BACTERIOLOGICAL, PHYSIOLOGICAL, ETC.

Estimation of Chlorides in Whole Blood. J. H. Austin and D. Van Slyke. (*J. Biol. Chem.*, 1920, **41**, 345-348.)—The method of Van Slyke and Donleavy for the estimation of chlorides in plasma (*J. Biol. Chem.*, 1919, **37**, 551) yields results from 30 to 40 per cent. too high when used with whole blood. The following modification is employed, and gives results in agreement with those obtained by the Carius method: 3 c.c. of blood and 15 c.c. of water are measured into a 60 c.c. flask, 30 c.c. of saturated picric acid solution are added, the whole diluted to 60 c.c., mixed, left to stand ten minutes, and filtered. To 40 c.c. of the filtrate 10 c.c. of a solution containing 5·812 grms. of silver nitrate and 250 c.c. of nitric acid per litre are added, the solutions mixed and allowed to stand overnight. The supernatant liquid is filtered, 20 c.c. are titrated, and the result calculated, as in the Van Slyke-Donleavy method. This modification also yields accurate results with plasma coloured by the hæmolysis of the blood cells, and which gives abnormal results by the original unmodified method. T. J. W.

Determination of Fibrin, Globulin, and Albumin Nitrogen in Blood Plasma. G. E. Cullen and D. Van Slyke. (*J. Biol. Chem.*, 1920, **41**, 587-597.)—*Fibrin nitrogen:* To 5 c.c. of plasma from oxalated blood are added 150 c.c. of 0·8 per cent. sodium chloride and 5 c.c. of 2·5 per cent. calcium chloride, and allowed to stand fifteen minutes and filtered. The residue is washed five times with 0·8 per

cent. sodium chloride, allowing each portion to remain in contact with the fibrin for ten minutes; then transferred to a Kjeldahl flask, and the nitrogen determined. *Filtrate nitrogen:* 20 c.c. of water and 25 c.c. of saturated ammonium sulphate are added to 5 c.c. of plasma, allowed to stand overnight, and filtered. Twenty c.c. of the filtrate are transferred to a Kjeldahl flask, 300 c.c. of 50 per cent. alcohol, 3 grms. of magnesium oxide, and 1 c.c. of white mineral oil are added, and the whole distilled until the distillate gives a negative test with red litmus-paper. The residue in the flask is digested with sulphuric acid and potassium sulphate to a light brown colour, washed down with a few c.c. of water, 10 c.c. more of sulphuric acid added, and the digestion continued for about three hours. The nitrogen is then estimated as usual. *Non-protein nitrogen:* A 50 c.c. flask is half filled with 2·5 per cent. trichloracetic acid solution and 5 c.c. of plasma are added, the flask filled to the mark with trichloracetic acid solution, the contents mixed, allowed to stand one hour, and filtered. The filtrate is measured, transferred to a Kjeldahl flask, and the nitrogen determined as above. *Total plasma nitrogen:* This is determined by the Gunning-Kjeldahl method, using 2 c.c. of plasma and digesting three hours after clearing. Corrections for the reagents used should be made. Albumin nitrogen is calculated by deducting the non-protein nitrogen from the "filtrate nitrogen." Globulin nitrogen is determined by subtracting the sum of the "filtrate nitrogen" and the fibrin nitrogen from the total nitrogen. Descriptions are given of the working out of these methods, and the results obtained are tabulated. T. J. W.

Determination of Sugar in Blood. O. Folin and H. Wu. (*J. Biol. Chem.*, 1920, **41**, 367-374.)—A modification of the method previously described by the authors (*J. Biol. Chem.*, 1919, **38**, 106), in which the phenol reagent is replaced by one containing molybdic acid and sodium tungstate. Two standard solutions are employed containing respectively 1 and 2 mgrms. of dextrose or invert sugar per 10 c.c. These are prepared by dilution of a 1 per cent. stock solution preserved with xylene or toluene. Two c.c. of the tungstic acid blood filtrate are added to a special form of test-tube, and the same volume of the standard solutions to two similar tubes. To each tube 2 c.c. of an alkaline copper solution (prepared by dissolving 40 grms. of pure anhydrous sodium carbonate in about 400 c.c. of water, adding 7·5 grms. of tartaric acid, 4·5 grms. crystallised copper sulphate, and diluting to 1 litre) are added, and the tubes heated in a boiling water bath for six minutes, followed by rapid cooling in cold water without shaking. Two c.c. of the molybdate-tungstate reagent (35 grms. of molybdic acid and 5 grms. of sodium tungstate in 400 c.c. of 5 per cent. sodium hydroxide: the solution is boiled vigorously for about thirty minutes, cooled, diluted to about 350 c.c., and 125 c.c. of concentrated 85 per cent. phosphoric acid added, finally diluting to 500 c.c.) are run into each tube to dissolve the cuprous oxide; the volume is then made up to 25 c.c. with water, the contents of each tube mixed, and the blue colours compared. A special form of test-tube is described which reduces the oxidation of the precipitated cuprous oxide by the air, and tables are provided showing the effect of various factors upon the results. The new modification yields results slightly lower than those given by the original method.

T. J. W.

Estimation of Magnesium in Blood. W. Denis. (*J. Biol. Chem.*, 1920, **41**, 363-365.)—Five c.c. of citrated plasma, serum, or whole blood are run into 15 c.c. of 6·5 per cent. trichloracetic acid solution, shaken, allowed to stand at least thirty minutes, and filtered. Ten c.c. of the filtrate are used for the estimation of calcium by the method of Lyman (*J. Biol. Chem.*, 1917, **29**, 169), and the supernatant liquid and washings are transferred to a platinum dish, 3 c.c. of 10 per cent. sulphuric acid added, evaporated to dryness, and ignited until white. The residue is dissolved in about 5 c.c. of water, and 10 per cent. hydrochloric acid added drop by drop until the solution is acid to methyl orange. The solution is transferred to a beaker, evaporated to 2 or 3 c.c., concentrated ammonia is gradually added until the solution is alkaline and 0·5 c.c. of 10 per cent. ammonium phosphate solution containing 50 c.c. of concentrated ammonia per litre, the mixture then being allowed to stand overnight. It is then transferred to a conical centrifuge tube, and the beaker washed with 20 per cent. alcohol containing 50 c.c. of ammonia per litre. After centrifuging, the liquid is removed and the beaker again washed with about 10 c.c. of the alcohol-ammonia mixture, this being repeated three times. After removing the last portion of washing liquid the tube is placed upon a water-bath until the ammonia present has evaporated, and the precipitate is dissolved in 10 c.c. of $\frac{N}{10}$ hydrochloric acid, transferred to a 100 c.c. flask, diluted to that volume with water, and mixed. Twenty-five c.c. of this solution are diluted to 50 c.c., 25 c.c. of strychnine molybdate reagent (see Bloor, *J. Biol. Chem.*, 1911, **32**, 34) are added, and, after standing five minutes, the volume of suspension is compared with that produced by 0·01 mgrm. of magnesium in 50 c.c. to which 26 c.c. of the strychnine molybdate solution is added, and which has been allowed to stand for the same time as the unknown. Estimations made by the above method with aqueous solutions, plasma, and serum containing from 0·02 to 0·1 mgrm. of magnesium, give an average recovery of 94 per cent. Estimations made on human blood serum, including pathological cases, give figures varying from 0·8 to 3·8 mgrm. per 100 c.c., and on normal serum from 1·6 to 3·5 mgrms. T. J. W.

Separation of Hydrocarbons by the Aid of Bacteria. J. Tausz and M. Peter. (*Zentralbl. f. Bakter. u. Parasitenk.*, 1919, **49**, 497-554; *Chem. Zentralbl.*, 1920, **91**, II., 264.)—Certain bacteria which attack naphthenes but not paraffins furnish a means hitherto wanting of separating these bodies. *B. aliphaticum*, *B. aliphaticum liquefaciens*, and the paraffin bacterium, are described; these were grown from garden mould in inorganic or organic media to which *n*-hexane, cyclohexane, or paraffin oil had been added. Paraffin bacteria are without action on naphthenes, benzenoid hydrocarbons, and some paraffins—for example, *n*-hexane and *n*-octane—but attack higher paraffins such as hexadecane, triacontane, and tetracontane. The other two bacteria are inert towards cyclic hydrocarbons and hexylene, but attack paraffins and *n*-caprylene and hexadecylene. The destruction is complete, even in presence of inert hydrocarbons. The presence of a very small proportion of aliphatic hydrocarbon in natural naphthenes, or of impurity in artificially prepared specimens, is shown by the clouding, due to bacterial growth, of media to which the hydrocarbon has been added. Naphthenes thus treated possess higher

constants. The method can be used for the detection of paraffins in crude oils and their products, and for the isolation of pure naphthenes. None of the bacteria showed the presence of urease; diffusible lipase and proteolytic enzymes were recognised in *B. aliphaticum* and the paraffin bacterium, diastase in the paraffin bacterium and *B. aliphaticum*, and catalase in both species. The following new values were obtained: 1·3-dimethylcyclohexane, b.-pt. 118°-120° C., D 20° C./4° C. 0·771, $[n]_{D\ 20°\ C.}$ 1·4258; 1·3·4-trimethylcyclohexane, b.-pt. 139°-140° C., D 20° C./4° C. 0·789, $[n]_{D\ 20°\ C.}$ 1·4330. O. E. M.

Braunstein's Modification of the Mörner-Sjöqvist Process for the Estimation of Urea. A. H. Todd. (*Biochem. J.*, 1920, **14**, 252.)—Braunstein suggested the use of crystalline or syrupy phosphoric acid in place of sulphuric acid at the "Kjeldahl" stage of the Mörner-Sjöqvist process (in order to prevent hippuric acid and creatinine escaping precipitation and appearing in the final result as urea). This assumes that urea is completely incinerated under the Braunstein conditions, viz., heating at 145° C. for four and a half hours. The author finds that urea-estimation is by no means quantitative under the original conditions, and the temperature is the most important factor. Experiment showed that incineration of the Mörner-Sjöqvist filtrate for fourteen hours at 185° C. with 15 grms. of Kahlbaum's phosphoric acid gives an accurate quantitative estimation of the urea and of the urea only. With creatine and hippuric acid *per se*, no trace of incineration with phosphoric acid occurred when working under these conditions. The actual heating is best performed in an oil-bath, while phosphoric acid may most conveniently be weighed out by melting it in a water oven, and pouring it out into a weighed glass evaporating basin. H. F. E. H.

Titration of Organic Acids in Urine. D. Van Slyke and W. W. Palmer. (*J. Biol. Chem.*, 1920, **41**, 567-585.)—One hundred c.c. of urine are mixed with 2 grms. of finely divided calcium hydroxide, allowed to stand with occasional stirring about fifteen minutes and filtered. To 25 c.c. of the filtrate 0·5 c.c. of 1 per cent. phenolphthaleïn is added, and $\frac{N}{5}$ hydrochloric acid run in until the pink colour just disappears, when 5 c.c. of 0·02 per cent. tropæolin 00 solution are added with continual shaking, followed by $\frac{N}{5}$ hydrochloric acid which is added gradually from a burette until the colour equals that of a standard solution in a similar tube containing 0·6 c.c. of $\frac{N}{5}$ hydrochloric acid, 5 c.c. of tropæolin 00 solution, and water to a total volume of 60 c.c. In calculating the required result, a correction is first made by deducting the value obtained in a blank experiment, and finally by deducting the equivalent in organic acids of the creatinine present in the urine. A 0·1 M solution of creatinine is equivalent to a $\frac{N}{10}$ solution of organic acid. Other indicators, including methyl orange, tetrabromophenolsulphonephthaleïn and dimethylamino-azobenzene, may be used in place of tropæolin 00. The average 24-hour excretion of healthy adult males is 6 c.c. of $\frac{N}{10}$ acid per kilo of body weight. The theoretical basis of the method is discussed and numerous tables are provided showing results obtained by the above method with various acids, bases, and salts contained in urine, together with determinations made on normal and pathological urines. T. J. W.