20. Gene Dosage Effect on Rh Blood Group Antigens
observed in Erythrocytes Heterozygous
for Rh⁻²⁹ ($\bar{r}$, ---) Gene

By Hayato HASEKURA,^{*)} Masao OTA,^{*)} Hirofumi FUKUSHIMA,^{*)}
Isamu YONEMURA,^{*)} and Takahira ISHIMORI^{**)}

(Communicated by Shoei ISEKI, M. J. A., Feb. 12, 1986)

Erythrocytes with different Rh blood group phenotypes differ in their reactivity against various Rh antibodies. This difference has been attributed to gene dosage effect or disturbing effect by other antigen constructions.¹⁾

We discovered earlier, the first Rh: -29, 38 ($\bar{r}h$, ---/---) propositus, a homozygote of the amorph gene R-29 ($\bar{r}$, ---), together with several heterozygotes in his family.²⁾ Comparison of these heterozygotes with homozygotes should provide critical samples in interpreting the difference of agglutination reactions, as the Rh antigens on these cells are products of single genes.

This report presents the data of comparative titration using classic agglutination reaction expressed in scores.

Materials and methods. *Erythrocytes.* The family members of H. A., the original Rh: -29, 38 ($\bar{r}h$, ---/---), including known heterozygotes of R-29 ($\bar{r}$, ---) gene, were bled to be tested (Fig. 1): 11 (MOTHER, R1 $\bar{r}$, CDe/---), 22 (WIFE, R1R1, CDe/CDe), 23 (BROTHER, R2 $\bar{r}$, cDE/---), 31 (SON-1, R1 $\bar{r}$, CDe/---), and 32 (SON-2, R1 $\bar{r}$, CDe/---). Also, family members of a disputed paternity case were included in the experiment (Fig. 2), where Child-3 (a three-year-old girl, R2 $\bar{r}$, cDE/---) was annulled by Father (R1 $\bar{r}$, CDe/---); she was excluded of legitimacy at the first trial on the basis that R1R1 (CCDee) man could not have an R2R2 (ccDEE) child. The second instance extended blood group tests to Child-1 (R2 $\bar{r}$, cDE/---) and Child-2 (R1R2, CDe/cDE) both undisputed on their legitimacy; Child-3 was then confirmed to be a legitimate, as Child-1, her brother, revealed the same Rh phenotype as hers.

Fig. 1. Pedigree of H. A., the original Rh: -29,38 ($\bar{r}h$, ---/---).

^{*)} Department of Legal Medicine, Shinshu University School of Medicine, Matsumoto, 390 Japan.

**) Funabashi Red Cross Blood Center, Funabashi, 274 Japan.

Agglutination scores against anti-Rh 2 (rh', C) and anti-Rh 5 (hr'', e) showed that R-29 ($\bar{R}$, ---) heterozygous cells possess decreased antigen reactivity compared

Table I. Agglutination scores of erythrocytes with Rh antisera

Antisera Methods	Rh 1(Rho, D)		Rh 2(rh', C)		Rh 5(hr'', e)		Rh 3(rh'', E)		Rh 4(hr', c)	
	Sal.	Brom.	Sal.	Brom.	Sal.	Brom.	Sal.	Brom.	Sal.	Brom.
Rh genotype										
R1R1, CDe/CDe	<u>23.0</u>	<u>65.7</u>	<u>33.7</u>	<u>62.0</u>	<u>30.7</u>	<u>64.0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
22 WIFE	23	65	33	46	17	63	0	0	0	0
Normal	29	81	30	61	22	67	0	0	0	0
Normal	17	51	38	79	53	62	0	0	0	0
R1 $\bar{r}$, CDe/---	<u>17.3</u>	<u>68.3</u>	<u>19.0</u>	<u>51.5</u>	<u>16.8</u>	<u>44.2</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11 MOTHER	20	66	19	50	12	45	0	0	0	0
31 SON-1	17	68	19	50	12	47	0	0	0	0
32 SON-2	17	68	19	53	12	47	0	0	0	0
42 FATHER	15	71	19	53	31	38	0	0	0	0
R2R2, cDE/cDE	<u>27.5</u>	<u>74.5</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>48.0</u>	<u>72.0</u>	<u>27.0</u>	<u>85.5</u>
41 MOTHER	32	88	0	0	0	0	48	66	27	81
Normal	23	61	0	0	0	0	48	78	27	90
R2 $\bar{r}$, cDE/---	<u>20.3</u>	<u>68.7</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>24.3</u>	<u>57.0</u>	<u>20.3</u>	<u>70.7</u>
23 BROTHER	17	64	0	0	0	0	45	67	19	77
51 CHILD-1	22	71	0	0	0	0	36	49	20	66
53 CHILD-3	22	71	0	0	0	0	33	55	22	69
R1R2, CDe/cDE	<u>16.5</u>	<u>52.5</u>	<u>23.0</u>	<u>49.5</u>	<u>19.7</u>	<u>37.0</u>	<u>31.5</u>	<u>45.0</u>	<u>15.5</u>	<u>62.5</u>
52 CHILD-2	13	55	23	52	28	40	28	37	17	64
Normal	20	50	23	47	8	43	35	53	14	61
Normal					23	28				

Underlined figures show average scores of the genotype.

Fig. 4

Fig. 5

Figs. 4-5. 4: Average Rh 2(rh', C) and Rh 5(hr'', e) antigen strength quantitated in agglutination scores. s, saline agglutination; b, bromelin agglutination. Suggestive dosage effect was observed. 5: Average Rh 3(rh'', E) and Rh 4(hr', c) antigen strength quantitated in agglutination scores. s, saline agglutination, b, bromelin agglutination. Suggestive dosage effect was observed.

with R1R1 (CDe/CDe) cells, thus resembling R1R2 (CDe/cDE) heterozygotes (Figs. 4-5, Table I).

Also anti-Rh 3 (rh'', E) and anti-Rh 4 (hr', c) agglutinated R⁻²⁹($\bar{r}$, ---) heterozygotes weaker than homozygous R2R2 (cDE/cDE) cells; reactivity of heterozygous R1R2 (CDe/cDE) cells was still weaker probably due to some disturbing effect on anti-Rh 1 (Rho, D) antibody reaction.

These results would suggest that gene dosage effect works on Rh 2-5 (rh', C; rh'', E; hr', c; hr'', e) antigens but not on Rh 1 (Rho, D) antigen; in other words, Rh 2-5 (rh', C; rh'', E; hr', c; hr'', e) antigens are incompletely dominant traits while Rh 1 (Rho, D) antigen is completely dominant.

Also in the German family including the second and the third case of $\bar{r}h$ (---/---) individuals, heterozygotes of the R⁻²⁹($\bar{r}$, ---) gene revealed the single dose antigen activity in agreement with the present study.⁴⁾

Summary. R⁻²⁹($\bar{r}$, ---) heterozygous erythrocytes were titrated against Rh blood grouping antisera in comparison with ordinary cells. Gene dosage effect was suggested to work on Rh 2-5 (rh', C; rh'', E; hr', c; hr'', e) antigens but not on Rh 1 (Rho, D) antigen.

References

- 1) R. R. Race and R. Sanger: *Blood Groups in Man*. 6th ed., Blackwell (Oxford), pp. 232-236 (1975).
- 2) T. Ishimori and H. Hasekura: A case of a Japanese blood with no detectable Rh blood group antigen. Discovery of a homozygote of deleted chromosomes or silent Rh alleles. *Proc. Japan Acad.*, **42**, 658-660 (1966).
- 3) W. L. Marsh: Scoring of hemagglutination reactions. *Transfusion*, **12**, 352-353 (1972).
- 4) S. Seidl, W. Spielmann, and H. Martin: Two siblings with Rh null disease. *Vox Sang.*, **23**, 182-189 (1972).