horse serum. 2. Killed timothy bacilli added to the water-in-oil emulsion promote both antibody formation and sensitization almost as effectively as killed tubercle bacilli. 3. Under the conditions of this experiment killed timothy bacilli sensitize to tuberculin.

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Agglutination of Circulating Leukocytes by Antileukocytic Sera.

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Several investigators had produced antisera against animal and human leukocytes,[1-6] but no successful attempt has been made to test and titrate the antileukocytic serum by agglutination. Some of the workers stated that the cytotoxic qualities of serum prevent a correct evaluation by agglutination.[2,4,7,8] Hueper and Russell[4] expressed the view that tissue culture is the only satisfactory method for correct titration of antileukocytic serum. In our experiments herein reported we were more successful and are able to present a method of agglutinating circulatory leukocytes by antileukocytic sera and of titrating the antisera.

Leukocytic agglutinins were produced in Chinchilla rabbits by intravenous injections of leukocytes secured from normal and leukemic individuals. Normal leukocytes were obtained from blood bank material used for preparation of plasma. The blood was centrifuged, the buff layer removed and washed several times. The animals received a total of 12 to 14 injections of 11,700,000 to 20,100,000 cells suspended in one cc of normal salt solution, twice weekly. Testing was done on the serum pooled from at least 3 animals. Blood was obtained by cardiac puncture in volumes of 10 to 15 cc. When agglutinins were demonstrated in a dilution of 1:2560 the antiserum was considered satisfactory for titration. Antisera were developed against lymphocytes and against cells of the granulocytic series of mature and immature types by the use of blood from normal individuals and from those with lymphoid and myeloid leukemia. Normal rabbit serum and normal salt solution were used as controls for the antisera.

Titrations were set up in a series of 10 x 75 mm test tubes. Blood was collected and mixed with a dry anticoagulant of ammonium and potassium oxalate, centrifuged and the buff layer pipetted off. The leukocytes were suspended in and the antisera diluted with normal salt solution. The necessity for the presence of electrolytes for the visible phase of agglutination has been demonstrated.[9,10] To ascertain the optimum number of leukocytes for an agglutination test, the cell concentration was varied and the volume of antiserum was kept constant. It was determined that 0.05 cc of normal salt solution containing 27,000 cells per cu mm constituted a satisfactory suspension. The leukocyte suspension was added to 0.5 cc volumes of inactivated antiserum diluted with normal salt solution in dilutions of from

[1] Ledingham, J. C. G., and Bedson, S. P., *Lancet*, 1915, **1**, 311.

[2] Lindström, G. A., *Acta Med. Scandinav.*, Supp., 1927, 22.

[3] Matsumo, M., *Tohoku J. Exp. Med.*, 1932, **19**, 168.

[4] Hueper, W. C., and Russell, M. A., *Arch. Path.*, 1932, **13**, 584.

[5] Chew, W. B., Stephens, D. J., and Lawrence, J. S., *J. Immunol.*, 1936, **30**, 301.

[6] Chew, W. B., and Lawrence, J. S., *J. Immunol.*, 1937, **33**, 271.

[7] Lambert, R. A., and Hanes, F. M., *J. Exp. Med.*, 1911, **14**, 453.

[8] Foot, N. C., *Centralbl. f. allg. Path. u. path. Anat.*, 1912, **23**, 578.

[9] Bordet, J., *Traité de l'Immunité*, Paris, 1920.

[10] Marrack, J. R., *The Chemistry of Antigens and Antibodies*, Med. Res. Council, Spec. Rep. Ser. 194, London, 1934.

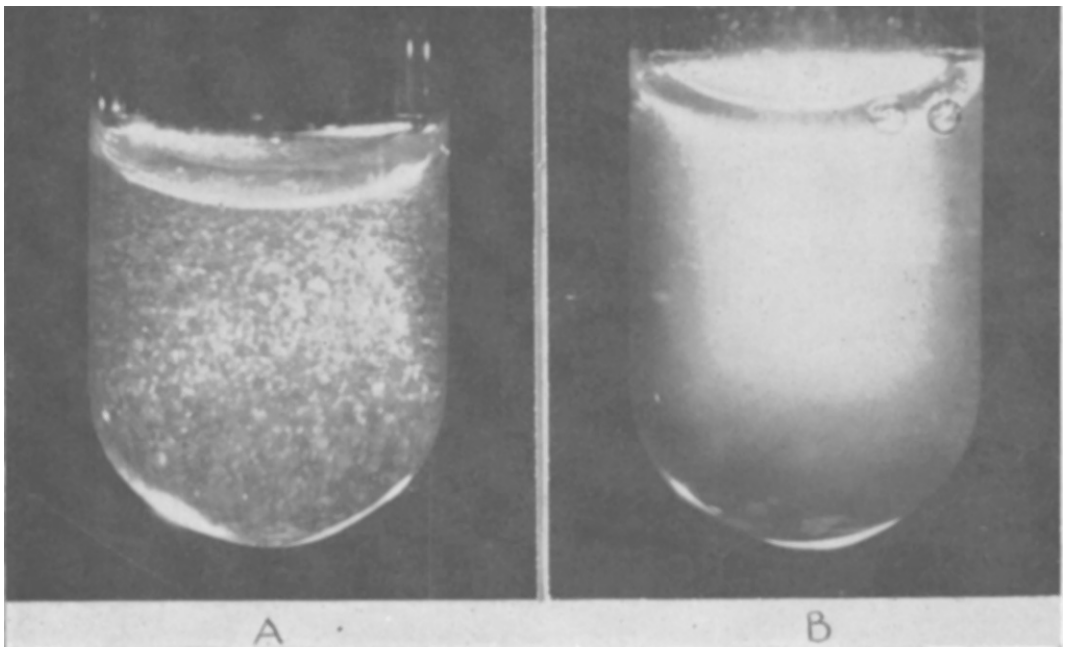

FIG. 1.
Gross appearance of agglutination of blood leukocytes by anti-leukocytic serum in a dilution of 1:2560. A. Positive reaction. B. Negative reaction.

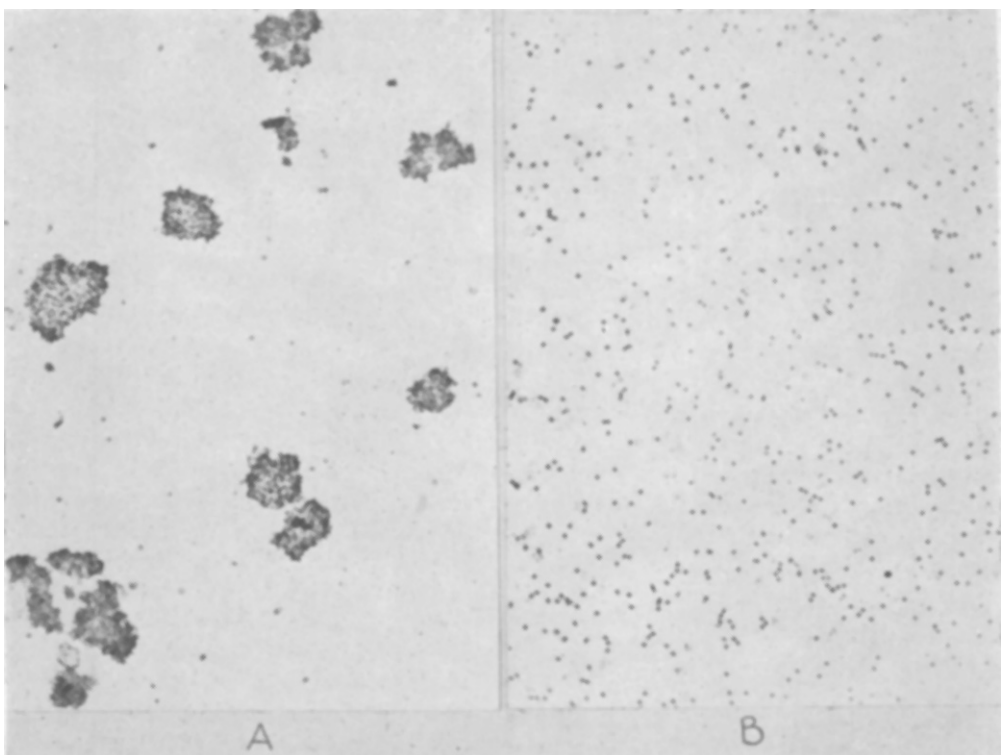

FIG. 2.
Microscopic appearance of agglutination of blood leukocytes by anti-leukocytic serum in a dilution of 1:2560. A. Positive reaction. B. Negative reaction.

1:10 to 1:5120. After shaking the test tubes for 3 minutes, they were placed in a water bath at 37°C for one hour. At the end of that time the tubes were shaken again and placed in the refrigerator over night.

Readings were made in the morning. After gentle agitation of the test tubes, presence of cell aggregates was determined first grossly under proper lighting conditions. A negative reaction was seen as a diffuse cloudiness. Agglutination varied in intensity depending on the original titer of the antiserum and the dilution. A strong reaction consisted in the presence of large clumps. As the agglutinin titer of the antiserum decreased, the cell aggregates became smaller and fewer.

Microscopic readings were made by pipetting a drop of the leukocyte-antiserum mixture onto a slide and examining it with a 16 mm objective. Clumping of the leukocytes with loss of identity of the individual cell constituted a positive reaction. Partial or complete disintegration of the cell architecture could be seen in the stronger reactions. In many instances the "prozone" phenomenon was encountered.

The technic of the test is not overly simple. The natural tendency of leukocytes, especially from normal people, to clump tends to produce false agglutination. After some experience, such reactions can be recognized grossly. In most instances of false agglutination, the leukocytes can be observed under the microscope to be held together loosely by strands of fibrin.

Summary. A method of testing and titrating antileukocytic sera by agglutination is presented. The procedure offers a relatively simple method of studying the immunochemical differences between normal and leukemic leukocytes and between lymphocytes and polymorphonuclear leukocytes.

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The Static Intrapelvic Pressure of the Hydronephrotic Kidney.

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Following complete ureteral obstruction the hydronephrotic kidney continues to secrete urine. The exact quantity of the glomerular filtrate elaborated and the mode of reabsorption is not known with certainty. The reabsorption of urine by pyelovenous refluxes or pyelotubular backflow is a possibility.[1,2,3] Ahlström[4] points out that with an increased intrapelvic pressure, rupture of the pelvis with an outpouring of its contents into the venous system is not, in itself, remarkable. Only when this reflux can be shown to occur at pressures which are maintained *in vitro* by urine secretion with obstruction to outflow can it be of pathogenic importance.[4] Despite the crucial importance of intrapelvic pressures, accurate determinations by physiologic methods have not been found in the literature, by the writer.

Experimental. By the use of the Null Point Manometer which is on occasion employed in various physiological laboratories we have determined the static intrapelvic pressure in 8 rabbits with unilateral hydronephrosis of varying intervals from 3 to 63 days. The left ureter of the rabbits was doubly tied and severed; intravenous sodium pentothal anesthesia, and aseptic surgical precautions were employed. The needle attached to the left arm of the Null Point Manometer System is inserted into the hydronephrotic sac through the thin wing of parenchyma near the edge

1 Hinman, F., and Lee-Brown, R. K., *J. Am. Med. Assn.*, 1924, **82**, 607.

2 Hinman, F., and Vecki, M., *J. Urol.*, 1926, **15**, 267.

3 Hinman, F., *Surg., Gynec. and Obstet.*, 1934, **58**, 356.

4 Ahlström, C. G., *Acta chir. Scandinav.*, 1934, **75**, 162.