References

1. J. Verduin, *Science* 121, 215 (1955).
2. A. Krogh, *J. Physiol.* 52, 391 (1919); *The Anatomy and Physiology of Capillaries* (Yale Univ. Press, New Haven, 1936).
3. H. A. Spoehr, *Photosynthesis* (Chemical Catalogue Co., New York, 1926).
4. T. Carlson, *J. Am. Chem. Soc.* 33, 1027 (1911).
5. G. Hüfner, *Wied. Ann.* 60, 134 (1897).
6. T. Carlson, in *International Critical Tables* (McGraw-Hill, New York, 1929), 5, p. 64.
7. R. Brdička and K. Wiesner, *Collection Czechoslovak Chem. Commun.* 12, 39 (1947).
8. I. M. Kolthoff and H. A. Laitinen, *Science* 92, 150 (1940).
9. I. M. Kolthoff and C. S. Miller, *J. Am. Chem. Soc.* 63, 1013 (1941).
10. P. W. Davies and F. Brink, Jr., *Rev. Sci. Instr.* 13, 524 (1942).
11. A. V. Hill, *Proc. Roy. Soc. London* B104, 39 (1929).
12. R. Höber *et al.*, *Physical Chemistry of Cells and Tissues* (J. and A. Churchill, London, 1947).
13. H. B. Bull, *Physical Biochemistry* (Wiley, New York, 1945).
14. I. M. Kolthoff and J. J. Lingane, *Polarography* (Interscience, New York, 1952), vol. 1.
15. W. M. Manning, *Ecology* 21, 509 (1940).
16. C. L. Prosser *et al.*, *Comparative Animal Physiology* (Saunders, Philadelphia, 1950).
17. G. E. Briggs and R. N. Robertson, *New Phytologist* 47, 265 (1948).
18. F. J. Roughton, *Proc. Roy. Soc. London* B140, 203 (1952).
19. W. O. James, *Plant Respiration* (Clarendon Press, Oxford, 1953).

13 June 1955

Correction

The ultrasonic dosages given in W. J. Fry *et al.*, "Ultrasonic lesions in the mammalian central nervous system" [*Science* **122**, 517 (1955)] should be corrected as follows: For the lesion illustrated in Fig. 1, the dosage was 40 atm acoustic pressure amplitude and $3.9(10^2)$ cm/sec acoustic particle velocity amplitude. For the lesions illustrated in Figs. 2 and 3, the dosage was 41 atm acoustic pressure amplitude and $4.0(10^2)$ cm/sec acoustic particle velocity amplitude.

W. J. Fry

Bioacoustics Laboratory, University of Illinois, Urbana

7 November 1955

Thomas Bradwardine His Tractatus de Proportionibus

I am sure both the author and the University of Wisconsin Press personnel welcomed the excellent review by Carl Boyer of H. Lamar Crosby, Jr.'s, recent volume on Thomas Bradwardine [*Science*, **122**, 562 (23 Sept. 1955)]. However, I believe one amendment to the review is in order. Boyer very graciously mentioned the work in medieval science being done at the University of Wisconsin, but in doing so he left the distinct impression that Crosby's volume was written here at Wisconsin in our department. As much as we would like to claim some part in the direction of the work that went into the writing of this volume, we must note that it was completed by Crosby under the stimulating guidance of Ernest Moody while the latter was at Columbia University. My only part in the volume was to recommend its consideration for publication by the University of Wisconsin Press and to add a foreword.

Marshall Clagett

Department of the History of Science, University of Wisconsin, Madison

24 October 1955

Separation of Complete and Incomplete Rh Antibodies by Centrifugation

The division of Rh antibodies into complete (saline agglutinins, bivalent) and incomplete (blocking, univalent) varieties is based on differences in their immunologic reactions. Physicochemical, electrophoretic, and biologic differences in these antibodies have been reviewed in recent publications from these laboratories, and additional data concerning their immunologic and electrophoretic nature have been presented (*1–3*). Although there are no experimental data dealing directly with the possibility of differences in molecular shape and size of Rh antibodies (*4*), Wiener has speculated that such differences probably exist (*5*). Previously, Deutsch *et al.* (*6*) and Pedersen (*7*), on the basis of ultracentrifuge studies of human serum, reported that β-isoagglutinins occurred to some extent in a "heavy" molecular weight fraction of serum globulin. In view of this work, an investigation was undertaken to determine whether Rh agglutinins could be separated from Rh incomplete antibodies by centrifugation. The present report presents the preliminary result of this investigation (*8*).

Serums were merely diluted with 1 vol of 1.0-percent sodium chloride solution, clarified by centrifugation at 4000 rev/min and then centrifuged for 7 hours at 40,000 rev/min in a Spinco No. 40 rotor with refrigeration. The first preparations consisted of four fractions representing four volumetric divisions of the 10-ml sample in each centrifuge tube. Fraction 1 consisted of the upper 3-ml portion, fraction 2 the next 3 ml, fraction 3 the next 3 ml and fraction 4 the remaining solution of about 1 ml plus the gelatinous pellet on the bottom.

The results of a typical fractionation in this preliminary series are shown in Table 1. The serum used in this run was prepared by mixing an incomplete anti-DE serum with a saline agglutinating anti-E serum in the ratio of 2.5 ml to 18.0 ml. The anti-DE serum was obtained from an O-Cde/cde individual who had been immunized to the Rh factor 13 years previously by a blood transfusion. Since then, there were repeated exposures to Rh antigen from pregnancies and more recently, following hysterectomy, from small injections of Rh positive blood as a volunteer donor. The serum had a titer in albumin of 1/4000 anti-D and 1/64 anti-E; there was no activity in saline. The anti-E saline agglutinating serum was obtained from an A-CDe/CDe individual immunized by cDE cells from blood transfusions and pregnancies. This serum's agglutinin titer was 1/512 in saline and 1/64 for incomplete antibodies, as estimated by the antiglobulin augmentation titer (*3*). The mixture of the two serums and the fractions produced by centrifugation resulted in titers shown in Table 1. Titrations were performed with O-CDe/cde, O-cdE/cde and B-cde/cde cells.

These preliminary results indicated that under the prescribed conditions of centrifugation, the saline agglutinating antibodies were sedimented more completely than were the incomplete antibodies. The saline agglutination reaction with the F-4 fraction was unusual in that the button of agglutinated cells could not be broken apart by the most vigorous shaking.

By repeated recycling of the F-4 fraction, and particularly the gelatinous pellet that separated out at the bottom of the centrifuge tube, it has been possible to separate the agglutinin from the incomplete antibody even more effectively.

Table 1. Antibody titers (expressed as dilutions of the original sample of serum) of serum fractions obtained by centrifugation.

Material	Protein concentration (%)	Agglutinins				
		Incomplete		Complete		
		anti-D	anti-E	anti-D	anti-E	anti-B
Serum	—	1/256	1/256	0	1/512	1/32
F-1	0.85	1/16	1/8	0	0	0
F-2	2.25	1/256	1/128	0	1/16	0
F-3	3.25	1/256	1/256	0	1/128	1/1
F-4	8.92	1/2000	±	0	1/4000	1/256

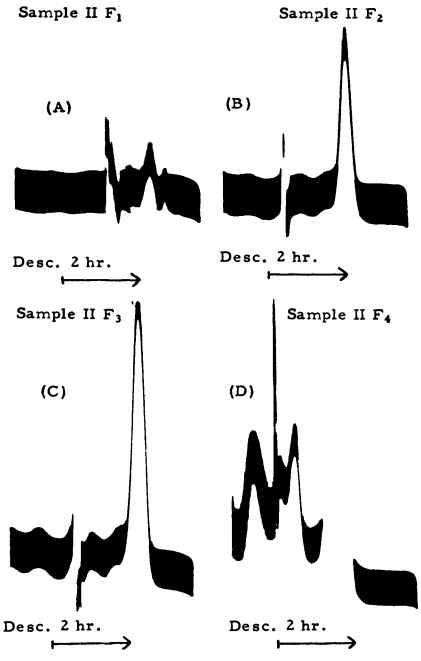

Fig. 1. Electrophoretic patterns of descending boundaries of human serum fractions obtained by centrifugation.

One serum from an O-cde/cde individual that failed to show any saline agglutinin antibodies was treated as described here. The pellet that formed after 7 hours at 40,000 rev/min, upon resolution and testing with O-CDe/cde cells, gave a saline agglutinin titer of 1/64. The pellet was redissolved in a volume of saline equal to the volume of the original diluted serum from which it was obtained, and the solution was centrifuged again. The sedimented material gave a saline agglutinin titer of 1/128 with stronger reactions in all dilutions.

Investigations of the physical properties of the various fractions have not been completed, but preliminary data showed the following properties. The F-1 fractions were colorless and had a slight opalescence. The remaining successive fractions had a yellow color that increased in intensity until it became dark yellowish-orange in F-4. The material that centrifuged out as a gelatinous pellet on the bottom was essentially colorless.

Electrophoretic patterns of the four fractions listed in Table 1 are shown in Fig. 1. The protein concentrations of the F-1, F-2, F-3, and F-4 fractions were 0.43, 1.13, 1.63 and 4.46 percent, respectively. The solution was in barbital buffer, *p*H 8.6 and $\mu = 0.1$. The F-1 fraction was obviously different from the original serum, but the remaining three fractions indicated no particular abnormalities except for the high protein concentration.

Sedimentation analysis was made of the F-4 fraction only. The pattern of a 4.5-percent protein solution in 1.0-percent sodium chloride is given in Fig. 2. This pattern was obtained at 40 minutes and 250,000 *g*. A heavy component comprising about 5 percent of the total protein with a sedimentation constant of about 17 S (uncorrected) separated at this time. The slower sedimenting material later separated into two components to give about 75 percent 4.5 S and 20 percent 7 S.

After the F-4 fraction was redissolved in 10 ml of 1.0-percent sodium chloride and was recentrifuged for 2 hours, and this step was repeated twice again, the principal electrophoretic components were alpha-2 globulin and albumin. The sedimentation pattern shown in Fig. 3 was taken at 24 minutes and 59,780 rev/min. The solution contained 0.7 percent protein. The $S_{20,w}$ for the fast component was 19.4 S. The slow component resolved later into equal amounts of two components having $S_{20,w}$ values of 6.8 S and 4.2 S, respectively.

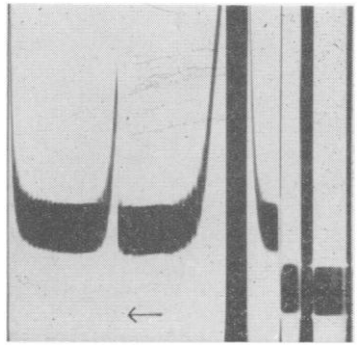

Fig. 2. A sedimentation velocity pattern of fraction 4 obtained by centrifugation of human serum.

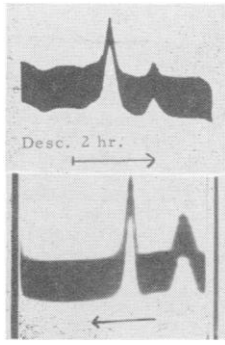

Fig. 3. (Top) Electrophoretic pattern of an F-4 fraction after three recyclings; (bottom) sedimentation pattern of same material.

These preliminary experiments clearly indicated that Rh saline agglutinins sedimented at a faster rate than the incomplete type of antibodies. They also suggest that practical separation of agglutinating and nonagglutinating types of antibodies can be accomplished by centrifugation.

DAN H. CAMPBELL
Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena
PHILLIP STURGEON*
Department of Research, Los Angeles Children's Hospital and *Department of Pediatrics, University of California School of Medicine, Los Angeles*
JEROME R. VINOGRAD
Gates and Crellin Laboratories of Chemistry

References and Notes

1. J. R. Cann *et al.*, *J. Immunol.* 68, 243 (1952).
2. P. Sturgeon and R. A. Brown, *ibid.* 68, 287 (1952).
3. P. Sturgeon, *ibid.* 73, 212 (1954).
4. P. Levine, *J. Heredity* 14, 251 (1954).
5. A. S. Wiener, *Ann. Allergy* 10, 535 (1952).
6. H. F. Deutsch *et al.*, *J. Immunol.* 56, 183 (1947).
7. K. O. Pedersen, *Ultracentrifugal Studies on Serum and Serum Fractions* (Almquist and Wiksell, Uppsala, 1945).
8. This work was supported by the National Institutes of Health. Contribution No. 2011. We wish to acknowledge the technical assistance of Alexander Kahn of Gates and Crellin Laboratories of Chemistry.
* Markle scholar in medical science.

14 July 1955

Charles G. Darwin, my predecessor in my Edinburgh Chair (of Natural Philosophy), once said something like this: "The ordinary man can see a thing an inch in front of his nose; a few can see things 2 inches distant; if anyone can see it at 3 inches, he is a man of genius."—MAX BORN, *Experiment and Theory in Physics* (Cambridge, 1943), p. 34.