rh positive. During the latter third of pregnancy there is a thinning of the chorionic villus, and it has been postulated that during this period minute amounts of fetal red blood cells carrying the Rh factor find their way into the maternal circulation. Under this stimulus the mother produces Rh antibodies which readily pass into the infant's circulation with subsequent destruction of the fetal Rh positive blood cells. This in turn brings about the death of the child.⁹

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9. The Rh Factor, Ortho Pharmaceutical Corporation, Raritan, New Jersey, pp. 20-21.

NEW BLOOD TESTS

A new quick method of determining a patient's blood group before transfusion is reported by Dr. William Thalhimer and Dr. Sophronia A. Myron, of the Manhattan Convalescent Serum Laboratory and the New York City Health Department Laboratories.¹⁰

With this method, an intern or technician can determine the patient's blood group in from five to thirty seconds. The test is made on a card or paper which becomes a permanent record that can be attached to the patient's hospital history.

The test resulted from the discovery of the New York doctors that blood group specific isoagglutinins for determining blood types can be greatly concentrated. These isoagglutinins are the substances in blood serum which make red blood cells of another's blood clump together if the two do not have the same type of blood.

It is difficult to get large amounts of group A and B serums suitable for determining blood groups because relatively few people in groups A and B have high concentrations of these isoagglutinins. By using chemical methods for concentrating the agglutinins, a satisfactory preparation is easily obtained for blood

^{10. &}quot;New Quick Method of Testing Blood Group", <u>Science</u> <u>News Letter</u>, February 7, 1942, p. 89.

grouping. This preparation is used in the new, quick and permanent test.

Light on the tragedy of childless marriages and babies dead before they taste the first breath of life is provided by a new British research.

A method of distinguishing incompatible couples who can never hope to have more than one normal healthy baby from others who have a somewhat better chance of having a family is reported by Dr. R. R. Race and G. L. Taylor, of the Galton Laboratory Serum unit, Medical Research council, in the British Journal, <u>Nature</u>.¹¹

In the tragic cases of Rh-caused stillbirth it is really the mother's own blood that kills the child. Although physicians do not understand how, there must be a little mingling of the blood of the unborn child with that of the mother.

When the baby has inherited from the father Rh positive blood and the mother has Rh negative blood, then the blood of the two cannot mingle safely. If the child is the first baby and the mother has not previously had a blood transfusion with the Rh positive blood, then the baby may come into the world alive although he is likely to have a bad case of jaundice.

By the time the second baby comes along, however,

^{11. &}quot;New Blood Test," <u>Science News Letter</u>, November 27, 1943, p. 338.

the mother has built up in her blood a resistance to the Rh positive factor. Now a mixing of the Rh positive blood with hers will probably mean death to the infant. Before the cause became known to physicians, this tragedy often developed into a double one. Since the childbirth in such cases was usually very difficult, the mother was often given a transfusion and her husband was naturally the one most likely to serve as donor. Typing of his blood might indicate that it was suitable, for until recently the Rh positive and Rh negative factors were not known and hence not looked for.

But the mother, already weakened by the battle against the lethal factor in her baby's blood could not stand another dose of it by transfusion. She would die, killed by the blood of her loved ones.

On the average, in the United States, thirteen out of every one hundred marriages are between a father with Rh positive blood and a mother who has Rh negative blood. Some of these marriages are doomed to childlessness or a series of tragedies. But others, although likely to suffer from some such incidents, still have a fair chance of producing healthy children. When the father with Rh blood is the child of parents both of whom had Rh positive blood, then the marriage has a poor chance of being blessed with more than one child. If, however, the father with Rh positive blood has

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inherited the Rh negative gene, there is a 50-50 chance that any child of his will be free from the Rh positive factor so that this will not be a barrier to safe birth.

Discovery of a new blood factor called "U" because of its almost universal distribution, has been announced by doctors at the University Hospital, New York City.

The new factor was discovered after a negro woman taken to the hospital with a bleeding ulcer, went into shock and died from reaction to blood given her by transfusion. A previous transfusion given her had to be stopped because of chills and fever. Both donors, however, had belonged to the same blood group, B, as the patient.¹²

After her death her blood was again examined. Tests showed that her blood contained an abnormal antibody that strongly clumped the cells of the two donors. Tests of 425 negroes and 690 whites showed the U factor present in all but four of the negroes.

Scientists say that the U factor is not related to the A B O, M N, Rh Hr, or K k systems, or to any other blood factor discovered to date.

Blood grouping is now a specialized science that only well trained persons can perform. Scientists feel

^{12. &}quot;New Blood Factor U Widely Distributed," <u>Science</u> <u>News Letter</u>, December 26, 1953, p. 406.

therefore that blood grouping should not be done by inexperienced interns but rather by an adequate blood grouping department of a reliable hospital or blood grouping laboratory.

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VARIOUS APPLICATIONS OF BLOOD TEST FINDINGS

Since one of the main objects of blood-group anthropology is to determine the history of past movements and mixings of mankind, it would be clearly a great advantage if we could ascertain the blood groups of dead bodies and skeletons by means of serological tests.

The A B O blood group substances are known to be present in most of the tissues and organs of the body, and are still present in the stroma after the lysis of red cells. They are known to be fairly resistant to chemical reagents and to survive for long periods in dried blood stains. In old bones there will be considerable quantities of dried blood in the marrow, apart from any blood group substances which may be present in the other organic components of the bone.

Considerations such as these have led various workers to perform tests for blood-group activity on tissue samples from corpses of archaeological and anthropological interest. The earliest experiments were those of Boyd which set a very high standard.¹³ These authors first showed that dried muscle tissue from human bodies of known blood groups could be used with accuracy to determine those blood groups. They then proceeded to determine those blood groups of tissues taken from both American and Egyptian mummies.

^{13.} A. E. Mourant, The Distribution of Human Blood Groups, Charles C. Thomas, August 1954, p. 152.

The technique depended upon the absorption from the testing fluid of the antibody or antibodies corresponding to the blood group of the tissue. The supernatant liquid was then tested for its remaining antibody content by agglutination tests with red cells of appropriate groups. The possibility of non-specific absorption by substances other than blood group antigens was considered, and in order to minimize this, tests were done both with natural human and immune rabbit agglutinating sera.

In the course of time these workers have reported on the apparent blood groups, as determined with muscle tissue of 300 mummies, American and Egyptian. Nearly every single specimen gave a clearcut result and both A and B groups were diagnosed for different specimens, indicating that distinct A and B substances had been detected. Some specimens absorbed neither antibody and were recorded as group 0, while others absorbed both and were regarded as AB.¹⁴

There is in Great Britian no specialist laboratory devoted to blood group work applied to maternity cases. In 1938 Lord Merthyr introduced his Bastardy Bill into the house of Lords. Early in 1939 it reached its second reading, and was sent to a Select Committee. The committee made some amendments to the bill which they then recommended should be "passed into law". Then the war

14. Mourant, op. cit., p. 153.

began and the bill lapsed. The proposed law would have made it possible for the court, or either party, to demand blood tests. The tests were to be done in Wales. The law would not have affected Scotland or Northern Ireland.

The proposal in the bill that only registered medical practitioners should be allowed to do the tests would exclude a number of workers of world wide reputation. The proposal dates from the time when blood grouping was a small branch of clinical pathology, a time which has now passed. Laws have since been established regarding such cases of paternity in most countries of the world.¹⁵

Dr. Alexander Wiener is an alert detective that uses his knowledge about heredity and blood groups to help solve many crimes of murder in the New York City area. He is the one who discovered the Rh factor of blood and as a result of his discovery many lives have been saved. Dr. Wiener needs less information and clues to solve a crime than does the ordinary detective.

Killers on several occasions have attempted to throw detectives off the trail by covering human blood with animal blood. The following is an actual account given by Dr. Wiener: A man's body was found near a

^{15.} Robert Russel Race, <u>Blood Groups in Man</u>, Blackwell Scientific Publications, 1950, p. 276.

tenement in Harlem and detectives suspected a woman who lived in the house. There was a large blood-stained area on her floor but she said she had killed a rat, and there was a dead rat in the garbage to back up her story. When Dr. Wiener finished his tests he agreed with the woman on part of her story. There was rat blood as she said but, there also was human blood underneath, and of the same grouping as that of the murdered man found on the street. The woman confessed to the crime.¹⁶

Another application of the knowledge of blood groups and inheritance was in the establishment of switched babies in a hospital. Parents in Europe left a hospital with twin boys. As the boys grew it was soon apparent that they were not identical twins. One day while visiting in another city the father of the twins noticed a child the same size as one of his sons and identical in appearance. Upon examination it was learned that the three boys were born the same day in the same hospital. Of course, the hospital would not admit their mistake at first, but blood typing established there was a mix up and the children were exchanged to their rightful parents.

Habitual criminals will stop at nothing to prevent detection and identification that will convict them.

16. "Invisible Clues That Trap Killers," <u>Science Digest</u>, September, 1950, pp. 4-6.

The following account shows how a criminal was identified and convicted on the knowledge and information of blood groups that was available.

Blood-grouping tests, had they been legal in Texas, might have shortened the time it took to prove that the tall, blond man who had given his name as Robert Pitts, but had no fingerprints on either hand, was Roscoe J. Pitts, an ex-convict wanted in North Carolina for burglary.

A painful operation, involving cutting the flesh from fingers on both hands, fastening each of those fingers to the man's body until they grew fast, and then cutting them away with some of the flesh from the torso adhering came to naught, however, because a man with no fingerprints is an impossibility, and their very lack was an accusation.¹?

A warning of occasional danger from using the blood of a universal donor is given by two Edmonton, Alberta doctors.

A universal donor is one who has group 0 blood. This usually may be safely given a patient of the same or another blood group. However, if the universal donor has had "shots" against typhoid, tetanus or some of the other diseases for which preventive vaccines are given,

^{17. &}quot;Blood Helps Trap Criminals," Science Digest, July, 1943, pp. 70-72.

his blood may be altered slightly so that it will not be compatible with all other blood groups.¹⁸

The Edmonton doctors discovered this when one of their patients died after a transfusion reaction and subsequent kidney failure. The patient had group A blood. During an operation for cancer she was given one pint of group A blood. Because no more A blood was immediately available and she was bleeding severly, she was given two pints of 0 blood from the blood bank.

When she failed to rally, further tests were made matching the bank blood with some of hers taken before the operation. One of the O bloods was compatible the other was not. This last, it was found came from a donor who four months previously had had inoculations against typhoid and paratyphoid fevers.

As early as 1910 Van Dungern pointed out the possible value of blood grouping to forensic medicine in cases of disputed paternity. It was not until 1934 that this suggestion was adopted, when the procedure was introduced into the German courts. The countries in which blood grouping has been applied medicolegally are: Denmark, Norway, Lithuania, Czechoslovakia, Holland, Sweden, Japan, Russia, Italy, Belgium and Ireland. In the United States, the tests have only rarely been used

^{18. &}quot;Universal Blood Donor May Be Dangerous," <u>Science</u> News Letter, November 21, 1953, p. 329.

until recent time.

Recently, however, the reaction of the legal profession to the blood grouping tests has become more favorable. In January, 1934, the tests were ordered for the first time by a higher court in this country. It was a case pending before the Supreme Court Justice Steinbrink of the State of New York. The adoption of the tests as a routine procedure in all cases of disputed paternity in the United States, is to be expected.

SUMMARY

The idea that necessity is the mother of invention holds in the scientific field as much if not more than in other fields of endeavor. Such has been the case in the establishment of blood grouping systems, tests for rapidly determining blood types, and the application of this array of knowledge to investigations of various types.

The development of blood grouping systems and even the realization for a need of grouping individuals has come about only yesterday in comparison to the many years that scientific investigation has been carried on.

Ever since man learned that life could be saved by blood transfusion, more and more research has been done in blood types and methods for quickly establishing the type or group to which an individual belongs.

This report certainly does not include all that is known at this time in the science of blood typing since the purpose of this report was not to give an extremely detailed scientific account but rather to present in a moderately scientific fashion the background of the beginning of this branch of science and how it has grown and become of paramount importance in our technical world of today. In recent years many applications have been made involving the knowledge of past research and investigation of the blood and the many secrets it holds.

We have come from a time when it would have been considered witch-craft to suggest such a thing as a blood transfusion to a time when it is considered a normal practice being carried out with great care and skill.

As long as there are individuals working in the fields of science that have a true interest in humanity and improving their well-being, the human race will indeed benefit.

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