12. Hunt, *Ind. and Eng. Chem.*, 1942, v24, 361, 836.

13. Kesten, H. D., Mulinos, M. G., and Posteran, L., *Arch. Path.*, 1939, v27, 447.

14. Latven, A. R., and Molitor, H., *J. Pharm. and Exp. Ther.*, 1939, v65, 89.

15. Laug, E. P., Calvery, H. O., Morris, H. J., and Woodward, G., *J. of Ind. Hyg. and Toxicol.*, 1939, v21, 173.

16. Launoy, M., *J. Pharm. et Chim.*, 1942, v9, 99.

17. Lehman, A. J., and Newman, H. W., *J. Pharm. and Exp. Ther.*, 1937, v60, 312.

18. Morris, H. J., Nelson, A. A., and Calvery, H. O., *J. Pharm. and Exp. Ther.*, 1942, v74, 266.

19. Newman, H. W., and Lehman, A., PROC. SOC. EXP. BIOL. AND MED., 1937, v35, 601.

20. Newman, H. W., Van Winkle, W., Kennedy, N. K., and Morton, M. C., *J. Pharm. and Exp. Ther.*, 1940, v68, 194.

21. Portella, A., in press.

22. Randolph, T. G., and Mallery, O. T., *J. Lab. Clin. Med.*, 1944, v29, 197.

23. Robertson, O. H., Loosli, C. G., Puck, Th. T., Wise, H., Lemon, H. M., and Lester, W., *J. Pharm. and Exp. Ther.*, 1947, v91, 52.

24. Seidenfeld, M. A., and Hanzlik, P. J., *J. Pharm. and Exp. Ther.*, 1932, v44, 109.

25. Smith, H. F., Seaton, J., Fischer, L., *Ind. Hygiene and Toxicol.*, 1941, v25, 259.

26. Winkle Van, N. J., *Am. J. Pharm.*, 1941, v72, 344.

27. Winkle Van, N. J., and Newman H. W., *Am. J. Pharm.*, 1937, v109, 478.

28. Weatherby, J. H., and Haag, H. B., *J. Am. Pharm. Assn.*, 1938, v27, 466.

29. Whitlock, G. P., Guerrant, N. B., and Dutcher, R. A., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 124.

Received June 22, 1951. P.S.E.B.M., 1951, v78.

Detection of Antibodies by Polyvinylpyrrolidone* II. Comparison of Sensitivity of Antihuman Globulin and PVP. (19178)

CRICHTON MCNEIL AND ELMER F. TRENTELMAN.
From the Laboratories of Holy Cross Hospital,† Salt Lake City, Utah.

This work, comparing the sensitivity of the Coomb's test and PVP titration to detect anti Rh antibody, is a natural outgrowth of our original publication concerning polyvinylpyrrolidone as a suitable antiserum diluent(1). Most serologists now concede that the Coomb's test excels other methods of incomplete antibody detection both in sharpness of reaction and sensitivity(2-6). Therefore, if PVP technics compare favorably with that of employing anti-human globulin for detection of antibodies, further expansion of the uses of PVP might be justified.

Materials. (1) Antisera—anti Rho' (CD) serum was used throughout the titrations. All of this reagent came from the same lot and has an albumin titer of approximately 1:128. The saline agglutinin titer was almost nil. (2) Red blood cell suspensions were obtained from a single donor whose type was O Rho' (CDe). The cells were drawn less than 5 days prior to use. 20% bovine albumin (Armour & Co.) in 0.9% saline was used as a diluent. (3) 10% Polyvinylpyrrolidone (PVP) was prepared as previously described (1) buffered to pH 6.9-7.0. (4) Anti-human globulin was obtained from a commercial source (Ortho) having passed the requirement for U. S. license. (5) Albumin 20% was prepared from 30% bovine albumin fraction II (Armour & Co.).

Methods. Titrations were carried out, using the serial dilution tube (size 75 x 10 mm) technic. In each titration, 0.2 ml of anti Rho' blocking serum was mixed with an equal quantity of diluent, as indicated in Table I, and 0.2 ml was transferred to the succeeding tube, which also contained an equal amount of diluent. The double dilution of serum was followed by the addition of 0.2 ml of a 4% fresh washed suspension of Rho' (CDe) red blood cells in saline or 20% albumin as indicated by the table. Mixing and incubation of tubes at 37°C water bath for one hour was

* Polyvinylpyrrolidone kindly furnished by General Aniline & Film Corp., Easton, Pa.

† Aided by Grant from Holy Cross Hospital Research Foundation.

TABLE I. Comparison of Sensitivity of Antibody Detection Using Indirect Coomb's Technic. Anti Rho' (CD) blocking serum diluted as follows:

Titer	Section 1, saline diluted serum					Section 2, 20% albumin diluted serum					Section 3, 10% PVP diluted serum				
	Cells in saline Tested with saline	Cells in saline Tested with albumin	Cells in saline Tested with PVP	Cells in saline Tested with antihuman globulin	Cells in albumin Washed with saline. Tested with saline	Cells in albumin Washed with saline. Tested with albumin	Cells in albumin Washed with saline. Tested with PVP	Cells in albumin Washed with saline. Tested with antihuman globulin	Cells in saline Tested with saline	Cells in saline Tested with albumin	Cells in saline Tested with PVP	Cells in saline Tested with antihuman globulin	Alternate technic. Serum diluted with PVP	Immed. centrif. 1 min
2	++	+	+	4	2+	2	2	4	2		3	4	4	4
4	++	|	+	4	++	+	+	4	2		3	4	4	4
8	++	|	+	4	++	+	+	4	2		3	4	4	4
16	++	|	+	4	++	+	+	4	2		3	4	4	4
32	++	|	+	4	++	+	+	4	2		3	4	4	4
64	++	|	+	4	++	+	+	4	2		3	4	4	4
128	++	|	+	4	++	+	+	4	2		3	4	4	4
256	+	|	+	3	+	+	+	3	2		3	4	4	4
512	|	|	|	2	|	+	|	2	|	|	|	4	4	4
1024	|	|	|	|	|	+	|	|	|	|	|	2	+	|
2048	|	|	|	|	|	+	|	|	|	|	|	|	|	|

Controls: 1. Rh neg (cde) cells in serum (CD)—Coomb's tested—neg. 2. Rh neg (cde) cells in PVP—PVP tested—neg. 3. Rh pos cells (CDe) in albumin—Coomb's tested—neg. 4. Rh pos cells (CDe) in saline—Coomb's tested—neg.

followed by repeated thorough washing (3x) of the cells with 0.9% saline. Finally each series of tubes received 2 drops of either saline, albumin, 10% PVP or antihuman globulin, as indicated in the table. Re-incubation for one hour and centrifugation 1 minute at 1500 r.p.m. followed by gently tapping the tube for the customary reading, completed the test.

Alternate PVP technic. A rapid tube technic utilizing PVP was developed for comparison with the indirect Coomb's technic. The same serum and cell suspension was used as follows: the serum was titered by the same double dilution serial technic, using 10% PVP as diluent. Each tube then received an equal volume (0.2 ml) of unwashed 4% saline suspended red cell antigen. The tubes were shaken to assure mixture and centrifuged immediately for 1 minute at 1500 r.p.m. Readings were made promptly after centrifugation. The entire test could be performed in less than 10 minutes.

Results. The table of results is expressed in 3 sections for simplicity and clarity. Section 1 shows the results of the quantitative Coomb's technic using saline as antiserum diluent. Each vertical column represents a different substance added at the end as a *"testing"* reagent. Section 2 represents antiserum diluted with albumin and in Section 3, PVP was used as diluent.

Inspection of the table will reveal the following facts:

Section 1—Cells "coated" by antisera diluted in saline react poorly in saline, fair in albumin, fair in PVP and good in antihuman globulin.

Section 2—Cells "coated" by antisera diluted in albumin react poorly in saline, fair in albumin, good in both PVP and anti-human globulin.

Section 3—Cells "coated" by antisera diluted in PVP react poorly in saline and albumin, good in both PVP and anti-human globulin.

Discussion. The sensitivity range of detecting antibody "coated" erythrocytes using PVP compares favorably with antihuman globulin, though slightly less potent. When the cells are incubated in saline medium the

action of PVP is poor, but in either albumin or PVP medium the sensitivity is good. The alternate simple technic given in Section 3 of the Table appears to give excellent results and avoids the cumbersome washing and incubation which is necessary in the indirect Coomb's test.

Although our case studies of sensitized mothers and infants carrying anti Rh antibodies is incomplete, the indication is that PVP detects these antibodies in rather high dilution even though albumin technics are negative or weakly positive. In addition, we have revealed an abnormal antibody to be present in the serum of a young woman who has been clinically diagnosed as lupus erythematosus. PVP revealed this antibody in equal titer to anti-human globulin, whereas saline and albumin titrations were completely negative. A similar case is reported by Callender(7). It appears that polyvinylpyrrilodone offers a simple, economical and sensitive method of Rh antibody detection.

Summary. (1) Using the indirect Coomb's technic we have found that 10% PVP offers results comparable to anti-human globulin when dilutions are carried out in albumin or PVP. (2) An alternate rapid PVP tube test (of equal sensitivity to antihuman globulin) is described. (3) In certain "collagen" or hypersensitivity diseases, PVP may detect an agglutinating antibody on homologous erythrocytes.

1. McNeil, C., Trentelman, E. F., in press.
2. Coombs, R. R., Mourant, A. F., and Race, R. R., *Lancet,* 1946, v250, 264.
3. Race, R. R., Sanger, Ruth, *Blood Groups in Man,* 1950. Charles C. Thomas, Springfield, Ill.
4. Stratton, F., and Ranton, P. H., *Brit. Med. J.,* 1949, v2, 682.
5. Hill, J. M., Haberman, Sol., and Jones, F., *Blood,* Special Issue No. 2, 1948.
6. Witebsky, E., Mohn, J. F., *J. Lab. and Clin. Med.,* 1948, v33, 1353.
7. Callender, Sheila T., and Race, R. R., *Ann. Engen., London,* 1946, v13, 102.

Received September 4, 1951. P.S.E.B.M., 1951, v78.

Effects of X Irradiation on Renal Function in Newly-Hatched Chicks. (19179)

S. P. STEARNER, E. J. B. CHRISTIAN, AND A. M. BRUES.
From the Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Ill.

The effects of X irradiation on young birds is dependent on dose rate as well as on total dose(1,2). Chicks and ducklings given 1000 r within a period of 30 minutes showed 75 to 80% mortality in the initial period (24 to 48 hours) after irradiation. When the same total dose was delivered over a 3-hour period, few deaths occurred until 5 to 7 days after exposure. Birds that died in the initial period showed necrosis of the renal tubules and accumulation of urate crystals in and around the tubules. A few individuals showed a urate polyserositis, in which a thin layer of urate crystals covered all serous membranes. Uric acid is the principal end product of protein metabolism in birds, and its precipitation throughout the body indicated a rapidly developing azotemia. This condition may be assumed to result either from renal failure or from an increased rate of nitrogen metabolism without a corresponding increase in excretion. The experiments reported here were concerned with the effects of X irradiation on renal function in the newly-hatched chick, as determined by estimations of blood urate levels and excretion of uric acid and phenol red.

Materials. White leghorn chicks, weighing 35 to 45 g, were treated 2 to 4 days after hatching. The irradiation dose was 1000 r, delivered at 6 r or at 43 r per minute. The conditions of irradiation were: 200 kv, 15 ma, 0.5 mm Cu and 3.0 mm Bakelite filters. Variation in dose rate was obtained by vary-