Summary. 1. The correlation between the blood level of sodium pentobarbital and the duration of anesthesia in rabbits was investigated.

2. The awakening level was found to be quite constant at about 1.2 mg %, and was uninfluenced by route of administration or dose of the drug in the range tested.

3. No relation could be demonstrated between duration of anesthesia and the barbiturate blood level 10 minutes after injection, or with awakening blood level.

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A Method of Preparing Collodion Particles for Serologic Agglutination.* (17344)

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The agglutination of collodion particles as a method of demonstrating union between antigen and antibody has been utilized by several investigators as a highly sensitive serologic technic.[1-3] However, difficulty in producing uniformly active pellets and the frequency of non-specific reactions have limited its general use. Cavelti[4] recently reviewed these points and suggested certain modifications of earlier methods to avoid their inherent difficulties. In the course of attempts made in this laboratory during the past two years to devise a serologic test for viral hepatitis, the use of the collodion particle agglutination technic was explored. A simple method of preparing collodion particles which give consistently reproducible results was devised. It is the object of this paper to describe this method.

Preparation of collodion particles. The general method of Loeb[5] modified by Cannon and Marshall[6] was used with certain alterations. All glassware used was sterile.

Stock solution. One and one-half pounds of collodion, U.S.P. Merck (non-flexible) is poured slowly into 2 liters of singly distilled water contained in a 4 liter glass beaker. The water is stirred constantly with a glass rod while the collodion is being added, and a mass of collodion separates. The water is decanted and the mass is then washed three times with distilled water, and is finally pressed by hand between several layers of bibulous paper to remove excess water. The mass of collodion is further dried in an incubator at a temperature of 37°C for 24 hours or until the odor of ether is no longer detectable. During the period of drying, the mass is broken up into smaller pieces to allow exposure of more surface. When dry, the mass of collodion is weighed, and a 5% solution in acetone is prepared in a water bath at a temperature of 37°C. Stirring with a glass rod expedites the solution. This stock solution is stored in the ice-box at 4° to 6°C.

Suspension of pellets. 150 ml of stock solution is poured into the glass container of a Waring Blendor. The base of the Blendor is wrapped with a damp towel, and another damp towel is draped over the open top of the glass container to avoid splashing. The apparatus is set before an open window in such a way that escaping fumes of acetone may be readily dispersed by currents of air. A glass funnel with the stem pulled to a fine capillary tip is placed on a ring stand so that the tip is approximately 2 cm below the top

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1 Goodner, K., *Science*, 1941, **94**, 241.

2 Saslaw, S., and Campbell, C. C., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 559.

3 Lange, K., Gold, M. M. A., Weiner, D., and Simon, V., *J. Clin. Invest.*, 1949, **28**, 50.

4 Cavelti, P. A., *J. Immunol.*, 1947, **57**, 141.

5 Loeb, J., *J. Gen. Physiol.*, 1922, **5**, 109.

6 Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

of the container and over the vortex of the agitated fluid. The Blendor is set in motion, and through the funnel is added 60 ml of a mixture of three parts distilled water and one part acetone. When all the water-acetone mixture has been added, the Blendor is turned off, and a heavy gelatinous precipitate separates and settles to the bottom of the glass container. The faintly cloudy supernate is decanted into a filter flask containing 300 cc of cold, doubly distilled water, and the resultant mixture becomes cloudy. The gelatinous precipitate in the glass container is redissolved in 150 ml of stock solution. The Blendor is again set in motion, 60 ml of a 3:1 water-acetone mixture is added, and the supernate is subsequently decanted from the gelatinous precipitate into the original filter flask containing the 300 cc of doubly distilled cold water and the first supernate. The gelatinous precipitate remaining in the glass container is redissolved in 100 ml of acetone, agitated with the Blendor, and precipitated with proportionate amounts of a 3:1 mixture of water and acetone. This procedure is repeated 2 or 3 times and the resultant supernates are decanted into the original filter flask containing the 300 cc of cold, doubly distilled water and the previous supernates.

The filter flask is then attached to a vacuum pump at a pressure of 25 lb until only a faint odor of acetone remains. This may be expedited by placing the filter flask in a water bath at 56°C. The cloudy suspension in the filter flask is then passed through a thin cotton filter to remove coarse collodion particles. The filtrate is centrifuged in an angle head centrifuge at 4000 r.p.m. for 20 minutes. The faintly cloudy supernate is decanted and discarded. The precipitate is resuspended and washed 3 times in doubly distilled water by centrifuging at a speed of 4000 r.p.m. for 10 minutes. The supernate after the last two washings should be almost clear. The washed particles are resuspended in doubly distilled water and made into a stock suspension of a density standardized so that a 1:10 dilution equals No. 2 of the McFarland Turbidity Scale.[7] Stock suspensions of pellets are kept in the ice-box. They may be stored at this temperature (4° to 6°C.) for 7 to 14 days, after which they tend to lose their capacity to enhance a known serologic response.

The suitability of each lot of pellets for use is determined by testing their capacity to be agglutinated by the interaction of Pneumococcus Type I polysaccharide and its homologous hyperimmune horse serum. To chemically clean, sterile, tubes (100 x 10 mm) containing 0.5 ml of polysaccharide in dilutions of 10^{-4} to 10^{-9} are added 0.1 ml of pellets, 0.3 ml of sterile 0.85% saline and 0.1 ml of a 1:5 dilution of hyperimmune horse serum. The mixture is shaken and allowed to stand one hour at room temperature, after which the tubes are spun at 1500 r.p.m. for 5 minutes in a horizontal head centrifuge. The tubes are then flipped, and the amount of agglutination is determined against a bright light with a No. 5 Magni-Focuser glass.† A control test is made in the same dilutions with normal horse serum. Characteristically the precipitin test as usually performed with Pneumococcus Type I polysaccharide and its homologous hyperimmune horse serum is positive in dilutions of antigen of 10^{-6}. The pellets must be agglutinated (at least 1+) in a dilution of antigen of 10^{-8} for them to be suitable for use as an enhancing mechanism in detecting a serologic response. Many lots of pellets are agglutinated in a dilution of antigen of 10^{-9}. No agglutination occurs in the normal horse serum control tests.

Summary. A simple and consistently reproducible method of preparing collodion particles in a Waring Blendor is described. Pellets made in this way are uniformly active for 7 to 14 days and give no non-specific reactions in the serologic system tested. In contrast to previously described methods which prohibit the use of metal agitators, the metal blades in the Waring Blendor caused no apparent difficulties in the preparation of pellets. Actually, the violence of the agitation produced by the high rotary speed of the blades appeared to assist in the production of more uniformly active and smaller pellets.

[7] McFarland, J., *J.A.M.A.*, 1907, **49**, 1176.

† Edroy Products Company, New York, N. Y.

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