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, t 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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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 and stained with PAS stain. The remaining kidney tissue was extracted with 0.5 N Sodium hydroxide for 4 days at 4C; 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 g 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 PASstaining 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. I 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-
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steroids 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 hyaluroniclase.

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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