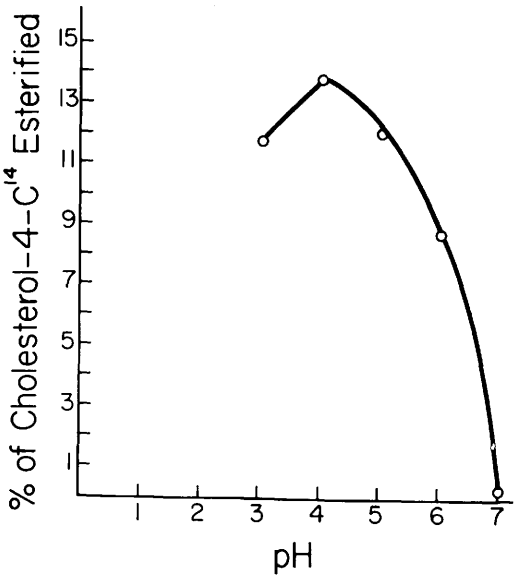

FIG. 1. Effect of pH on esterification of free cholesterol-4-C^{14} by acetone powder extracts of hog adrenal gland.

pH range between 3 and 5, with maximum activity observed at pH 4.0. No esterification was observed at pH 7.0.

Summary. It is demonstrated that extracts of acetone powders of hog adrenal glands do esterify free cholesterol and hydrolyze esterified cholesterol. The optimum pH range for esterification was between 3 and 5, and no esterification was observed at pH 7.4. Hydrolysis of the ester was observed at pH 7.4 and to a lesser extent below pH 6.

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Received September 27, 1963. P.S.E.B.M., 1963, v114.

Renal Clearance of Glucosamine in the Rat.* (28799)

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Glucosamine (2-amino-2-deoxy-D-glucose) is an amino sugar widely distributed throughout all tissues of the body and exists almost entirely as a bound component of mucoproteins, nucleic acids, and mucopolysaccharides(1). Carter and Peters found that the renal clearance of exogenous glucosamine in dogs was equal to the glomerular filtration rate (GFR), measured by creatinine clearances, over a wide range of glucosamine plasma concentrations(2). Coulson and Hernandez, in testing the hyperglycemic effect of glucosamine in rats and alligators, found that the glucosamine excreted by the kidney was identical to that injected and was not a metabolic product(3). However, no information is available on renal clearance of glucosamine in rats. Glasser(4) has shown that creatinine clearances in rats are inaccurate as a measure of GFR if plasma levels are below 150 mg%, because of tubular secretion of creatinine in this species. Therefore, in the experiments reported here, glucosamine clearances in rats were compared with inulin clearances since it is difficult to maintain such a high plasma level of creatinine.

Materials and methods. Male Sprague-Dawley rats, weighing 400 to 500 g, were used. After being anesthetized with pentobarbital sodium, 35 mg/kg, i.p., the rats were prepared as follows: a tracheal cannula (PE 240-320) was inserted, the jugular vein was cannulated (PE 50) for infusion pur-

* This work was supported in part by training and research grants from USPHS.

poses, both ureters were cannulated (PE 10) through a midline abdominal incision, and the femoral vein was cannulated (PE 50) for collection of blood samples. The rats were infused with a solution containing para-aminohippuric acid (PAH), inulin and glucosamine.† The concentrations of these substances in the infusion mixture are given in Table I. At the low concentration of glucosamine (1%), mannitol was added to the mixture to insure good diuresis. Since 10% glucosamine is hypertonic, no other osmotic agent was added to the mixture at this or higher concentrations of glucosamine. The pH of the glucosamine infusion solution was adjusted to 7.4. The rats were infused *via* the jugular vein at a rate of 0.097 ml/min by means of a Harvard Variable Speed Infusion/Withdrawal Pump. The infusion was started approximately one hour before clearances commenced. During the experiment 2-3 blood samples, usually 1-2 ml, were drawn, taking 15-20 minutes for each sample. After centrifugation of the blood samples, the plasma was removed. Urine from both kidneys was collected in the same graduated conical centrifuge tube over 5 to 15 minute intervals. Urine and plasma samples were frozen until the chemical analyses were carried out. Glucosamine concentrations were determined in plasma filtrates, obtained by $ZnSO_4$-NaOH precipitation of plasma samples(5), and in urine samples by the specific method of Dische and Borenfreund(6). Glucose does not interfere with this method. The estimation of GFR and effective renal plasma flow (ERPF) was made by determination of the concentration of inulin and PAH in plasma filtrates and urine samples. The method of Schreiner(7), a modification of the Roe, Epstein, and Goldstein method(8), was used for inulin and the method of Smith *et al* for PAH(9).

Results. Renal clearance of exogenous glucosamine was measured 37 times using 7 rats and compared to inulin clearances during the same time intervals. The results are summarized in Table I. The values given for ERPF, urine flow, clearances of glucosamine and inulin are averages for the number of clearance periods performed on each rat. In calculating the clearance of glucosamine and inulin the values for the plasma sample closest to the urine sample being analyzed were used. All of the plasma concentrations measured for glucosamine and inulin are given. The data show that the ratio of the clearance of glucosamine to that of inulin is essentially unity at all concentrations of glucosamine used. Statistical analysis of the mean of the ratios compared to unity reveal that the mean ratio was not significantly different from unity. Student's "t" test was used(10).

Discussion and summary. That the clearance of exogenous glucosamine in the rat occurs at the same rate as that of inulin suggests that glucosamine might be a substance which after glomerular filtration does not undergo subsequent tubular reabsorption or secretion. The inability to change the renal clearance of glucosamine after elevation of plasma glucosamine concentrations from approximately 2 to 70 mg% indicates that excretion of glucosamine is probably independent of tubular secretion or reabsorption.

Renal excretion of glucosamine in dogs was shown by Carter and Peters to be only by glomerular filtration. The clearance of glucosamine was identical to that of creatinine, and glucosamine did not interfere with the glucose reabsorption (glucose Tm) or with the secretion of N′-methylnicotinamide by the dog or chicken kidney tubule(2).

Equilibrium dialysis experiments showed that glucosamine was not bound to plasma proteins(2). Attempts to determine endogenous glucosamine or other hexosamine by the Dische and Borenfreund method in plasma and urine samples of 3 normal non-perfused rats revealed that endogenous glucosamine levels are not measurable by this method. Therefore, in view of the above and the fact that Coulson and Hernandez(3) found that glucosamine excreted by the rat kidney was not a metabolic product, the glucosamine clearances do not require correction for any of these factors; *i.e.*, plasma binding, en-

† D (+) Glucosamine Hydrochloride was kindly supplied through the courtesy of Dr. D. G. Iezzoni, Charles Pfizer & Co., Inc.

TABLE I. Renal Clearance of Glucosamine Compared to Clearance of Inulin in Rats.

Rat No.	Infusion mixture	No. of clearance periods	ERPF, ml/min	Urine flow, ml/min	Plasma conc, mg/ml Glucosamine	Plasma conc, mg/ml Inulin	C_{Glu}, ml/min	C_{In}, ml/min	C_{Glu}/C_{In}
1	1% glucosamine 2% inulin .03% PAH 6% mannitol	5	4.08	.065	.022 .022 .035	.731 .719 1.410	1.61	1.67	.97
2	10% glucosamine 2% inulin .03% PAH	5	7.60	.126	.200 .253 .296	.622 .742 .930	1.86	1.85	1.00
3	10% glucosamine 2% inulin .03% PAH	3	4.46	.081	.154 .161	.741 .712	2.06	1.99	1.03
4	10% glucosamine 2% inulin .03% PAH	3	5.32	.083	.394 .232	1.410 .979	1.16	1.26	.92
5	20% glucosamine 1.5% inulin .03% PAH	6	2.08	.158	.370 .493 .693	.802 1.072 1.448	1.41	1.26	1.12
6	20% glucosamine 1.5% inulin .03% PAH	10	3.34	.216	.342 .572	.782 1.192	2.22	1.91	1.16
7	20% glucosamine 1.5% inulin .03% PAH	5	4.47	.151	.354 .414	.640 .900	1.79	1.75	1.02
Means:			4.48				1.73	1.67	1.03*

C = clearance, Glu = glucosamine, In = inulin, ERPF = effective renal plasma flow, PAH = para-aminohippuric acid.

* Statistical analysis showed that the mean of the ratios was not significantly different from unity. (Student's "t" test, $.4<P<.5$.)

dogenous levels, or metabolism of the glucosamine.

Glucosamine appears to be another substance in addition to inulin that can be used accurately to measure glomerular filtration rate in the rat.

The authors wish to express their thanks to Miss Karen Weigel for able assistance in these experiments.

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Received September 30, 1963. P.S.E.B.M., 1963, v114.