That the nephridium possesses a true excretory function was indicated by its composition. On the fresh basis, it contained 0.5 percent uric acid (Folin-Denis method) and 0.016 percent of creatine + creatinine (Folin-Benedict method).

The rate of glycogen consumption was determined in liver and muscle while the mollusks were held without food. The glycogen content of the muscle, as determined by Pflüger's method, decreased from the normal of about 30 percent (dry basis) to 19.4 percent after the animals had been held at room temperature without food for fifteen days. In another lot, held in the refrigerator fifteen days, the muscle glycogen dropped to 5.4 percent.

The liver glycogen decreased from the normal of 11 percent to a minimum of about 1 percent after the animals had been held at room temperature for six days. It remained at this level until the experiment was discontinued on the fifteenth day. Essentially the same changes in liver glycogen were noted in those animals held in the refrigerator. It appears from these observations that the liver glycogen of this mollusk is the reserve which is first drawn upon, while the muscle glycogen consititutes a secondary reserve.

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A micromethod for nephelometric estimation of uric acid and purine bases.

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In an endeavor to develop a microchemical method for the determination of uric acid and purine bases it was found that uric acid was quantitatively precipitated, when in solutions as dilute as 1/10,000 by a modified Salkowski reagent. Graves and Kober, for nephelometric determinations, suspended similar silver purine

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precipitates in egg albumin. This procedure was found unsatisfactory. Efforts to maintain a uniform suspension by reducing the surface tension and augmenting the viscosity were also unsuccessful. In the absence of other salts it was found, however, that a silver-urate-precipitate remained uniformly suspended for one hour, which permitted satisfactory nephelometric determinations.

Technique for Determinations:

(Reagent. 40 cc. of a 5 percent ammoniacal silver nitrate solution is placed in a 250 cc. volumetric flask with 40 cc. of either 5 percent ammonium chloride or 8 percent sodium chloride. Ammonia is then added until the silver chloride is dissolved plus an excess of 10 cc., and the whole made up to volume.)

Uric acid, in a lithium carbonate solution, is delivered in amounts ranging from 0.1 to 2 mg. into centrifuge tubes, to these are added 5 to 10 cc. of the reagent. The resulting precipitate is separated by centrifugalization, the liquid drained off and the inner lip of the tube dried with filter paper. Concentrated HCl is then added drop by drop until the silver urate is entirely in solution. The addition of water to this mixture produces a turbidity which is quantitative for the amount of uric acid present. The dilution must be made in a strictly uniform way. The most satisfactory method is to allow the desired amount of water (15, 20 or 25 cc.) to rush in suddenly from a burette. These solutions are then ready for nephelometric readings. The results can not be calculated by colormetric formulae. The following equation may be used:

$$x = \frac{10 \times St}{R + a(R-10)}$$

where the standard is set at 10, St represents the concentration of the standard, R the reading of the unknown, and a is a factor to be determined for each nephelometer.

Results obtained.

Expected	Found
0.38 mg.	0.40 mg.
0.54	0.60
1.29	1.295
1.29	1.23

Application: In biological solutions the proteins are best removed at the boiling point by means of colloidal iron and sodium

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.