References and Notes

1. J. Kopoldova, J. Kolousek, A. Babicky, J. Liebster, *Nature* **182**, 1074 (1958); D. Cavallini, B. Mondovì, B. Giovanella, C. De Marco, *ibid.* **184**, 61 (1959).
2. B. Shapiro and L. Eldjarn, *Radiation Research* **3**, 255, 393 (1955).
3. R. S. Yaslow, *Proc. 1st Natl. Biophys. Conf.* (1959), p. 169.
4. The cystamine dihydrochloride used in this study was prepared from commercial mercaptoethylamine by the procedure of A. H. Nathan and M. T. Bogert [*J. Am. Chem. Soc.* **63**, 2361 (1941)].
5. D. Cavallini, C. De Marco, B. Mondovì, *J. Biol. Chem.* **234**, 854 (1959).
6. C. E. Dent, *Biochem. J.* **43**, 590 (1947).
7. A. P. Ryle and F. Sanger, *ibid.* **60**, 535 (1955); R. Benesh and R. Benesh, *J. Am. Chem. Soc.* **80**, 1666 (1958).
8. C. Huggins, D. F. Tapley, V. Jensen, *Nature* **167**, 592 (1951); W. Kauzmann and R. G. Douglas, *Arch. Biochem. Biophys.* **65**, 106 (1956).
9. C. Ressler, *Science* **128**, 1281 (1958).
10. This work was supported by grants from the Consiglio Nazionale delle Ricerche and from the Comitato Nazionale Ricerche Nucleari.

23 November 1959

$Rh_0(D)$ Genotype and Red Cell $Rh_0(D)$ Antigen Content

Abstract. The $Rh_0(D)$ content of red cells obtained from different individuals as determined with I^{131} anti-$Rh_0(D)$ showed a bimodal distribution. Family studies indicate that the cells with the lower antigen content represent the heterozygous $Rh_0(D)$ state and that the cells with 1.6 times more $Rh_0(D)$ correspond to the homozygous state.

The blood group antigens on the stroma of the red cell represent specific immunochemical molecules whose synthesis is genetically determined (*1*). If the one gene–one product hypothesis applies to the blood group antigens, the red cell derived from a homozygous individual should have twice the antigen content found on a red cell derived from a heterozygous individual.

The $Rh_0(D)$ antigen content of the human red cell was determined with incomplete I^{131} trace labeled anti-$Rh_0(D)$ and the methods described previously (*2*). The method of approach was to determine the frequency distribution of the $Rh_0(D)$ antigen content in a population of different Caucasian red cells (*3*). The heterozygous state in this distribution then was identified by study of genetically defined $Rh_0(D)$ heterozygous cells obtained from family studies. The use of this experimental approach was necessitated because of the inability to differentiate in advance of the experiment the heterozygous from the homozygous $Rh_0(D)$ red cell by conventional techniques (*4*).

Red cells were obtained by selection from the donor population entering the Blood Bank (*5*) and were typed for the ABO and Rh antigens with commercially obtained antisera. Only a few $Rh_0(D)$ negative red cells were used as controls. The red cells from 199 individuals were studied, but only the results obtained on rh′(C) negative, $Rh_0(D)$ positive cells are presented in this report. The results are expressed as quantity of antibody nitrogen bound to 0.01 ml of centrifuged red cells as determined by the microhematocrit technique (*6*) using the International microhematocrit centrifuge (5 minutes at $g_{max} = 12{,}000$). The mean number of red cells in 0.01 ml of centrifuged red cells was determined for a number of cell suspensions with the Coulter electronic counter (*7*, *8*) and was found to equal $9.01 \pm 1.48 \times 10^7$ cells (*9*).

The over-all precision of the technique was evaluated by the following experiment. Red cells from a given individual were stored in Alsever's solution at 4°C and on subsequent days an aliquot was reconstituted and reacted with the I^{131} anti-$Rh_0(D)$. A fresh specimen of cells obtained from the same individual was run in parallel with the stored cells. The mean and standard error of the mean for repeated determinations on the stored cells over a period of 11 days was 1.85 ± 0.13, 10^{-2} μg of N per 0.01 ml of red blood cells. The corresponding value for fresh unstored red cells was 1.85 ± 0.15, 10^{-2} μg of N. There was no significant difference between these two values ($t = 0.18$, $n = 14$). $Rh_0(D)$ negative red cells took up less than 3 to 7 percent of the I^{131} bound to $Rh_0(D)$ positive red cells. The amount of nitrogen bound was independent of both the Rh and ABO phenotype of the $Rh_0(D)$ negative cell. All the red cell suspensions were reacted in an excess of antibody in order to obtain maximum saturation of the $Rh_0(D)$ antigen sites. Antibody excess was determined by demonstrating free I^{131} anti-$Rh_0(D)$ in the supernatants after reaction and by re-reacting the I^{131}-sensitized red cells with additional I^{131} anti-$Rh_0(D)$ to show that the available antigen sites were saturated. There was less than 10 percent increase in the amount of antibody bound to the sensitized red cell after a second 60-minute incubation with the I^{131} anti-$Rh_0(D)$.

The absolute frequency of the antibody nitrogen bound to 47 $Rh_0(D)$ positive, rh′(C) negative cells is shown in Fig. 1. It can be seen that the cells segregate into two groups, one with a mean of 2.44 and the other with a mean of 3.95 (10^{-2} μg of N per 0.01 ml of red blood cells). The cells in the higher nitrogen value group take up 1.62 times more antibody nitrogen than do the cells in the lower group. Known heterozygous $Rh_0(D)$ positive red cells obtained from families with a history of Rh hemolytic diseases of the newborn (*10*) were employed to identify the heterozygous value in this bimodal distribution. Eight genetically determined

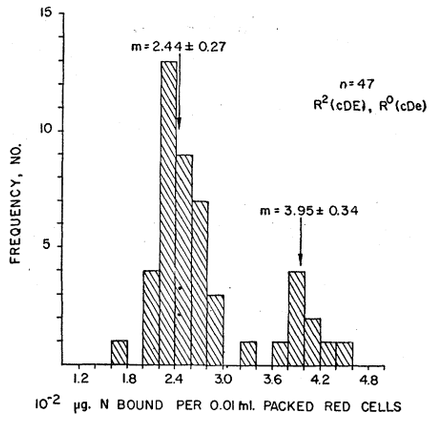

Fig. 1. Distribution of I^{131} anti-$Rh_0(D)$ nitrogen bound to 47 different $Rh_0(D)$ positive, rh′(C) negative red cells. The values shown are in units of 10^{-2} μg of nitrogen per 0.01 ml of centrifuged red cells with the mean and standard error of the mean shown for each group.

heterozygous $Rh_0(D)$ positive red cells had values which ranged from 1.47 to 2.48 (10^{-2} μg of N per 0.01 ml of red blood cells). These results indicate that the 2.44 antibody nitrogen peak represents the heterozygous state [$Rh_0(D)$, rh(d)] and that the 3.95 value corresponds to the homozygous state [$Rh_0(D)$, $Rh_0(D)$]. If certain assumptions are granted, namely uniformity of iodination of the globulins in the gamma globulin fraction and a molecular weight of 1.6×10^5 for the anti-$Rh_0(D)$, then the heterozygous $Rh_0(D)$ red cell contains about 6400 $Rh_0(D)$ antigen sites per cell and the homozygous cell about 10,300 per cell (*11*).

S. P. Masouredis
Department of Preventive Medicine and Cancer Research Institute, University of California Medical Center, San Francisco

References and Notes

1. R. R. Race and R. Sanger, *Brit. Med. Bull.* **15**, 99 (1959).
2. S. P. Masouredis, *J. Clin. Invest.* **38**, 279 (1959).
3. S. P. Masouredis, *ibid.* **38**, 1024 (1959).
4. R. R. Race and R. Sanger, *Blood Groups in Man* (Blackwell, Oxford, England, ed. 3, 1958).
5. Central Blood Bank of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, Pa., where this study was initiated.
6. J. J. McGovern, A. R. Jones, A. G. Steinberg, *New Engl. J. Med.* **253**, 308 (1955).
7. C. F. T. Mattern, F. S. Brackett, B. J. Olson, *J. Appl. Physiol.* **10**, 56 (1957).
8. The interest of Dr. R. C. Hamilton and Sister Marietta of St. Francis Hospital, Pittsburgh, in performing these determinations is gratefully acknowledged.
9. Mean and standard error of the mean are used throughout this report.
10. Dr. Paul Gaffney and Dr. William Chapman of Pittsburgh, Pa., generously made available their patients for this study.
11. The technical assistance of Cecile Ann Chi and Elizabeth Ferguson is gratefully acknowledged. This study was supported by grant H-5071 from the National Heart Institute, U.S. Public Health Service.

22 January 1960