Microchemical

Carbohydrates. I. Micro-Determination of Carbohydrates in Pure Solutions, and in Animal Material. Z. Dische. (*Mikrochem.*, 1931, **10**, 129–189.)—A comparative description of the different micro methods of sugar determination in use, with complete practical details and references is given. The methods include: A. **Reduction Methods.** 1. *Reduction of Cupric Salts.*—The reduced copper oxide is filtered off, and may be determined titrimetically by dissolving in an oxidising acid reagent and titrating with potassium permanganate, as in the method of Fontes and Thivolle (*Bull. Soc. Chim. biol.*, 1927, **9**, 353). The reduced copper may also be determined iodimetrically by the Shaffer–Hartmann method (*J. Biol. Chem.*, 1920, **45**, 365; Somogyi, *ibid.*, 1926, **70**, 599; and Thompsett, *Biochem. J.*, 1930, **24**, 1148). Alternatively, the unreduced copper may be determined iodimetrically by the MacLean method, modified by Bang (*Biochem. Z.*, 1918, **87**, 248; 1918, **92**, 344), titrating finally with $N/1000$ thiosulphate solution. The reduced copper may also be determined colorimetrically by the Folin–Wu method (*J. Biol. Chem.*, 1919, **38**, 81; **41**, 367; Folin, *ibid.*, 1930, **82**, 83; *ibid.*, 1926, **67**, 357; Benedict, *ibid.*, 1926, **68**, 759; and 1928, **76**, 457).

2. *Reduction of Mercury Salts.*—The method has been worked out on the micro scale for biological material by Baudouin and Lewin (*Bull. Soc. Chim. biol.*, 1927, **9**, 280), and appears to be more rapid and more simple than the copper salt methods.

3. *Reduction of Ferricyanide.*—The reduction of ferricyanide is not so susceptible to back oxidation in the air as the copper salt methods, but is less specific. In the Hagedorn–Jensen method (*Biochem. Z.*, 1923, **135**, 46) the reduced ferricyanide is determined iodimetrically. Flatow (*Biochem. Z.*, 1928, **194**, 132) determines the excess ferricyanide by titrating with 0·2 per cent. indigo carmine solution, and Jonescu (*Bull. Soc. Chim. biol.*, 1928, **10**, 252) determines the reduced ferricyanide by titration with permanganate. The unreduced ferricyanide may be determined colorimetrically by Folin's method (*J. Biol. Chem.*, 1928, **77**, 421; 1929, **81**, 231; 1929, **83**, 115; ANALYST, 1928, **53**, 392; 1929, **54**, 246), or by the method of Van Slyke and Hawkins (*J. Biol. Chem.*, 1929, **84**, 69). The unreduced ferricyanide may also be determined gasometrically by the method of Van Slyke and Hawkins (*J. Biol. Chem.*, 1929, **79**, 739), in which the nitrogen developed by the action of alkaline hydrazine on potassium ferricyanide is measured.

4. *Methods Depending on the Reduction of Organic Substances.*—Chief of these methods is the reduction of acetone-picric acid, in the method of Benedict (*J. Biol. Chem.*, 1929, **48**, 50), and that of Sumner (*ibid.*, 1921, **47**, 5; 1925, **62**, 287; and 1925, **65**, 393). The colour developed is compared with a standard of similar sugar content. In Milroy's method (*Biochem. J.*, 1925, **19**, 746) nitro-anthraquinone sulphonic acid is reduced by the sugar to a red amino compound, in 10 per cent. potassium carbonate solution. Eisenhardt's method (*Münch. med. Wochenschr.*, 1920, p. 1382) depends on the reduction of methylene blue.

B. **Methods Depending on the Fermentation of Sugar.**—Sugar is fermented by yeast and micro-organisms, such as *B. coli*, to carbon dioxide. The method of Wagner (*J. Metabol. Res.*, 1925, **5**, 353) measures, in a Van Slyke apparatus, the carbon dioxide formed, and Grafe and Jorgenfrei (*Deutsch. Arch. klin. Med.*, 1924, **145**, 294), whose method is adapted for blood, use a Barcroft manometer.

C. **Osazone Methods.**—These methods are all colorimetric, and have been used on the micro scale by Dische and Popper (*Biochem. Z.*, 1926, **175**, 371), Dische [*Mikrochem.*, 1929, **1** (Neue Folg.), 1], Glassmann (*Z. physiol. Chem.*, 1925, **150**, 16), and Tillmans and Philippi (*Biochem. Z.*, 1929, **215**, 36).

D. **Methods Depending on Carbohydrate Reactions.**—Methods are based on the characteristic reactions of carbohydrates when heated with different organic substances, such as naphthol, indole, diphenylamine, thymol, phloroglucinol, in concentrated mineral acids. In these methods are also included special methods for determining different carbohydrates in admixture with each other.

II. DETERMINATION OF CARBOHYDRATES IN DIFFERENT ANIMAL MATERIALS.—The application of the methods to various kinds of biological material is discussed in detail, including different methods of removing the protein before the determination. J. W. B.

Micro-Determination of Phosphoric and Arsenic Acids with "Molybdenum-blue." S. Zinzadge. (*Z. für Pflanzenern., Düng. u. Bodenk.*, 1932, **23**, 447–454.)—The method is also described in other Continental journals, *e.g. Ann. agronomiques*, 1931, **43**, 321, *Bull. Soc. Chim.*, 1931, **49**, 872. It depends on

the use of a special "molybdenum-blue" reagent, and differs from the existing methods for phosphoric and arsenic acids involving the use of molybdenum compounds in that the reagent and the blue colour produced with these acids are both relatively stable. The reagent can be purchased in a proprietory form from Schering-Kahlbaum, A.G., of Berlin, or the Société des Usines Chimiques Rhone-Poulenc, of Paris, or may be prepared as follows: 120 c.c. of sulphuric acid (sp.gr. 1·785) are well mixed with 6·02 grms. of pure molybdic oxide powder in a porcelain dish; the mixture is heated and stirred until the molybdic oxide is dissolved; after cooling, 70 c.c. of water are added, the liquid is cooled and diluted to 200 c.c. (Solution 1). One hundred c.c. of this solution are boiled for 10 minutes after the addition of 0·28 grms. of pure molybdenum metal powder, cooled and diluted to 100 c.c. (Solution 2). The "molybdenum-blue" reagent is made by mixing solutions 1 and 2 in such a proportion that 2·51 c.c. of the mixture will just decolorise $0·20 \pm 0·01$ c.c. of 0·1 *N* permanganate solution. *Method.*—One to 30 c.c. of the solution to be tested (containing 0·0005 to 0·5 mgrm. of P_2O_5 or As_2O_5) are placed in a small 50 c.c. conical flask, neutralised with dilute soda solution or sulphuric acid, 2 drops of saturated aqueous 1, 2, 6-dinitrophenol being used as indicator (slight yellow colour at end-point), diluted to 40 c.c., and the "molybdenum-blue" reagent is added. The solution is boiled gently for 5 minutes, kept for a further 15 to 20 minutes, cooled and diluted to 50 c.c. The intensity of the blue colour is compared with that of a standard phosphate solution, prepared from potassium dihydrogen phosphate, which has been treated in the same manner. The quantity of "molybdenum-blue" reagent to be added depends on the amount of phosphate present, and must be established by preliminary trial: for 0·0005 to 0·1 mgrm. of P_2O_5, 0·3 c.c., and for 0·1 to 0·5 mgrm. of P_2O_5, 0·6 c.c., is required. Various colorimeters are recommended for the colorimetric comparison, but for approximate results a colorimeter is not necessary. Arsenic acid reacts with the reagent similarly to phosphoric acid, and may be determined in the same way, a standard comparison solution prepared from arsenic acid being used. Silica does not interfere. S. G. C.

Micro Melting-Point Determinations. H. Linser. (*Mikrochem.*, 1931, **9**, 255–268.)—Klein's melting-point apparatus (*Mikrochem. Pregl-Festschrift*, 1929, 192) for the observation of melting points under the microscope is used to determine the temperature of sublimation, melting point, temperature of decomposition and other properties of a number of substances. Before use, a new instrument must be heated several times to 400° C., and maintained at that temperature, so that all the insulating material is thoroughly dried, before reproducible results are obtainable. A calibration curve should be made for each instrument by determining the readings for a number of test substances of known m.pt., when heated at a velocity of 3 to 6° per minute. The m.pt. is taken as the temperature at which the smallest crystals and particles liquefy and the corners of the larger crystals begin to melt. This is not necessarily the same temperature as the macro m.pt. The observed m.pt. is lowered by increasing the velocity of heating. For a number of substances it was found that $y = 3·1x$, where x represents melting-point displacement and y the velocity in degrees per minute. Therefore, the velocity

of heating should be observed and the correction applied. A further correction table is advisable, in which the corrected value of the melting points of a number of substances is compared with the known value of their melting points. For most instruments this last correction table is redundant, and the true and calculated values coincide. J. W. B.