sters treated with cortisone. These changes are similar qualitatively to those which have been reported in human diabetic glomerulosclerosis(5). The abnormal substance was felt to be a mucopolysaccharide, since it is removed by incubation with hyaluronidase.

These findings coupled with those described above indicate an influence of adrenal steroids on the carbohydrate content of the kidney which may represent a link in the pathogenesis of glomerulosclerosis in human diabetes.

Summary. The changes induced in concentration of rhamnose, mannose, fucose, glucose and galactose in the kidney of rabbits treated with various steroids were studied using a semi-quantitative chromatographic method. The findings are discussed in relation to the pathogenesis of diabetic glomerulosclerosis.

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A New Colorimetric Reagent for Micro Determination of Ammonia.* (22831)

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This paper describes a new colorimetric procedure for the measurement of ammonia in quantities of 0.05 to 0.5 micromole. The method has proved to be more rapid and convenient than the usual microtitration, and the precision appears to be at least as good. The microdiffusion method of Conway(1) is employed to separate the ammonia from interfering substances. The subsequent measurement is based on the well-known reaction of ammonia with hypobromite. A carefully measured volume of hypobromite solution is added, enough so that an excess is present. The hypobromite remaining after the oxidation of the ammonia is then measured by its power to decolorize a dilute solution of phenosafranin. Previously described methods based on the reaction of ammonia with hypobromite depend upon iodometric titration of the excess hypobromite. In one such procedure, that of Levy and Palmer(2), as little as 0.36 micromole of ammonia is oxidized by hypobromite in a volume of 2 ml. For studies of ammonia in blood and tissues, however, it is desirable to work at still greater dilutions. As a first step, therefore, the iodometric method was used to test the reaction at greater dilutions. It was thus established that the oxidation of ammonia by hypobromite is stoichiometric at ammonia concentrations at least as low as .03 micromole per ml.

The choice of phenosafranin as a colorimetric reagent was based on tests of a number of dyes and stains. Although hypobromite has a decolorizing action on many such intensely colored substances, it was found that in most instances the decolorizing action is not directly proportional to the hypobromite concentration. With phenosafranin, however, a strictly linear relationship obtains.

Methods. Borate buffer, 0.5 *M,* pH about 9.8. A 16 g quantity of sodium hydroxide is dissolved in 800 ml water, 31 g boric acid is added and dissolved, and the solution is diluted to 1 l. *Phenosafranin,* approximately 0.006%. It is convenient to prepare a liter of 0.02% solution and to make dilutions as required. The concentration should be so ad-

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justed that when 1 ml is added to 7.5 ml water and 1 ml of borate buffer, the optical density at 515 mμ is about 0.9. The phenosafranin solution is very stable. *Bromine solution.* While this can be prepared from liquid bromine and potassium bromide, a more convenient method is as follows: 16 g potassium bromide and 1.67 g potassium bromate are dissolved in 450 ml water. An 8.5 ml volume of 10 *N* sulfuric acid is added and the solution is diluted to 500 ml. It should be stored in a dark place. The bromine concentration slowly decreases. *Hypobromite reagent.* To 25 ml of the borate buffer is added about 0.5 ml of the bromine solution. The concentration should be so adjusted that when 1 ml of phenosafranin is added to a mixture of 2.5 ml water and 1 ml of hypobromite, with subsequent dilution to 9.5 ml, the optical density at 515 mμ is between 0.1 and 0.2. The hypobromite should be freshly prepared on the day of use. A slow change to the inactive bromate occurs, amounting to about 1% per hour.

Procedure. Microdiffusion is carried out as described by Conway(1), ammonia being absorbed in 1.5 ml of 0.01 *N* sulfuric acid in the central chamber. At end of diffusion period the contents of this chamber are transferred to colorimeter tube by a dropper. The chamber is washed with 1 ml water which is transferred to the colorimeter tube as completely as possible. To the solution in the tube (volume 2.5 ml) is added 1 ml of hypobromite solution. After at least 2 minutes, 1 ml of phenosafranin is added. After at least one minute, 5 ml of water is added. The solution is very thoroughly mixed after each addition. The optical density is read at 515 mμ. Results are calculated from a calibration curve prepared from standard ammonium sulfate solutions subjected to microdiffusion procedure. All readings are taken with instrument set so that distilled water gives an optical density of zero (100% transmittance). Blank determinations are run in triplicate with each group of samples, and for each ammonia determination the average blank value is subtracted from the reading. Care must be taken to insure that amount of ammonia in the aliquot of sample is less than

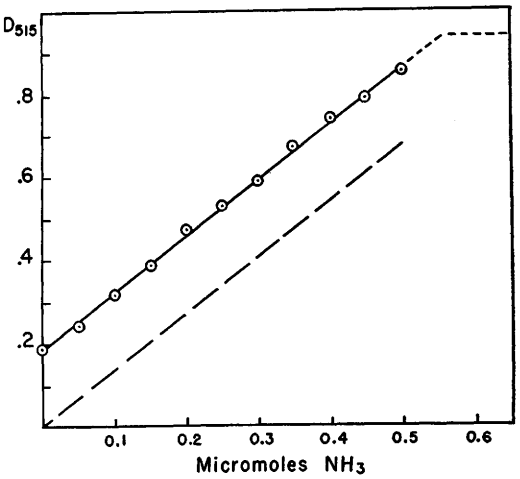

FIG. 1. Standardization curve. Horizontal portion at upper end of curve (dotted line) indicates color intensity of phenosafranin when no hypobromite is added, or when all of the hypobromite is reduced by ammonia. Dashed line represents curve after subtraction of the blank from each reading.

the amount of hypobromite added, since the curve becomes a horizontal line when the hypobromite is completely reduced by ammonia (Fig. 1). If the reading approaches the upper limit, the determination should be repeated, if possible, on a smaller aliquot.

Results. Fig. 1 shows a standardization curve done on ammonium sulfate solutions directly, without microdiffusion. Readings were taken with a Coleman 6 B instrument. The dashed line represents the curve obtained after subtraction of the blank value from each reading. The points on the curve represent individual determinations done in one group, and the small deviations from the curve suggest the degree of precision which can be attained. The magnitude of the blank varies from one run to another, being dependent upon the concentration of hypobromite, but the slope of the curve is highly reproducible.

Application to brain tissue. The tissue (obtained after freezing the brain *in situ*) is ground and extracted with cold 10% trichloroacetic acid, using approximately 5 ml/g. After filtration, 1 ml aliquots of the extract are used for the determination. The microdiffusion must be started immediately, and is carried on for a 90-minute period. Correction for the slow formation of ammonia from glutamine during microdiffusion is made as

described by Richter and Dawson(3).

Application to blood. For measuring blood ammonia it is desirable to reduce the range of the method. The concentrations of hypobromite and of phenosafranin are reduced by about one-third. The volumes of reagents used are not changed, but the final dilution is made with only 1.5 ml water, giving a final volume of 6 ml. Blood is treated with ammonia-free potassium oxalate to prevent clotting, and the determinations are carried out on 1 ml aliquots. These may be pipetted into the Conway units and the diffusion started within 5 minutes after the blood is drawn, or more elaborate precautions may be taken to avoid ammonia formation after shedding(1). The diffusion period is limited to 20 or 30 minutes, in order to minimize ammonia formation due to action of the alkali on other blood constituents. The calculations are based on standards treated in the same way, since recovery is much below 100% in these short diffusion periods. Conway's(1) correction factors may be applied to allow for ammonia formation after shedding and for that due to action of the alkali, and for the slightly lower rate of diffusion from blood-carbonate mixtures as compared with water-carbonate standards.

Glutamine. The colorimetric method has been applied to the measurement of glutamine in brain tissue. One ml of the trichloroacetic acid extract is diluted with 4 ml of 10% trichloroacetic acid, stoppered loosely, and heated at 70° for 75 minutes. The glutamine is thereby converted to ammonia and pyrrolidonecarboxylic acid (method of Richter and Dawson(3)). The total ammonia is determined on 1 ml aliquots, and the value for preformed ammonia is subtracted.

Non-protein nitrogen. The method has been applied to the direct determination of non-protein nitrogen without microdiffusion. A suitable aliquot of a trichloroacetic acid filtrate of blood or tissue is digested by the Koch-McMeekin method with the precautions recommended by Miller and Miller(4). After the last addition of hydrogen peroxide, the heating is continued for 40 minutes to insure complete removal of hydrogen peroxide. (This is a necessary precaution, since any hydrogen peroxide remaining would react with the hypobromite). After cooling, the digest is diluted and enough sodium hydroxide is added to almost, but not quite, neutralize the sulfuric acid present. (The amount of sodium hydroxide necessary may be determined on a blank digest.) The solution is then made up to a definite volume and aliquots are taken for the determination. Blanks are run on the digestion procedure, and the calculations are based on standards not subjected to microdiffusion.

Summary. A new colorimetric procedure is substituted for microtitration in the measurement of ammonia by the Conway method. The ammonia is oxidized by addition of hypobromite, and the excess hypobromite determined by its power to decolorize phenosafranin. The range of the method as described is 0.05 to 0.5 micromole. The procedure has been applied to brain tissue and to blood, and to the measurement of glutamine. It has also been used for the determination of non-protein nitrogen after Koch-McMeekin digestion, the microdiffusion being omitted.

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