ance data than was previously possible in those cases where the fraction of ions associated is small. Work on this problem and on the case of unsymmetrical electrolytes is in progress.

* Contribution No. 1275.

[1] L. Onsager, *Physik. Z.*, **27**, 388, 1926.

[2] L. Onsager, *ibid.*, **28**, 277, 1927.

[3] L. Onsager and R. M. Fuoss, *J. Phys. Chem.*, **36**, 2689, 1932. In order to save space, material in pp. 2689–2704 and 2735–2744 of this reference will be used without rederivation. A review of this work is available in H. S. Harned and B. B. Owen, *Physical Chemistry of Electrolytic Solutions*, (New York: Reinhold Publishing Corp., 1950), pp. 75–85.

[4] P. Debye and E. Hückel, *Physik. Z.*, **24**, 185, 1923.

[5] P. Debye and E. Hückel, *Ibid.*, **24**, 305, 1923.

[6] We plan to present the details of this computation later.

[7] H. Falkenhagen, M. Leist, and G. Kelbg, *Ann. Physik*, 6th ser., **11**, 51, 1952.

[8] M. Eigen and E. Wicke, *Naturwissenschaften*, **38**, 453, 1951.

[9] R. M. Fuoss, *J. Phys. Chem.*. **58**, 682, 1954.

[10] T. Shedlovsky, *J. Am. Chem. Soc.*, **54**, 1411, 1932.

AN INTERACTION BETWEEN ALLELES AT THE Rh LOCUS IN MAN WHICH WEAKENS THE REACTIVITY OF THE Rh_0 FACTOR (D^u)

BY R. CEPPELLINI, L. C. DUNN, AND M. TURRI

INSTITUTE FOR THE STUDY OF HUMAN VARIATION, COLUMBIA UNIVERSITY, NEW YORK; ISTITUTO DI GENETICA AND ISTITUTO SIEROTERAPICO MILANESE S. BELFANTI, UNIVERSITÀ DI MILANO

Communicated March 25, 1955

Occasionally bloods are found which, when tested with different anti-Rh_0 (anti-D) sera, give some but not all the serological reactions expected from the presence of the Rh_0 (D) factor, or the intensity of such reactions is consistently weaker than the average. Owing to the clinical importance of the Rh_0 factor, on which the condition of Rh-positivity or Rh-negativity depends, such "intermediate variants" (Wiener[1]), usually symbolized as D^u (Stratton[2]) or $\mathfrak{R}h_0$ (Wiener[3]), represent a difficult problem for blood-grouping laboratories. For a review of the subject we refer to Race and Sanger.[4]

From a genetical point of view, a number of family investigations, actually not very large (Stratton and Renton;[5] Dunsford;[6, 7] Race, Sanger, and Lawler[8]), have led to the conclusion that the D^u variants are to be regarded as products of mutation of the *D* allele and are thus inherited like the other Rh blood factors. Owing to the fact that often the D^u variants in members of the same family show identical serological peculiarities, while they may differ broadly between different families, a series of D^u alleles has been supposed. This view received additional indirect support from the knowledge that generally blood groups behave as a direct, not mediate, product of the determining gene and are little influenced by other environmental or genetical agents.

While the interpretation given by the British authors is certainly true in some and may be true in the majority of D^u cases, other genetical interpretations cannot be disregarded.

In 1952 one of us,[9] discussing the data on some of the individuals now presented in family A (Fig. 1 and Table 2), stated that "the maternal chromosome *CDe* seems to produce in the offspring a normal D antigen when paired with the paternal chromosome *cde*, but a D^u antigen when paired with the paternal chromosome *Cde*." Thus the D^u phenotypes in that family appeared to be not the direct product of a mutant D^u (or $\Re^1$) gene but the result of interaction of the *D* chromosome with the partner *Cde* chromosome.

This observation was in agreement with the excess of CCD^ue phenotypes noted in their material by Race *et al.*,[8] who, following their hypothesis of an uncomplicated inheritance of D^u, were forced to attribute to such phenotypes the rare CD^ue/Cde genotype (the other possible alternative CD^ue/CD^ue should be exceedingly rare) because, on a serological level and in the absence of the hypothetical antiserum, D^u must be regarded to be dominant to *d* but recessive to *D* in the same way as A_2 is dominant to *O* but recessive to A_1. The authors concluded "that the relatively large number of CD^ue/Cde bloods demands an explanation but we have not any really satisfactory one to offer."[8]

Eldon[10] also observed an excess of CCD^ue individuals and suggested the existence of a modifying gene *U*. Although Eldon's views are not easily discussed on the basis of the scanty genetical and serological data which he presents, the hypothesis of a modifier linked with the Rh locus or independent of it cannot be discarded on the basis of present evidence.

An unusual opportunity for investigating this problem was presented when we found in a Roman Jewish community[11] an unusually high frequency of the *Cde* chromosome (*r'*gene), over 4 per cent as compared with less than 1 per cent in most Italian samples. Owing to the fact that we were dealing mainly with complete families, the genotypes were often ascertained unambiguously; out of 642 individuals tested, the *Cde* chromosome was present at least in the 43 following combinations: 24 *Cde/cde;* 16 *Cde/CDe;* 2 *Cde/cDE;* 1 *Cde/cDe.*

Moreover, according to the first Rh typing, where three different anti-D sera were routinely used in parallel, besides 545 Rh-positive and 83 Rh-negative persons, unequivocally classified as such, in fourteen cases a weakened or discordant D-positivity toward the three sera was observed, which was taken as sufficient evidence for classifying these bloods as D^u. In all cases these reactions, confirmed by quantitative titrations with several anti-D sera, were found in families in which the *Cde* chromosome was present, and thus Ceppellini's suggestion, previously quoted, was proved to be of more general value. Eleven such families (eight from the Roman Jewish community and three from other Italian districts) have now been studied. Six of these families were chosen because a propositus was identified as D^u in the first typing; in the other five cases the families were chosen through a Ccde propositus.

The presence of any weakly reacting form of the D factor was excluded for all bloods finally classified as Rh-negative (cde or Ccde) by means of the indirect antiglobulin test after sensitization with at least three (sometimes as many as nine) potent incomplete anti-D sera.

The level at which a D^u classification is justified is rather an arbitrary one; it is thus of fundamental importance in presenting such an investigation to state exactly the serological criteria and techniques used. For lack of space, such details will

be given only in the full account of our study, to be published elsewhere. In this preliminary note we shall present only a few examples of some of the families studied.

In Table 1 an example of titration is reported in detail for one family against one anti-D serum: according to the intensity of the agglutination, different point

TABLE 1

TITRATION AGAINST ONE ANTI-D SERUM (v) OF THE Rh-POSITIVE MEMBERS OF FAMILY A*

FAMILY MEMBERS	DILUTION OF ANTI-D SERUM (v) 1	2	4	8	16	32	64	128	SCORE
I-2	+	++^{v}	++^{c}	++^{c}	++^{v}	++	±	w	43
II-6	−	−	++	+	−	−	−	−	8
II-8	++	++^{v}	++^{c}	++^{v}	++^{v}	+	w	−	39
I-4	−	w	+	w	−	−	−	−	5
II-10	++	++^{c}	++^{c}	++^{c}	++^{v}	+	±	−	44

AGGLUTINATION SYMBOLS AND SCORING

++^{c}	=	Complete agglutination into a few solid clumps	= 9 points
++^{v}	=	Clumps easily recognized with the naked eye	= 7 points
++	=	Large clumps seen under low-power mag.; rare unagglutinated cells	= 5 points
+	=	Clumps of 8–15 elements, some unagglutinated cells	= 3 points
±	=	Rare clumps of 4–6 elements, many unagglutinated cells	= 2 points
w	=	Occasional clumps of 2–4 elements, majority of cells unagglutinated	= 1 point

* See Figure 1 and Table 2.
Note the prozone, most marked with the two weakly reacting bloods (II-6 and I-4).

scores are attributed for each dilution (from 10 to 1), and the sum of points makes up the individual score for a given serum. Usually at least five anti-D saline agglutinating sera for each set of experiments were used; the sera were selected only inasmuch as they did not contain any antibody other than anti-D; strict precautions for avoiding bias in reading were taken.

In Table 2 the scores for the members of family A (Fig. 1) are presented: the total scores (sum of the scores for the six different antisera) show that the reac-

TABLE 2

FAMILY A (FIG. 1): INDIVIDUAL SCORES OF THE Rh-POSITIVE MEMBERS AGAINST 6 DIFFERENT ANTI-D SALINE SERA (ALL Rh-NEGATIVE INDIVIDUALS RECHECKED WITH INDIRECT ANTIGLOBULIN TEST)

	PHENOTYPE*	GENOTYPE†	ANTI-D SERA (i)	(ii)	(iii)	(iv)	(v)	(vi)	TOTAL
I-1	Ccde	*Cde/cde*	...	...	..	..	..	..	0
I-2	CcDe	***CDe/cde***	115	108	75	62	43	37	440
II-5	Ccde	*Cde/**cde***	...	...	..	..	..	..	0
II-6‡	CCD^{u}e	*Cde/**CDe***	64	62	23	44	8	18	219
II-7	cde	*cde/**cde***	...	...	..	..	..	..	0
II-8	CcDe	*cde/**CDe***	108	110	80	74	39	39	450
I-3	cde	***cde/cde***	...	...	..	..	..	..	0
I-4	CCD^{u}e	*Cde/CDe*	60	71	19	48	5	12	215
II-9	Ccde	*Cde/**cde***	...	...	..	..	..	..	0
II-10	CcDe	*CDe/**cde***	121	110	69	59	44	41	444

* Phenotypes symbolized by the letters corresponding to the blood factors actually found by using the 5 Rh anti-sera: anti-C, anti-c, anti-D, anti-E, anti-e; d symbolizes negativity toward anti-D. D^{u} reported when on the first typing an abnormally weak reaction of D was observed.
† Genotypes unambiguously determined from the family segregations; in the offspring the chromosome inherited from the father is printed in italic; the chromosome from the mother, in italic boldface.
‡ Propositus.

tivity of the D antigens produced by the same *CDe* chromosomes (2, 6, 8 and 4, 10) is markedly changed according to whether they are paired with a *cde* or a *Cde* chromosome.

It is worth while to emphasize that we aimed mainly at investigating whether the presence of a *Cde* chromosome in the genotype quantitatively depressed the reactivity of the D antigen, a fact which may not be related to the appearance of a true D^u phenotype as originally defined by Race *et al.*[8] Actually, while a few of our *D/Cde* individuals (see Tables 1 and 2) show a markedly different reactivity toward different anti-D sera (a typical feature of D^u phenotypes, which seems to be of a qualitative order), the majority of them merely score lower than normal D bloods with all anti-D sera. This suggests that the phenomenon here described is in the main a quantitative one.

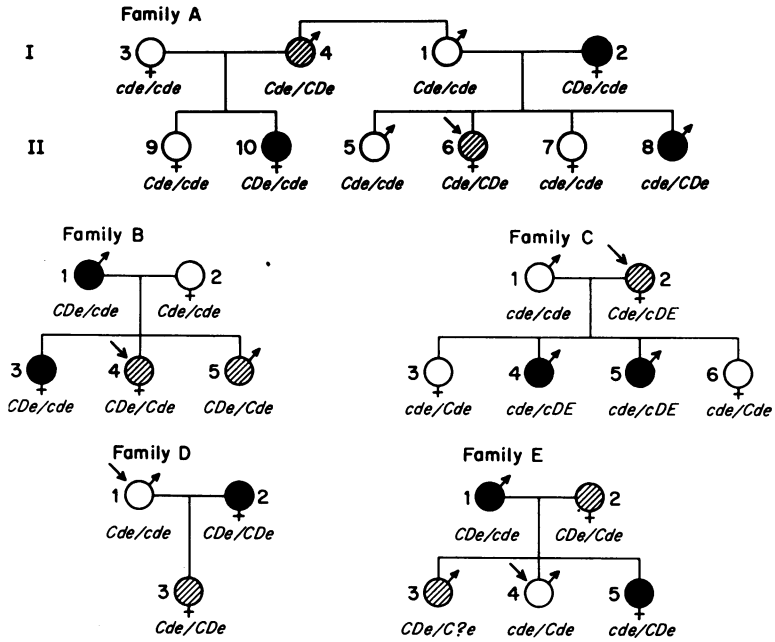

FIG. 1.—● = D-positive blood, of normal strength when titrated against several anti-D sera. ● = D-positive blood, significantly weaker than average on titration (like a D^u variant). ○ = D-negative blood (I.C.T.-negative, with several strong anti-D). → = Propositus. For family A see Table 2. For families B, C, D, and E see Table 3. In genotypes of offspring paternal chromosome is written first (*from Ceppellini et al.*[15]).

A proper answer to such a quantitative problem can only be obtained by subjecting the data to an analysis of variance, as presented in Table 3. The Rh-positive members of four families were titrated on two different days (experiments I and II) against the same five anti-D sera. The twelve individuals are divided into two classes, according to whether, in their genotype, unambiguously defined by family segregations, a *Cde* chromosome is present or not. Variability of reactions between individuals of the same genotype with respect to *Cde*–not *Cde*, tested on the same day, with the same serum, was used as the error: a t with 8 degrees of freedom is thus calculated for each serum (Table 3).

The statistical analysis proves beyond any doubt that in this experiment the five *D/Cde* genotypes show a lower D reactivity than the seven *D*/non-*Cde* geno-

types; the five different antisera show no significantly different discriminating abilities ($F_{4,40} = 1.93$).

Four similar sets of experiments have been analyzed, with the same results, that is, altogether, a total of 19 certain *D/Cde*, 20 *D*/not-*Cde*, and 4 *D/?* genotypes (detailed data to be published elsewhere).

We conclude that the presence of *Cde* paired to a normal *D* chromosome (in our investigation 15 *Cde/CDe*, 3 *Cde/cDE*, and 1 *Cde/cDe* genotypes were studied) produces a significant weakening of the D reactivity, which in extreme cases may be of the same order as the serological behavior shown by typical D^u bloods. Therefore, D^u phenotypes are not always due to the same genetical mechanism.

Such interpretation applies also to certain of the families reported by Race *et al.*[8] (families Lowe, Green, Curtis; probably also the two individuals Gillham and Pope—the last one a typical example of low-grade D^u) and by Eldon.[10]

TABLE 3

STATISTICAL ANALYSIS OF DATA

		Individual Scores against 5 Different Anti-D Sera					
		I	II	III	IV	V	Total Score
Class D/Cde:							
1st day	D-3	62	43	18	43	56	222
	E-2	50	41	30	26	45	201
2d day	B-4	39	26	28	40	56	189
	B-5	37	34	32	36	66	206
	C-2	39	25	29	39	56	188
	$\bar{m}$	47.20	33.81	27.40	36.80	55.8	201.00
Class D/not-Cde:							
1st day	D-2	86	71	56	49	65	325
	E-1	68	56	42	51	67	284
	E-5	80	64	52	52	67	315
2d day	B-1	51	42	50	51	71	265
	B-3	66	45	45	55	62	273
	C-4	57	42	53	50	72	274
	C-5	54	41	52	52	72	271
	$\bar{m}$	66.00	51.57	49.71	51.43	68.00	286.71
	t (8 d.f.)	5.23*	6.54*	7.58*	5.39*	4.16†	11.21*

Statistical analysis of the scores representing the strength of agglutination of 12 Rh-positive individuals belonging to 4 families against 5 different anti-D saline sera (families B, C, D, E [Fig. 1]). The same panel of anti-D sera was used in the two experiments carried on on two different days. The individuals have been grouped into two classes according to the presence or absence of *Cde* in their genotype (*ascertained unambiguously through family segregations*). D-2 comes from two CcDe parents and has a cde sib. E-3, who scores 52, 35, 32, 34, 44 (total 197), has not been included in the analysis because of the uncertain genotype.

* Significant at .001 *P* level.

† Significant at .01 *P* level.

We do not discuss here the obvious practical implications of our findings, for instance, with regard to the use of Rh blood groups in paternity cases. We wish only to point out that, for the blood groups as for other characters, the phenotype cannot be regarded in any case as a direct reflection of the genotype.

From a formal point of view the phenomenon described could be explained in a variety of ways. We mention only the interpretations for which at least a basis for discussion exists:

a) If the weakening effect of *Cde* were confined to the CCD^ue phenotype, it could be regarded as due to a dosage effect between competing Rh alleles: two *C* alleles versus only one *D* produce D^u. The fact that the same phenomenon has been observed in *Cde/cDE* and *Cde/cDe* as well as in *CDe/Cde* genotypes does not justify such a hypothesis.

b) The weakening effect could be produced not by the *Cde* chromosome as a whole but by a mutant form of *d* (say, d^w) capable of interacting with a partner allele D. Up to now, this would have been recognized only in the Cd^we combination. It should be investigated whether a cd^we chromosome exists, that is to say, whether some CcD^ue or cD^ue phenotypes are to be regarded as CDe/cd^we or cDe/cd^we. Surprisingly enough, a number of published D^u families do not contradict, at least from a formal point of view, this possibility.

In conclusion, the weakly reacting D phenotype which occurs when a *Cde* chromosome is paired to a normal *D* chromosome is, in our opinion, one instance of the many interactions between alleles taking place at the Rh locus. Similar interactions between *CDe* and *cDE* (for example) have been assumed by Cameron *et al.*[12] to produce a depression of the anti-E reactivity. This kind of interaction appears more reasonable if we regard Rh as a *complex* locus, inherited as a unit but built up from more than one site of mutation (Ceppellini;[13] Dunn[14]).

Summary.—In eleven Italian families, eight from the "ghetto community" of Rome, a weakened reaction of the D antigen has been shown to be associated with the simultaneous presence of the chromosome *Cde* (*r'*) in the genotype. This is assumed to be due to interaction between *D* and the combined effects of *Cde* in juxtaposition or to other peculiarities of particular *Cde* chromosomes.

[1] A. S. Wiener, *Science*, **100**, 595, 1944.

[2] F. Stratton, *Nature*, **155**, 542, 1946.

[3] A. S. Wiener, *An Rh-Hr Syllabus* (New York: Grune & Stratton, 1954).

[4] R. R. Race and R. Sanger, *Blood Groups in Man* ("Blackwell Scientific Publications" [2d ed.; Oxford, 1954]).

[5] P. H. Renton and F. Stratton, *Ann. Eugen.* (London), **15**, 189, 1950.

[6] I. Dunsford, *Ann. Eugen.* (London), **14**, 142, 1948.

[7] I. Dunsford, *ibid.*, **17**, 283, 1953.

[8] R. R. Race, R. Sanger, S. D. Lawler, *Ann. Eugen.* (London), **14**, 171, 1948.

[9] R. Ceppellini, "Le Varianti Rh," in R. Ceppellini, S. Nasso, and F. Tezilacich, *La Malattia emolitica del neonato* (Milan: Ed. I.S.M.S.B., 1952), p. 140.

[10] K. Eldon, *Rev. d'hématol.*, **5**, 294, 1950.

[11] L. C. Dunn, *Am. J. Phys. Anthropol.* 1955 (in press).

[12] R. R. Race, R. Sanger, P. Levine, R. T. McGee, J. J. van Loghem, M. van der Hart, and C. Cameron, *Nature*, **174**, 460, 1954.

[13] R. Ceppellini, "La Natura dei geni Rh," in R. Ceppellini, S. Nasso, and F. Tezilacich, *La Malattia emolitica del neonato* (Milan: Ed. I.S.M.S.B., 1952), p. 169.

[14] L. C. Dunn, *Caryologia*, suppl. vol., p. 155, 1954.

[15] R. Ceppellini, L. C. Dunn, M. Turri. Report to 7th Int. Cong. Blood Transfusion, Paris, Sept. 11, 1954.