which both anti-M and anti-Rh were produced by an incompatible transfusion, and another discovered by Stratton (personal communication), with both antibodies present in pregnancy, do not negative this hypothesis.

Secondly, clinical and serological manifestations of haemolytic disease of the newborn were entirely absent in the case of Mrs. A, in whom the antibody was known to have increased in titre during the pregnancy and to have become active against the cells of the foetus and its father at body temperature. Similar findings with an immune iso-agglutinin are almost invariably followed by affection of the newborn.

Finally, a technique of differentiating immune and naturally occurring antibodies was applied to these sera. This technique, described by Boorman and Dodd (1947), is the comparison of the titre of the agglutinin in saline with the titre of the agglutinin when human serum, lacking the antigen to the antibody under examination, is used as diluent in place of saline. Boorman and Dodd found enhancement of titre in serum in which immune antibodies were present, whereas naturally occurring antibodies showed no enhancement. In the cases of Mrs. A and Mrs. B there was no evidence of titre enhancement, further confirming the theory that these anti-M agglutinins were of natural occurrence.

Reason must be given why in the case of Mrs. A the titre of the antibody rose during the intrauterine life of the foetus if the antibody was of natural occurrence. This phenomenon has been reported by Jonsson (1936) and others in the case of naturally occurring anti-A and anti-B, and not infrequently observed by one of us (I.D.). Similar observations have been made in Rh-negative women who, having produced Rh antibodies in previous pregnancies, reproduce these antibodies in increasing titre in a subsequent pregnancy, even though carrying a normal Rh-negative foetus. This can be explained by the supposition that there has been a non-specific stimulation of already existing antibodies during the course of pregnancy.

In the case of Mrs. A an explanation of the alteration of the antibody activity so that agglutination of the infant's and of the father's cells occurred at 37° C. is found in the work of Kettel (1930), who proved that the thermal amplitude of a cold agglutinin—e.g., anti-M—is directly proportional to its titre. The titre of 1:128 of Mrs. A's anti-M is the first reported to have reached sufficient strength to react at body temperature.

Tovey (1944) has suggested that anti-M or anti-N agglutinins will appear only rarely in the serum of a mother who is carrying a baby of dissimilar M or N type. This he suggests is due to the pronounced non-specific absorptive capacity of human tissues for these iso-agglutinins. This may explain why Mrs. A's anti-M, though active at 37° C., did not cause haemolytic disease of the newborn.

Summary

Two cases in which the agglutinin anti-M was associated with pregnancy are reported, and the literature on the subject is reviewed.

Reasons are suggested for considering these anti-M agglutinins to be naturally occurring antibodies.

The relation of anti-M to the aetiology of haemolytic disease of the newborn is discussed.

We wish to thank Mr. J. E. Stacey and Mr. L. B. Patrick for permission to quote their cases whilst one of us (C.C.B.) was at the Jessop Hospital for Women, Sheffield; also Dr. E. F. Aubert (late regional transfusion officer) and his successor, Lieutenant-Colonel R. H. Malone, for their valuable advice and permission to publish this note. Dr. A. E. Mourant, of the Ministry of Health Blood

Group Reference Laboratory, was kind enough to confirm the findings of Mrs. A's serum.

Note.—An anti-M with the same thermal amplitude as Mrs. A's has been reported to us by Dr. Tovey, of the Regional Blood Transfusion Laboratory, Bristol, and one of us (I.D.) has recently found another anti-M associated with pregnancy, in which case the child, though M-positive, was clinically normal.

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THE RHESUS FACTOR D^u

A NOTE ON ITS PRACTICAL IMPORTANCE

BY

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Rh testing is being undertaken in many laboratories either as a test in antenatal cases or for selecting Rh-negative blood donors. Fisher has suggested that the reactions of the various Rh types should be explained in terms of three pairs of allelic genes, C-c, D-d, and E-e, controlling the presence of the corresponding antigens.

Additional alleles, C^w and D^u, have been detected at the C-c and D-d loci, and it is with D^u at the D-d locus that this paper deals. This theory is fully explained by Mollison, Mourant, and Race (1948).

The D antigen is the most important of the Rh antigens, and a person is now referred to as being "D-positive" or "D-negative" according to whether his erythrocytes do or do not possess the D antigen. In the Manchester area there are about 18% of D-negative and about 82% of D-positive persons, so that, if the rarer subgroups are excluded from our consideration for the moment, it will be seen that this classification roughly corresponds to "Rh-positive" and "Rh-negative."

The D^u Group

When blood cells were being typed for Rh it was noticed that some anti-Rh (anti-D) testing sera reacted with certain blood samples positively, whereas others, apparently equally good testing sera, failed to react with these cell samples. Further examination of these cells revealed that they reacted with up to 45% of anti-D sera only; these cell samples may of course also react positively, or negatively, with anti-C and anti-E sera in the usual way. This special type of D antigen was tentatively called D^u (Stratton, 1946); subsequently cells of types CD^ue/cde, cD^ue/cde, and cD^uE/cde were discovered and recognized (Race, Sanger, and Lawler, 1948a, 1949b; Stratton and Renton, 1948).

It was later found that some types of D^u cell were so weak in their reactions that they would give saline agglutination with no anti-D typing sera, or in some cases with only a very few. Nevertheless, it was found that these cells would

give a positive Coombs reaction when tested with certain strong incomplete anti-D sera, but they failed to give a positive albumin reaction in the same circumstances.

It should not be imagined that the difference between D^u and D is one of strength of reaction only; we have conclusively shown that there is a qualitative difference between D and D^u and between one D^u blood and another. Moreover, we were able to show that D^u is an inherited allele or modification of D.

It will be observed that the anti-D sera which react with D^u are really anti-D + D^u sera, whereas those which fail to react with D^u are anti-D sera alone. The term D^u therefore defines a series of alleles or modifications of the main antigen D at the D-d locus which differ qualitatively and quantitatively from D, and in many cases from each other. The reactions of D^u may therefore be summarized as follows:

1. They react with anything from 0-45% of anti-D sera (anti-D + D^u) by saline agglutination; those that react with none are referred to as "low-grade" type D^u and those that react with many as "high-grade."

2. They all give positive Coombs reactions when examined with certain strong incomplete anti-D sera.

3. With the exception of a few of the high-grade D^us, they all fail to give positive albumin reactions with incomplete anti-D sera.

Thus the reactions of D^u-positive cells vary from those scarcely distinguishable from D right down to those detectable only by the Coombs test, using special incomplete anti-D sera. These reactions are shown in the Table.

Table Showing Percentage of Anti-D Sera Giving Positive Results (the Actual Percentage of Positive Results Depends on the Individual Cell Sample Tested)

Test	Antigen in Cells			
	D	High-grade D ^u	Low-grade D ^u	d
Agglutination in albumin* ..	60-90%	0-10%	Nil	Nil
Agglutination in saline† ..	60-100%	5-45%	Nil	Nil
Coombs's reaction* ..	90-100%	70-100%	20-60%	Nil

* The sera tested were the strongest available. † The sera tested were a random selection.

It was found by examining large numbers of cell samples that about half the cells apparently of genotype Cde/cde are in reality CD^ue/cde and about one in nine of those apparently cdE/cde are really cD^uE/cde. The proportions are probably greater than this, since our study was made especially into low-grade forms of D^u-containing cells.

Practical Importance

When testing blood samples for Rh the nature and extent of the test depend upon the reason for its performance. If the test is being done in the case of antenatal patients or other persons to determine whether or not they can be immunized to Rh, then it is necessary to determine only whether the person is D-positive or D-negative.

On the other hand, when testing a person's cells to ascertain whether he is Rh-negative for the purpose of being a blood donor, one must make sure that the donor is not only D-negative but C-negative and E-negative as well. This is because the recipient's serum may contain not only anti-D but anti-C and, rarely, anti-E antibodies as well.

We have examined several blood samples from mothers of genotypes Cde/cde and cdE/cde whose sera contained anti-D antibodies. Careful examination failed to show the presence of D^u in any of these cell samples, and consequently, so far as we have ascertained at the present time,

the presence of D^u indicates that the cell sample is D-positive. Therefore, in the first instance mentioned above, when testing antenatal patients, anti-D + D^u sera may be used and a D^u person considered to be D-positive.

A child suffering from hypoplastic anaemia has been treated by Professor Gaisford with multiple blood transfusions from the age of 10 months; he is now 6 years of age, and during this period he has been transfused approximately six times yearly and has received about 100 pints (57 litres) of D-positive blood. Recently he was found to be of group CD^ue/cde, but no abnormal antibodies have been detected in his serum. In addition, he has more recently received a few pints of Rh-negative blood until the nature of the D^u group was more fully understood, but it seems that this child has failed to respond to the D antigen.

That a child of this age can respond to antigenic stimuli is shown by another case. A boy suffering from a similar condition was seen by one of us (F.S.) when about 8 years of age. He was Rh-negative and had strong Rh antibodies in his serum. He had likewise been treated over a period of about three years with multiple transfusions.

Van Loghem (1947, 1948) showed that D^u may become antigenic to D-negative persons, since immunization of an Rh-negative volunteer with blood of CD^ue/cde resulted in the formation of incomplete anti-D antibodies; whilst in Mollison's case (personal communication) a CD^ue/cde baby suffered from haemolytic disease of the newborn caused by incomplete anti-D antibodies in the maternal serum.

In the second instance, therefore, in which we are selecting Rh-negative donors for transfusion purposes, it is necessary to exclude the D^u-containing groups. The most practical way to avoid these is by using anti-C and anti-E sera in addition to anti-D when typing the blood. This will not only eliminate D-positive blood but also Cde/cde and cdE/cde as well as CD^ue/cde and cD^uE/cde—that is to say, the vast majority of D^u-positive blood. In certain populations—for example, negro—where the incidence of cDe/cde may be as high as 45%, this will not suffice, and it will be necessary to do a Coombs test, using incomplete anti-D sera to exclude group cD^ue/cde, which has a very low frequency in England (probably about 0.015%).

Furthermore, D^u is of practical importance when it is proposed to use Cde/cde and cdE/cde cells for determining the presence of anti-C and/or anti-E agglutinins respectively in Rh antisera. If these cells are used but in reality contain the D^u group and are CD^ue/cde and cD^uE/cde, the Rh antisera will falsely be thought to contain anti-C or anti-E, whereas the reaction may be due to the presence of anti-D reacting with the D^u component of the cells. This is of very great importance if the sera are to be used subsequently as test sera, and considerable confusion will result.

In the cross-matching test it is as well to remember that, should one encounter a donor whose cells contain D^u and a recipient's serum containing incomplete anti-D antibodies, the albumin test, often recommended for cross-matching, will be negative but the Coombs test will reveal the incompatibility. In general, when investigating sera for atypical antibodies both the Coombs and the albumin tests should be used, in case the antibody be of a type which reacts in only one of these tests. For our own part we are led to believe that the Coombs test is the one to be preferred in the detection of antibodies where only a single test can be performed.

Conclusion

In conclusion it should be emphasized that only a proportion of anti-Rh agglutinating sera react well with D^u-positive cells, and, since D^u bloods differ from one another

and from D qualitatively as well as quantitatively, sera giving the strongest agglutination with Du cells are not always those with the highest titres when tested against D-positive cells. In carrying out an examination of Du cells, using incomplete anti-D sera and the Coombs test or albumin test, the sera have also to be specially selected for this purpose.

The observations made in this paper apply, in the main, to the differentiation of Du from d. The determination of Du cells and their isolation and differentiation from the D-containing erythrocytes, on the other hand, is a difficult and complicated matter. The differing reactions of Du blood and the difficulties of knowing precisely what antibodies anti-Rh sera contain add to these difficulties.

To overcome this it is necessary to examine large numbers of cells of all types containing both the D antigen and the Du antigen combined with C and/or E, as well as cells containing the C and E antigens alone (i.e., types Cde/cde and cdE/cde); each type should be examined with very many varieties of anti-Rh agglutinating sera and incomplete antisera. The results should be tabulated and compared one with another, and any new cell should be put through this process and its reactions compared with those of other cells; by this means it is possible to give the type of the new cell which has been tested. In making our comprehensive table, for example, we used 49 Rh-agglutinating sera and 12 incomplete Rh antisera.

Full theoretical and explanatory results are not given in this short paper, but a further publication will show these and the manner in which they have been worked out.

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New regulations have been introduced by the Government to improve the standard of purity of milk. The Minister of Agriculture and Fisheries becomes responsible for the registration of dairy farmers and farms, the inspection of cattle on dairy farms, and the enforcement of the regulations on dairy farms (unless they relate to diseases communicable to man). If the Minister proposes to refuse or cancel a registration, provision is made for objections to be referred to a tribunal and for representations to be made to the Minister. Local authorities are still responsible for the provisions which apply to milk distributors other than dairy farmers and for the registration of these milk distributors and of dairies. Local authorities also continue to be responsible for the provisions relating to diseases communicable to man. The present powers relating to infected milk are extended to enable a medical officer of health to stop the sale of milk, or to require milk to be diverted for heat treatment, if there is evidence that it is infected with organisms of disease communicable to man. A medical officer of health may also require milk to be diverted for heat treatment if he has reasonable grounds for suspecting that it is so infected. There are a number of minor changes in the regulations relating to buildings, the cleansing of vessels and utensils, and the distribution of milk and its protection against contamination. In particular, provision is made for modern practices in dairying, including the use of mechanical refrigeration for cooling and approved chemical agents for the cleansing of appliances. A Central Advisory Committee and County Advisory Committee will be set up to review and make recommendations on the operation and administration of the regulations.

NOTES ON AN EXPERIMENTAL STUDY OF INTELLECTUAL DETERIORATION

BY

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The term "intellectual deterioration" means for the clinician a decline in intelligence as a result of a brain lesion, chronic mental illness, or old age. The psychologist has been satisfied to define intellectual deterioration operationally in terms of the tests he uses to measure it. These tests have been designed on the assumption made originally by Babcock (1930) that vocabulary level is a valid index of the pre-morbid intelligence of the patient. By a comparison of the score on a vocabulary test with the score on a pure test of intelligence an assessment of the degree of deterioration is made.

Hunt (1944), however, has questioned the assumption that vocabulary level is a valid index of pre-morbid ability. He points out that vocabulary may suffer as a consequence of the disorder; also that for unintelligent subjects vocabulary may be an imperfect indicator of ability.

The theory underlying the design of tests of intellectual deterioration can be verified only by a large-scale study of individuals suffering from a disorder known to cause intellectual deterioration which responds to clinical treatment. The present investigation was performed as a preliminary to the design of a large-scale investigation, and the results seemed of sufficient interest to report.

The Investigation

The subjects tested were three women and one man suffering from myxoedema. This disease seemed suitable for the investigation, as it causes apparent intellectual deterioration and responds rapidly to treatment by thyroxine. The main disadvantage from the point of view of research is its rarity. In the course of over a year only four subjects were found at the endocrine clinic of a large hospital. These were tested before starting treatment and three months later, when by clinical criteria they had recovered.

Tests Used

1. *Raven's Progressive Matrices Test*.—This is a non-verbal intelligence test. The untimed version was given.

2. *The Mill Hill Vocabulary Scale*.—This is a 32-item vocabulary test of the synonyms type. The scores on both these tests were converted into intelligence quotients (S.D. 16 points) by means of a table constructed in this department. An index of deterioration is obtained from these two tests by the formula:

$$\frac{\text{Matrices I.Q.}}{\text{Vocabulary I.Q.}} \times 100.$$

A quotient below 90 implies a certain degree of intellectual deterioration.

3. *The Shipley (1940) Test*.—There are two subtests: (a) A 20-item "abstractions" intelligence test consisting of a series of problems graded in difficulty in which the subject fills in a missing letter or figure. The first of these is: 1, 2, 3, 4, 5, — and the reasoning becomes more complex as the test progresses. There is a ten-minute time limit and the score is expressed as an abstractions mental age. (b) The second part of the Shipley test is a 40-item vocabulary test of the synonyms type, with a ten-minute time limit, from which a vocabulary mental age is obtained. From a comparison of the abstractions and vocabulary raw scores Shipley gives an index of deterioration (called by him a conceptual quotient) which is designed on virtually the same principle as the matrices/Mill Hill vocabulary