sulphate. Chlorides and phosphates are determined by a preliminary titration with 0.5 percent silver nitrate, and if present the following procedure is used. The precipitation is made as usual but it is treated with 1 percent HCl instead of the concentrated HCl and held in a water bath for a few minutes. Purine bases and uric acid alone pass into solution and can by this means be separated.

The method will be applied to the determination of purine bases as soon as they can be procured in pure state; however, no doubt exists that the method will be applicable for their determination.

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The production of a hemolytic substance in young liquid cultures of Cl. botulinum.

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A clear zone of hemolysis is usually noted around each colony of Cl. botulinum when the organism is grown anaerobically on blood agar medium at 35° C. for 3 to 4 days. The blood cells within the hemolyzed area are entirely dissolved. Preliminary experiments to demonstrate the production of a soluble hemolytic substance in 24 hour sheep's serum veal broth cultures were unsuccessful. Subsequent tests indicate that the hemolytic substance is formed during the first stages of growth; it is in all probability destroyed by the 24th hour.

The demonstration of the lytic substance depends on (1) the composition of the culture medium, (2) the age of the culture, and (3) the strain. Plain veal infusion broth is unfavorable for production of the lytic agent. The addition of small amounts of glucose or of 20 percent heated (56° C.—30°) sterile sheep's serum stimulates its formation. The first visible signs of growth in a 1 percent glucose veal broth medium, inoculated with spore forms (3-4 day beef heart cultures heated at 80° C.

for 10 minutes) are usually noted after 14 to 16 hours incubation at 35° C. Hemolysin can be demonstrated in such a culture after 16 to 18 hours growth and usually persists until the 22nd or the 26th hour, depending on the composition of the medium. The lytic substance invariably disappears from the culture by the 38th hour of growth. After the inoculation of young actively growing vegetative forms (12 to 14 hour cultures), the first signs of turbidity usually appear after 4 to 6 hours incubation. About 2 hours later hemolysin can be demonstrated. The peak of hemolysin production is reached after 8 to 10 hours growth; by the twelfth hour the concentration of the lytic agent has either decreased or entirely disappeared. Twenty-four hour cultures are non-hemolytic.

Estimations of the viable organisms made simultaneously with the hemolysin tests reveal that the greatest outpouring of the lytic substance takes place during the logarithmic period of growth. Multiplication *per se* is not the only prerequisite of hemolysin production, since certain vigorously growing cultures may remain entirely inactive to red cells. However, it undoubtedly plays an important part in the accumulation of the substance in the medium.

The determination of the hemolytic activity of a number of Type A and Type B strains has shown that individual variations may exist. Two Type A strains grown in suitable culture mediums invariably failed to form hemolysin. Very young growths (4 to 8 hours) were indifferent to the red blood cells, while older ones darkened but did not dissolve the erythrocytes. However, one of these cultures tested on a blood plate produced a small zone of hemolysis. The failure to demonstrate the lytic agent during the growth of the organism in liquid mediums has as yet not been explained. The data at hand indicate that Type A are less active in hemolysin production than Type B strains.

The hemolysin is very sensitive to the action of heat, light and mechanical agitation. It is readily adsorbed by diatomaceous earth. Exposure to a temperature of 60° C. for 10 minutes destroys it completely. At room temperature the lytic substance gradually becomes weaker; the rate of disappearance depends on the age of the culture which is exposed to the deleterious influences. Centrifugalization at high speed may destroy it; occasionally a clear supernatant dissolves the red blood cells. The detailed results will be published in the near future.