The situation in *E. patella*(15) differed from that in *Paramecium* in that selfing was common in *Euplotes* and it was shown that some of the pairs found in conjugating mixtures consisted of 2 animals from the same clone. Also, animal-free fluid from a culture of one mating type induced selfing among animals of other mating types. Because of these differences, Sonneborn(3) concluded that the conception of a mating type in *Euplotes* was different from that in *Paramecium*. Selfing was uncommon in the 155 clones reported in this paper, but no method was found to determine the clonal origins of the animals associated in pairs. It is, therefore, not possible to state which of the two concepts of mating type applies to *S. putrina*.

Summary. Two sexually isolated varieties were found among 155 clones of *Stylonychia putrina* collected in Southern California plus 5 clones produced in the laboratory. In Variety I, at least 15 mating types have been found; in Variety II, 11 mating types.

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1. Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, 1937, v23, 378.
2. ———, *Anat. Rec.*, 1942, v84, 542.
3. ———, *Advances in Genetics*, 1947, v1, 263.
4. Beale, G. H., and Schneller, M., *J. Gen. Microbiol.*, 1954, v11, 57.
5. Jennings, H. S., *Genetics*, 1939, v24, 202.
6. Jennings, H. S., and Opitz, P., *ibid.*, 1944, v29, 576.
7. Chen, T. T., *Proc. Nat. Acad. Sci.*, 1946, v32, 173.
8. Giese, A. C., *Anat. Rec.*, 1941, v81, supplement, 131.
9. Giese, A. C., and Arkoosh, M., *Physiol. Zool.*, 1939, v12, 70.
10. Gilman, L. C., *Biol. Bull.*, 1949, v97, 239.
11. ———, *ibid.*, 1950, v99, 348.
12. Hiwatashi, K., *Sci. Rept. Tohoku Univ. 4th Ser.*, 1949, v18, 137.
13. Sonneborn, T. M., *Collecting Net*, 1939, v14, 77.
14. Wichterman, R., *Proc. Penna. Acad. Sci.*, 1951, v25, 51.
15. Kimball, R. F., *Am. Nat.*, 1939, v73, 451.
16. Elliott, A. M., and Gruchy, D. F., *Biol. Bull.*, 1952, v103, 301.
17. Elliott, A. M., and Hayes, R. E., *J. Protozool.*, 1955, v2, 75.
18. Gruchy, D. F., *ibid.*, 1955, v2, 178.
19. Downs, L. E., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 605.
20. Siegel, R. W., *Biol. Bull.*, 1956, v110, 352.

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Influence of Steroids on Carbohydrate Content of Rabbit Kidney: A Chromatographic Study.* (22830)

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Intraglomerular kidney lesions produced in rabbits by the short-term administration of cortisone(1), prednisone and prednisolone are stained by the periodic acid-Schiff (PAS) method, a reaction of carbohydrates possessing free 1,2-glycol and amino alcohol groups (2). In the course of a systematic investigation into the nature of these renal lesions, chromatographic studies were designed to demonstrate the effects of cortisone acetate,† prednisone,‡ and desoxycorticosterone acetate on concentrations of certain carbohydrates extractable from rabbit kidneys. The sugars studied, rhamnose, mannose, fucose, glucose and galactose, are known to stain with PAS (2).

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‡ Prednisone was supplied through the courtesy of Mr. J. W. Brisick, Schering Corp. Ltd. of Canada.

Materials and methods. New Zealand white male rabbits ranging from 2 to 2.8 kg were maintained for a control period of 7 to 14 days on Sherwood rabbit pellets *ad libitum*. The left kidney was removed under nembutal and ether anesthesia. Postoperatively, the animals were provided with the same diet, but drinking water was replaced by 1% sodium chloride solution. This regimen was continued for 3 weeks during which the animals were divided into the following treatment groups: Group A: Control; Group B: 5 mg of desoxycorticosterone acetate intramuscularly day; Group C: 5 mg of desoxycorticosterone acetate and 7.5 mg of cortisone acetate intramuscularly day; Group D: 5 mg of prednisone suspension intraperitoneally day. After 21 days, the animals were killed by overdose of nembutal and were autopsied. Portions of right kidney were fixed in Zenker-Formol, sectioned at 2.5 μ and stained with PAS stain. The remaining kidney tissue was extracted with 0.5 N sodium hydroxide for 4 days at 4°C; after neutralization, the extract was treated with 2 volumes of cold ethanol to precipitate acid mucopolysaccharides (fraction 1). A second precipitate (fraction 2) was obtained by increasing the ethanol concentration to 84%. Deproteinization of these extracts was not attempted. The 2 fractions were hydrolysed with a cation exchange resin (Permutit Q), and hydrolysates subjected to paper chromatography as described by Glegg and Eidinger (2). In this technic, hexosamines and hexuronic acid were either destroyed or adsorbed by the resin and did not appear on the chromatograms. After development of the chromatograms and location of sugar areas with aniline hydrogen oxalate, the quantities of the various sugars present were estimated by comparing their spot intensities under ultraviolet light (3660 Angstrom units) with those resulting from subjecting 125 μg of standard sugars to the chromatographic procedure. In this manner it was possible to make a crude estimation of the quantities of sugar in milligrams per hundred grams of fresh kidney.

Results. Several areas in all kidneys were observed to stain positively with the PAS technic: (a) capillary basement membrane of the glomeruli (b) the brush border of cells lining the proximal convoluted tubules and (c) the cytoplasm of cells lining the convoluted and collecting tubules. In the control group and in animals treated with desoxycorticosterone acetate alone, no further staining areas were identified. In the groups which received cortisone and prednisone, characteristic nodular glomerular lesions, some of which stained with PAS, were observed. A moderate increase in the quantity of PAS-staining material was noted in the capillary walls and in intratubular casts. These lesions were identical to those described by others(1,3). The pathological alterations were more numerous and severe in the group treated with prednisone.

Fig. 1 indicates the concentrations of rhamnose, mannose, fucose, glucose and galactose in fractions 1 and 2 in each of the groups. The most notable alterations were the disappearance of rhamnose from the kidneys of the groups treated with cortisone acetate or prednisone and a diminution in concentration in those treated with DCA. There was an increase in mannose and galactose content in all groups treated with steroids, but the levels of fucose and glucose were not significantly changed.

Discussion. Sommers and Haley(4) noted the accumulation of an abnormal substance in the glomerular stroma, capillaries, and walls of kidney arterioles in humans and ham-

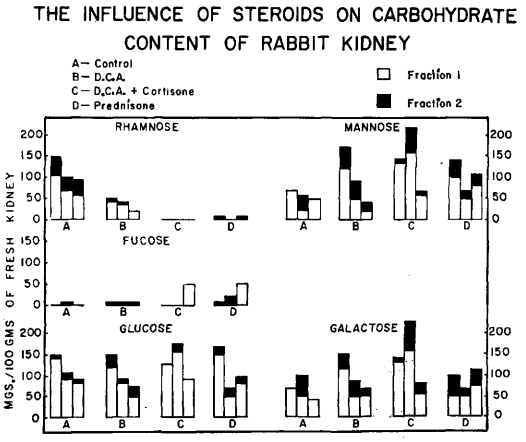

FIG. 1.

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.

1. Wilens, L. S., Stumpf, H. H., *Am. J. Path.*, 1952, v31, 275.

2. Glegg, R. E., Eidinger, D., LeBlond, C. P., *Science*, 1954, v120, 839.

3. Bloodworth, J. M. B., Hamwi, G. J., *Am. J. Path.*, 1955, v31, 167.

4. Sommers, S. C., Haley, K. H., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 919.

5. Sommers, S. C., Crozier, R., Warren, S., *Am. J. Path.*, 1954, v30, 919.

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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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