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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.

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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.

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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