A Serologic and Genetic Analysis of an *r'r'* (*dCe/dCe*) Patient Producing Anti-D and Anti-c

By Philip Levine, Mary B. Cooper and Elizabeth A. Koch

A HITHERTO UNDESCRIBED combination of antibodies was observed recently in an Rh-negative woman of genotype *r'r'* whose husband was also Rh negative (*rr*). This patient, DvH, Italian by birth, had two normal full-term pregnancies and one miscarriage in 1944 followed by a transfusion; in 1950 she delivered a macerated fetus of genotype *r'r*. This fetus had hemolytic disease as evidenced by a strong direct antiglobulin reaction. In the course of this fourth pregnancy, antibodies of unknown specificity were observed which reacted on all Rh-negative and Rh-positive bloods. An order to transfuse the patient had to be canceled because of difficulty of finding a compatible donor. When the patient was in her fourth month of her fifth pregnancy, blood specimens of the patient and her family were submitted for more detailed studies.

The serological findings and obstetrical history are summarized in table 1. Except for the blood of the donor which became available only recently the findings in this table were made by one of us (MBC) and fully confirmed in the more recent tests.

The unusual feature in this mating is its rarity, the patient's genotype *dCe/dCe* occurring but once in 10,000 in a random population. The incidence of chromosome *dCe* is about 1:100 so that the frequency of the genotype is $(1:100)^2$ or 1:10,000. In the general population, the frequency of this mating is $2 \times 1:10,000 \times 13/100$ or 1:38,461; and this specific mating in which the female partner is *dCe/dCe* has one-half the above incidence or 1:76,923.

Tests of the patient's serum on a panel of test cells showed the presence of an incomplete antibody which reacted with all of the two hundred random bloods, Rh positive or Rh negative. Titrations of the serum with a number of red cells of specific antigenic constitution revealed a fairly uniform titer of about 1:64–1:128 on all bloods (see table 2.). It was obvious that the mating was incompatible for **c** (**hr'**) but not for **D**, and it was suspected that the reactions with Rh positive bloods might be due to an antibody, perhaps anti-**D**, stimulated by the transfusion following the miscarriage of 1944. Another possibility was the presence of a high-incidence antibody acting either by itself or along with anti-**c** and/or anti-**D**.

Antibodies with a high incidence of positive reactions, such as anti-**k** (anti-Cellano), anti-**e** (anti-**rh''**), anti-**Tj**^{a} (anti-Jay),[2] and another newly discovered high-incidence antibody now under investigation* could be excluded by the

From the Rh Blood Testing Laboratory, Ortho Research Foundation, Raritan, N. J., and the Laboratory of the General Hospital of Syracuse, Syracuse, N. Y.

Submitted November 4, 1953; accepted for publication December 17, 1953.

The authors are indebted to Drs. R. H. Rowner, R. C. Schwartz, and F. N. Roberts for making available their clinical observations and other pertinent data regarding the patient and her infant.

* The blood specimen in this case was submitted by Dr. Herbert Derman, Director of the City of Kingston Laboratories, Kingston, N. Y.

TABLE 1.—*Serologic Findings and Obstetric History*

		D	C	E	c	e	Genotypes (alternate terminologies)	
SvH, husband	O	o	o	o	+	+	*dce/dce*	*rr*
DvH, wife	O	o	+	o	o	+	*dCe/dCe*	*r'r'*
1. 1941, living and well	O	o	+	o	+	+	*dCe/dce*	*r'r*
2. 1944, miscarriage and transfusion*								
3. 1949, living and well	O	o	+	o	+	+	*dCe/dce*	*r'r*
4. 1950, macerated fetus	O	o	+	o	+	+	*dCe/dce*	*r'r*
5. edc. April 1953								
* donor	O	+	o	o	+	+	*Dce/dce*	*R''r*

TABLE 2.—*Demonstration of Anti-D and Anti-c by Cross Absorption*

Serum DvH was diluted 1:2 and absorbed once with equal volumes of washed packed red cells sediment for one hour at 37 C. The supernatants and the unabsorbed serum were titrated and tested by the antiglobulin technic of Coombs.

	Titration with		Specificity
	DCe/DCe	*dce/dce*	
Absorbed with *DCe/DCe*	o	1:128	anti-**c**
Absorbed with *dce/dce*	1:64	o	anti-**D**
Unabsorbed	1:64	1:128	anti-**D** + anti-**c**

The specificities of the two supernatants were confirmed in tests with numerous bloods of varying Rh-Hr antigens.

demonstration that the patient's red cells contained the corresponding blood factors. The normal behavior of the red cells with antibodies of the CDE system and particularly the failure of the red cells to react with anti-**D** excluded the unusual combination of antibodies associated with the so-called deletion effect characterized by the genotype *D—/D—*.[3]

The only alternative was the presence of anti-**c** along with persisting anti-**D** produced by the transfusion in 1944 of group O blood subsequently shown to be Rh positive. This possibility was put to the test by crossabsorption with selected bloods and the findings presented in table 2 give clear-cut evidence indicating the presence of both antibodies.

In the current pregnancy, there were indications that the fetus was not too active, and in view of the facts that the husband was homozygous for **c** (genotype *cc*), and the preceding pregnancy ended in a macerated fetus, the prognosis for a viable infant who could be salvaged by immediate replacement was not too favorable. In the event of transfusion requirement for the patient or her affected infant, only group O, *dCe/dCe* blood may be used, a type of blood which occurs 42 per cent for group O and .01 per cent for *dCe/dCe* i.e. 42/100 × 1/10,000 or 1:23,800. Accordingly, prior to her expected delivery, some of the patient's blood was drawn in ACD mixture so that her plasma-free cells were available.

In addition, her parents and siblings were tested with the hope of finding

TABLE 3.—*The Rh-Hr Factors in the Family of the Propositus, D.v.H.*

Red blood cells of		Tests with anti-				serum DvH	Genotypes alternate terminologies	
		D	C	E	c			
Mr. deF., father	B	o	+	o	(+)	*	*dCe/dce*	*rr'*
Mrs. deF., mother	B	o	+	o	+	*	*dCe/dce*	*rr'*
1. DvH., propositus	O	o	+	o	o	o	*dCe/dCe*	*r'r'*
2. J.deF., brother	O	o	o	o	+	+	*dce/dce*	*rr*
3. N.deF., brother	B	o	+	o	o	*	*dCe/dCe*	*r'r'*
4. W.E.R., sister	B	o	o	o	+	*	*dce/dce*	*rr*
5. E.deF., sister	O	o	+	o	o	o	*dCe/dCe*	*r'r'*

The presence of the **c** factor in the father's blood, indicated by parenthesis, is implied from the genetic analysis described in the text.

* Not tested because of B-O incompatibility.

group O compatible blood other than that of the propositus and these findings are presented in table 3.

The blood of the father, since expired, was tested by Dr. Dreyfuss, of the Vassar Brothers Hospital, Poughkeepsie, and his findings with only three of the four sera used in the above tests, were made available to the authors. The mother's blood was tested only recently, but the Rh genotypes of both parents could be anticipated on the basis of the findings with the five siblings. Two siblings (2 and 4) are of genotype *dce/dce* so that each parent transmits the chromosome *dce*. The other chromosome in the two parents must be *dCe* because the propositus and two siblings (1, 3, and 5) are of genotype *dCe/dCe*. Thus, both parents must be heterozygous and of genotype *dCe/dce*. In a double heterozygous mating one expects the three different genotypes *dCe/dCe*, *dCe/dce*, and *dce/dce* in a ratio of 1:2:1 respectively. It is curious that only the two less frequent homozygotes emerge from this mating while none of the 50 per cent expected heterozygous parental types is represented in the five siblings. Incidentally, both parents must be heterozygous also for B, i.e. *BO*, and the observed ratio of B:O i.e. 2:3 also deviates from the expected 3:1. The theoretic ratios which hold for numerous matings of any given type need not be expected in any one family.

These studies yielded also the significant information that sibling 5, E.deF. of group O, was completely compatible with anti-**D** and anti-**c** present in her sister's serum, and her blood was successfully employed in a replacement transfusion of the severely affected infant. Unfortunately, sibling N.deF., although compatible with the patient's anti-**D** and anti-**c**, is in group B and could not donate to the group O patient.

It has been shown recently that individuals homozygous for a rare chromosome or gene are more apt to result from consanguineous than from random matings.[3-6] This applies to the rare genotypes Tj^bTj^b with concomitant anti-**Tj**^{a}, O_cO_c with its associated anti-**H** (active at 37 C.) and to each of three instances of *D—/D—*. With these facts in mind, the propositus was interviewed and she stated that her parents are indeed related and the degree of their common ancestry is that of fourth cousins or closer.

TABLE 4.—*Titrations with Antiglobulin Technic*

Test cells	Serum		Specificity
	maternal	cord	
DCe/DCe	1:64	1:64	anti-**D**
dcE/dce	1:64	1:16	anti-**c**
dce/dce	1:128	1:16	anti-**c**

The propositus delivered spontaneously on April 16, 1953, a full-term male infant weighing 8 lbs. and 4 oz. The infant was apathetic, listless, moderately cyanotic, and had marked hepatomegaly and moderate splenomegaly. One hour after birth jaundice was noted and this rapidly increased in intensity. The direct Coombs test was strongly positive and cord serum contained 6.3 mg. percent bilirubin. As expected, the infant was in group O and his genotype was *dCe/dce*. A study of peripheral blood drawn one and one-half hours after birth showed the following: hemoglobin, 10 Gm. per cent; red cell count, 3.0 million; nucleated r.b.c., 46/100 w.b.c.; stab-form, 15 per cent; segmented, 48 per cent; eosinophils, 15 per cent; monocytes, 7 per cent; lymphocytes, 25 per cent.

Blood smears showed marked macrocytosis, moderate polychromasia, and an occasional megaloblast and erythrophagocyte.[7] Unfortunately, no studies were made to determine the reticulocyte count (cf. Pickles[8]).

Titration studies of the two antibodies in the maternal and cord serums are presented in table 4.

All of the maternal anti-**D** was passively transferred into the infant's circulation, but this antibody could not induce hemolysis since the pregnancy was compatible for the **D** factor. There was, however, a considerable portion of the incompatible maternal anti-**c** in the cord serum. A replacement transfusion via the umbilical vein was started four hours after birth with the mother's blood drawn nineteen days previously from which the plasma was withdrawn and replaced prior to the transfusion with 18 hour old group O plasma.* Because of the desperate condition of the infant, the catheter was left in the umbilical vein for further transfusion therapy. Ten hours later or fourteen hours after birth, a second replacement transfusion was carried out with 240 cc. group O blood of the compatible sibling, E.deF. Soon after the birth, this blood was drawn at Vassar Brothers Hospital, Poughkeepsie, and transported to Syracuse through the facilities of the American Red Cross.

At 48 hours the infant was considerably improved and his blood showed 11.5 Gm. percent of hemoglobin and a red cell count of 4.4 million. No further transfusions were required and the infant made an uneventful recovery. Continuous follow-up of the infant, now five months old, by Drs. Robert C. Schwartz and Frederick N. Roberts showed normal weight gain, no physical abnormalities, and no evidence of kernicterus.

* Failure of the patient to cooperate fully made it difficult to obtain her blood at a time more favorable for replacement therapy, i.e. just prior to delivery. Another factor was the uncertainty of the expected date of confinement.

Discussion

One of the problems this case presented was the identification of one or more antibodies in the serum which reacted on all random bloods tested. The contrast of antigens in the red cells of the propositus and her husband, and the history of a previous transfusion suggested an incompatibility of **c** (**hr′**) and therefore the presence of anti-**c** along with anti-**D**, confirmed by cross-absorption experiments. Had the propositus been transfused in 1944 with Rh-negative blood, there would not be too much difficulty in finding compatible blood for the affected male infant. In the presence of anti-**c** alone, Rh-positive blood of the rather frequent genotype *DCe/DCe* (R^1R^1) could have been given to the infant even though this would violate the rule that the infant be transfused with blood of the same Rh genotype as the immunized mother. The problem is not solved, however, for a female Rh negative affected infant of genotype *dCe/dce* (*r′r*) because this could initiate antibody production of anti-**D**, and thus handicap the infant's future capacity to bear normal Rh-positive children.

It is generally stated that the newborn infant lacks the property of antibody production, but the normal survival of transfused compatible blood for one hundred to one hundred and twenty days would make it necessary to establish resistance to antibody production also on the part of the 3 to 4 month infant.

With a previous history of fetal hydrops or macerated fetus, the chances are that the fetus in the subsequent pregnancy will die in utero before there is an opportunity to induce labor at 37 to 38 weeks and to institute therapy immediately. Remarkably enough, the most recent pregnancy went to term and the critically affected infant was saved by replacement transfusions. Fortunately, the rare compatible blood was made available and administered soon after delivery and before irreversible brain damage occurred. The question arises whether or not a second replacement would have been necessary if the mother's freshly drawn blood had been available instead of her nineteen day old blood. As a rule, blood preserved for not more than three days should be employed for replacement therapy.

Had the propositus herself required multiple or massive transfusions in the event of postpartum bleeding, it would not have been possible to supply more than one or two units of compatible blood. If the patient had produced only anti-**c**, assuming a transfusion in 1944 of Rh-negative blood, sufficient blood of genotype *DCe/DCe*, compatible with anti-**c**, would have been available. This would, however, create the present status of anti-**D** plus anti-**c** and thus only postpone the problem of selecting rare compatible donors for future blood requirement.

At any rate, the success in finding rare compatible donors in this case depended upon a complete antigenic and genetic analysis in the family of the propositus well in advance of transfusion requirement. The same problem arises in patients who may produce (1) anti-**k** (99.8 per cent positive reactions), (2) anti-**Tj^a**, or (3) combinations of antibodies such as anti-**E** and anti-**e** present in the serum of individuals of genotype *D—/D—*. In the two latter instances, experience has shown that even with a 100 per cent incidence of incompatibilities among random individuals, compatible donors may be found in the patient's siblings or other relatives. The chances for finding compatible donors of identical genotypes are

greater in siblings than in parents as illustrated in the case here reported. This is attributable to the fact that the homozygous individual who produces antibodies for the corresponding allelic factors frequently has parents each of whom is heterozygous and transmits the rare gene or chromosome. Under such conditions, 25 per cent of the offspring may receive from each parent the rare gene or chromosome. The importance of consanguineous marriages of couples carrying a rare gene producing pathologic conditions such as amaurotic family idiocy has been emphasized by human geneticists. As illustrated in the present case, the identical genetic principles apply to rare genes in general, and it would seem that the significance of consanguineous marriages is directly proportional to the rarity of the genes involved.*

For the country at large with a caucasoid population of about 150,000,000, one may expect 15,000 individuals of the rare genotype *dCe/dCe* and somewhat more than 7500 exposed to the antigenic stimuli of pregnancies and/or transfusions with potential capacity to produce the same combinations of antibodies, i.e. anti-**D** and anti-**c**.

For the protection of patients genetically endowed with this or other rare types of blood, it is important to select from the Rh-negative list the rare homozygotes for **C** and for **E**, i.e. genotypes *dCe/dCe* or *dcE/dcE*. In the latter case, assuming isoimmunization by factors **D** and **e**, the incidence of random compatible blood is 1:7100 not taking into account the blood groups. Unfortunately, the procedure in most blood banks is to classify the 1.5 to 2.0 per cent Rh-negative individuals having factors **C** and/or **E**, with the Rh-positive group. This results from the common practice of screening donors with an anti-**D** serum containing anti-**C** and frequently also anti-**E**. It has always been the policy of the authors to screen all donors and patients with anti-**D** only. Further tests are carried out with anti-**C** and anti-**E** for the detection of subtypes dCe (rh′) and dcE (rh″). Once individuals of genotypes *dCe/dCe* or *dcE/dcE* are detected with the use of anti-**c** or anti-**e**, respectively, it becomes important to study the bloods of their siblings and other relatives for selection of still other individuals of identical genotypes. At the same time, inquiry regarding consanguinity of the parents should become a routine procedure.

If several of the larger blood banks adopt this procedure, it will be possible to have a list of such individuals (at least groups O and A) who can serve as donors for selected cases as described above. These donors may then be listed with a central clearing bureau organized first at a national level and later extended, perhaps through the facilities of the World Health Organization. Undoubtedly, specialists in the field who have lists of individuals homozygous for other rare factors will make them available to the central clearing bureau. Unless these measures are adopted, workers in the field of transfusions will be faced with the prospect of failure to provide compatible donors although the antibodies could be identified.

Summary and Conclusions

A most unusual combination of antibodies, anti-**D** and anti-**c** produced by a transfusion and pregnancy, respectively, was identified by cross-absorption

* This subject will be discussed at length in a future publication.

experiments. The immunized mother (propositus), whose husband was of genotype *dce/dce*, possessed the very rare genotype *dCe/dCe*, each of her parents, who were related to each other as fourth cousins, contributing the rare chromosome *dCe*.

The critically affected male infant was successfully treated by early replacement transfusion with the mother's plasma-free red cells and her sibling's rare compatible blood. The infant made a complete recovery although the preceding pregnancy ended in a macerated fetus.

Summario in Interlingua

In un femina pregnante un rarissime combination de anticorpores—anti-**D** plus anti-**c** (producite respectivemente per un transfusion de sanguine e per le pregnantia)—esseva identificate per experimentos selectional. Le marito del patiente esseva del genotypo *dce/dce*. Le patiente mesme esseva del rarissime genotypo *dCe/dCe*. Tanto su patre como etiam su matre (affin como cosinos al quarte grado) habeva contribuite le rar chromosoma *dCe*. Illa pareva a termino un infante mascule. Le infante esseva criticamente afficite, sed ille respondeva satisfactorimente a prompte transfusiones substitutional de erythrocytos del matre (con plasma ab un altere donator) e de compatibile sanguine integre prendite ab un del sorores del matre. Le infante habeva un complete recuperation ben que le previe pregnantia habeva resultate in un feto macerate.

REFERENCES

[1] Race, R. R. and Sanger, R.: Blood Groups in Man, Springfield, Ill., C. C Thomas, 1950.

[2] Levine, P., Bobbitt, O. B., Waller, R. K., and Kuhmichel, A.: Isoimmunization by a new blood factor in tumor cells. Proc. Soc. Exper. Biol. & Med. *77:* 403–405, 1951.

[3] Race, R. R., Sanger, R., and Selwyn, J. G.: A possible deletion in a human Rh chromosome: a serological and genetical study. Brit. J. Exper. Path. *32:* 124–135, 1951.

[4] Waller, R. K., Sanger, R., and Bobbitt, O. B.: Two examples of the D--/D-- genotype in an American family. Brit. M. J. *1:* 198–201, 1953.

[5] Bhende, Y. M., Deshpande, C. K., Bhatia, H. M., Sanger, R., Race, R. R., Morgan, W. T. J., and Watkins, W. M.: A "new" blood-group character related to the ABO system. Lancet *1:* 903–906, 1952.

[6] Levine, P.: Genetics of the newer human blood factors, in: Advances in Genetics, New York, Academic Press, 1953.

[7] Cooper, M. B.: Erythrophagocytosis in hemolytic disease of the newborn: a report of twenty-five cases. J. Hemat. *5:* 678–683, 1950.

[8] Pickles, M. M.: Haemolytic Disease of the Newborn, Oxford, Blackwell Scientific Publications, 1949.