Hydrocortisone produced higher and more prolonged plasma 17-OHCS elevations than did equivalent doses of hydrocortisone administered orally. The half-life of Δ^1-hydrocortisone was found to be 1.37 times that of hydrocortisone. Intravenous administration of these steroids as the hemisuccinates gave lower but somewhat more prolonged elevations in plasma 17-OHCS concentrations than the corresponding free alcohols. It was concluded that Δ^1-hydrocortisone was metabolized more slowly than hydrocortisone and suggested that this may explain, at least in part, the enhanced potency of the Δ^1-steroid.

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Titration of Influenza Virus and Viral Antibodies by Preserved Human Erythrocytes.* (22760)

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Procedures for titration of influenza viruses and their antibodies by use of chicken and human erythrocytes in tube hemagglutination (HA) and hemagglutination inhibition (HAI) tests have been well established(1-8). Most of the presently used tube HA and HAI titrations are read by the pattern method(5) and employ freshly washed chicken or human erythrocyte suspensions. Flick(9) reported the use of formalin treated human erythrocytes in studying the hemagglutinating capacity of influenza A virus; however, these cells exhibited spontaneous agglutination and could not be read by the pattern technic. Previous work in this laboratory(10) has demonstrated effective preservation of sheep erythrocytes for use in various serological systems. This report describes the preparation and use of preserved human erythrocytes in the titration of influenza viruses and their antibodies.

Materials and methods. Diluent used in all serological tests consisted of 0.137 M NaCl, 0.01 M Na_2HPO_4, and 0.003 M KH_2PO_4 in distilled water, the pH being 7.2 $\pm$ 0.05. Blood group O, Rh+ human erythrocytes were used in all tests. Those used as fresh cell suspensions were obtained by bleeding into an equal volume of Alsever's solution, and before use they were washed 3 times in 40 volumes of diluent and resuspended to a final concentration of 0.5%. Egg-adapted departmental strains of the type A (PR8 and IA43),

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type A' (Benson and USD 2/53), and type B (Lee) influenza viruses were used throughout this study, representing pools of chorioallantoic and amniotic fluid viruses respectively. IA43 strain was originally obtained from Dr. A. P. McKee, University of Iowa, and the Benson strain from Dr. Jonas Salk, University of Pittsburgh. Virus preparations were stored at −20°C. Antiviral sera were prepared as pools from several immunized rabbits and stored at −20°C. Normal rabbit serum was prepared in a similar fashion from non-immunized rabbits. Portions of pre-immunization sera, as well as immune sera, were treated with receptor-destroying enzymes (RDE) prepared from filtrates of the 4Z strain of *Vibrio cholerae* according to the method suggested by the Expert Committee on Influenza(8). Following treatment with RDE all sera were inactivated by heat at 56°C for one hour. *Preparation of Preserved Human Erythrocytes (PHE).* PHE were prepared in a manner similar to that previously reported for preparation of sheep erythrocytes(10), with two major exceptions. Defibrinated group O, Rh+ human blood was always used, and erythrocytes were exposed to formalin twice. Forty ml of 50% formalin-diluent (equal volumes of formalin and twice concentrated diluent above) were rapidly mixed with 100 ml defibrinated human blood previously diluted with an equal volume of diluent. Formalinized blood was placed in a 37°C waterbath for 1½ hours with frequent mixing. Cells were then centrifuged and washed twice in at least 50 volumes of diluent each time, taking care to remove and discard by aspiration the top thin light colored coat after the first centrifugation. The sediment was reconstituted to 100 ml in diluent, again rapidly mixed with 40 ml of 50% formalin diluent, and held in the 37°C waterbath for another 1½ hour interval. The cells were then centrifuged and washed 6 times in at least 50 volumes of diluent each time. The washed and packed erythrocytes were then resuspended in diluent containing 0.3% formalin to approximately 10% by volume. Cell volume was then more accurately adjusted to 4% by centrifuging 5.0 ml of the 10% suspension in a Kolmer or vaccine tube and determining the volume of packed cells. As an additional precaution against contamination during use of the cells after preparation, 0.25 ml of a dye mixture (1 part of 1% aqueous crystal violet plus 2 parts of 1% aqueous brilliant green) was added and mixed with each 100 ml of 4% suspension. Addition of the dye also facilitated reading. PHE was dispensed in screw-cap bottles or dropper-vials and stored in the refrigerator. Tube HA and HAI titrations were carried out with 0.5% suspensions of both freshly washed human erythrocytes and PHE as described by the Expert Committee on Influenza(8), with the exception that HAI titers are expressed as reciprocals of final dilutions of sera. *Rapid Plate Titration of Influenza Virus.* CAF virus, undiluted and diluted 10^{-1} and 10^{-2}, was pipetted onto appropriate 2 cm rings on a glass plate, each virus dilution in volumes of .08, .04, .02, and .01 ml from left to right. Virus was pipetted with Bangs pipettes or 0.2 ml pipettes graduated in 0.01 ml divisions, and 1 drop (.025 ml) of PHE was immediately added to each ring. All 4 volumes of each virus dilution were mixed with PHE with a separate applicator stick from right to left, rotated for 1 to 2 minutes by hand and read macroscopically against a dark background over a strong oblique light in the usual manner of reading rapid plate bacterial agglutination tests. Each virus was run simultaneously with a diluent or CAF control. Undiluted virus volumes of .08, .04, .02, and .01 ml were found to correspond to virus dilutions of 1:100, 1:200, 1:400, and 1:800 respectively in the tube HA procedure; volumes of virus diluted 10^{-1} corresponded to tube HA virus dilutions 10 times those with undiluted virus, etc. HA titer of the virus was expressed as the highest corresponding dilution showing definite clumping, with negative diluent or CAF controls showing fine suspensions of cells, smoothly dispersed. Thus, if .02 ml of virus diluted 10^{-1} was the smallest amount showing definite clumping, the titer would be 1:4000. *Rapid Plate Titration of Virus Antibody.* All viral antisera were titrated against dilutions of CAF virus calculated to contain 5 HA units per .02 ml dose. Thus, if the virus titer were 1:2000 (2000 HA/ml), 5 HA would be contained in .02 ml

undiluted virus; if virus titer were 1:8000, .02 ml virus diluted 1:4 would contain 5 HA. Inactivated serum, undiluted and diluted 10^{-1} and 10^{-2}, was pipetted onto appropriate rings on a glass plate, each serum dilution in volumes of .08, .04, .02, and .01 ml. Five HA units virus in .02 ml were then delivered to one side of each quantity of serum and 1 drop of PHE added. Reagents were mixed and read as indicated above. Undiluted serum volumes were found to correspond to tube HAI serum dilutions of 1:20, 1:40, 1:80, and 1:160 respectively, and decimal dilutions of serum corresponded to 10 fold tube HAI serum dilutions. Serum titer was expressed as highest corresponding dilution showing complete inhibition of agglutination, with diluent or negative serum controls showing marked agglutination.

Results. Preliminary observations on preparation of PHE paralleled those with preserved sheep erythrocytes(10). Two exposures to a final concentration of 14% formalin seemed optimal. Microscopically, in both stained and diluent preparations, the preserved erythrocytes stained well with some erythrocytes showing evidence of crenation. The addition of formalin diluent to whole blood rather than washed erythrocytes seemed advantageous; when washed erythrocytes were used, as reported by Flick(9), considerable lysis occurred. Furthermore, PHE showed considerable tendency to autoagglutinate when washed erythrocytes were exposed to final concentrations of formalin approaching 50%. PHE was found to be stable for several months, both from the standpoint of hemolysis and reactivity with influenza virus. Stable suspensions of PHE have been consistently prepared and maintained for a minimum of one year in the refrigerator. PHE suspensions were resistant to lysis in distilled water. A study of the optimal concentration of PHE for use in rapid plate procedures revealed that much less than a 4% suspension was too light to be easily read and too sensitive in the sense that the suspension seemed too granular. Much greater than 4% suspension was too heavy for easy reading and proved insufficiently sensitive with respect to the assigned virus dilutions when titrated by the rapid plate technic against known viruses. A 4% suspension was consistently found to yield end points in the rapid plate procedure in close agreement with tube hemagglutination titers, when virus dilutions were assigned to PHE-virus mixtures as described in above procedure for rapid plate titration of virus. PHE was used as 0.5% suspensions in all tube HA and HAI tests, in conformity with established procedures employing freshly washed human erythrocytes.

TABLE I. Comparison between PHE and Fresh Human Erythrocytes in Tube HA Titrations.

Virus	Virus pool	HA, units/ml — Fresh RBC	PHE
PR8 (A)	1	2560	1280
	2	6400	6400
	3	5620	7680
IA43 (A)	1	2560	5120
	2	9600	12800
	3	15260	30520
IA43 (A) inact.*	2	3200	6400
Benson (A′)	1	1920	2560
	2	3200	6400
USD 2/53 (A′)		300	400
Lee (B)	1	120	120
	2	1600	2400
	3	1920	1280

* Inactivated at 56°C for 1 hr.

It is apparent (Table I) that PHE compared favorably with suspensions of freshly washed human erythrocytes in titrating various influenza viruses. In like manner, PHE and fresh human erythrocytes showed comparable activity in HAI titrations of antiviral sera produced in rabbits (Table II). The rapid plate technic employing PHE showed good correlation with tube procedures in both HA and HAI titrations (Tables III, IV).

TABLE II. Comparison between PHE and Fresh Human Erythrocytes in Tube HAI Titrations.

Antivirus*	RBC	Virus — PR8	Benson	Lee
PR8 (A)	Fresh	10240†	1280	320
	PHE	20480	1280	320
Benson (A′)	Fresh	1280	5120	640
	PHE	640	5120	640
Lee (B)	Fresh	1280	640	10240
	PHE	640	1280	10240

* Antisera inactivated at 56°C for 30 min., but not RDE treated.

† Titers expressed as HAI units/ml.

TABLE III. Comparison between Tube and Rapid Plate HA Titrations Using PHE.

Virus	Virus pool	HA, units/ml Tube	Rapid
PR8 (A)	5	2560	2000
	6	6400	4000
	3	7680	8000
Lee (B)	5	2560	2000
	6	6400	4000
	3	1280	1000
JA43 (A)	3	30520	20000
Benson (A')	3	2560	2000

Discussion. Data in Table I illustrate typical findings in many tube HA titrations using both PHE and fresh human erythrocytes, discrepancies between which rarely have exceeded 1 full tube dilution. Good correlation exists when titrating A, A', and B types of influenza virus, and it is interesting to note that PHE is equally susceptible to agglutination by influenza virus inactivated by heat. PHE shows equally good correlation with freshly washed human erythrocytes in both sensitivity and specificity in reciprocal HAI titrations of antiviral sera untreated (Table II) and treated with RDE (Table IV). Additional experiments involving tube and rapid plate HA and HAI titrations with both PHE and freshly washed human erythrocytes on serially diluted virus and antiviral sera have shown equally good correlation. PHE offers the distinct advantages of employing constant cellular antigens and eliminating the daily washing of erythrocytes in viral and antiviral titrations. In our experience the presently described rapid plate procedure using PHE provides an extremely rapid and sufficiently accurate method for titrating influenza virus and viral antibodies.

TABLE IV. Comparison between Tube and Rapid Plate HAI Titrations Using PHE.

Antivirus*	Titration	Virus: PR8	Virus: Lee
PR8 (A)	Tube	10240†	< 40
	Rapid	4000	<100
Lee (B)	Tube	< 40	5120
	Rapid	<100	4000
Normal rabbit serum	Tube	< 40	< 40
	Rapid	<100	<100

* All sera RDE treated and inactivated at 56°C for 1 hr.

† Titers expressed as HAI units/ml.

Summary. The preparation of PHE and their use in both tube and rapid plate HA and HAI procedures for the titration of influenza virus and viral antibodies are described. Such preserved cells have been stable, with respect to both resistance to lysis and sensitivity to hemagglutination by influenza virus, for a year. PHE showed good correlation in sensitivity with freshly washed human erythrocytes, and the rapid plate technic using PHE correlated well with tube HA and HAI procedures.

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