serum concentration following a single parenteral injection of oxacillin supports this thesis since little or no drug is detectable 4 to 6 hours after injection(4).

Conclusion. The renal clearance of oxacillin has been determined in normal males and found to be approximately 45% that of penicillin G. Implications regarding protein binding of oxacillin are discussed.

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Hexosamine Analysis of Renal Papillae in Diuretic and Antidiuretic Rats.* (29140)

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Ginetzinsky(1) has suggested that the action of anti-diuretic hormone (ADH) is mediated by hyaluronidase which depolymerizes the mucopolysaccharides surrounding the collecting ducts in the renal papillae, thus allowing for greater reabsorption of water from the tubules into the interstitia. He reported that the urine of rats in the anti-diuretic state contained large amounts of hyaluronidase. He also reported histological differences in the rat renal papillae between the diuretic and antidiuretic states. During diuretic states cells of the collecting tubules were plump with abundant cytoplasm and the interstitia stained very metachromatically. During anti-diuretic states cells of the tubules were flattened and seemed to have lost most of their cytoplasm, and the interstitia did not stain as metachromatically as during the diuretic state. Berlyne(2) however failed to show any relationship between states of hydration and urinary hyaluronidase. Dicker and Eggleton(3) on the other hand have confirmed the relationship between states of hydration and hyaluronidase. Thorn(4) has produced an anti-diuresis in rats by injections of large doses of hyaluronidase. Ginetzinsky's morphological findings have been disputed by Breddy *et al*(5) and have been confirmed by Stolarczyk and Manitus(6). Ivanova and Vinogradov(7) have also confirmed the histological changes and have suggested that the mucopolysaccharide involved is chondroitin sulfate C. Farber *et al*(8) have isolated acid mucopolysaccharides from beef and rabbit renal papillae.

The mucopolysaccharides isolated from the renal papillae, chondroitin sulfate and hyaluronate, contain hexosamine as one of their two repeating units. Thus the hexosamine content of the renal papillae should correlate well with the amount of acid mucopolysaccharides in the renal papillae.

This study shows that the hexosamine content of the renal papillae is correlated with the histochemical findings and with the states of diuresis and anti-diuresis of the rat. During diuresis the hexosamine content increased while during anti-diuresis the hexosamine content decreased.

Materials and methods. Over 100 white female Wistar rats, 250-300 g, fed a normal diet were divided into the following 7 groups:

Group 1 (N): normally hydrated rats, water *ad lib.*

Group 2 (D): normally hydrated rats (N) dehydrated for 24-48 hours.

Group 3 (NH): normally hydrated rats (N) further hydrated by water, (5% of body

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weight) given by stomach tube.

Group 4 (NA): normally hydrated rats (N) injected with aqueous ADH, 100 to 1000 milliunits dose.

Group 5 (DH): rats dehydrated for 24-48 hours. (D) hydrated by water (5% of body weight) given by stomach tube.

Group 6 (DA): rats dehydrated for 24-48 hours. (D) injected with aqueous ADH, 1000 milliunits dose.

Group 7 (DHA): normally hydrated rats (N) simultaneously hydrated as in group 3 and injected with ADH as in group 4.

All rats were sacrificed by ether anesthesia at times ranging from 15 minutes to 48 hours as noted in the results, after having been made diuretic or anti-diuretic. The kidneys were removed immediately after sacrifice and the renal papillae and/or cortex-medulla were dissected out. Care was taken to obtain about the same amount of renal tissue from each rat; in all cases where the papillae were analyzed the papillary tip was included. The method of Boas(9) was employed for hexosamine analysis following hydrolysis with 4 N HCl for 15 hours. All values for hexosamine are expressed as gamma hexosamine per mg of dry weight. Differences are considered statistically significant using the tables of t to p value of less than 0.02.

Histologic sections were made from transverse slices of kidneys placed in 10% formalin. Hematoxylin and eosin, Hale's stain, and toluidine blue stains were employed.

Results. Chemical analysis revealed significant differences in individual groups between the hexosamine (hex) content of the renal medulla-cortex and papillae. In the normally hydrated rat (N), the medulla-cortex averaged 7.3 γ hex, while the papillae averaged 14.6 ($p < 0.001$). In the dehydrated rat (D), the medulla-cortex averaged 6.8, while the papillae averaged 12.4 ($p < 0.001$). The difference between the papillae in the above 2 groups was significant ($p < 0.001$), but the difference between the medulla-cortex in the same 2 groups was not significant ($0.05 > p > 0.04$) (Table I).

Significant differences were found between the many groups in the hex content of the renal papillae. Dehydration for 24-48 hours (D) caused a lowering to 12.4, $p < 0.001$ when paired with normally hydrated rat (N). Hydration of the normal rat (NH) caused an increase to 16.1, $p < 0.002$ when paired with normal rat (N). Giving ADH to the hydrated rat (DHA) lowered the content to 12.1, $p < 0.001$ when paired with hydrated rat (NH). While giving ADH to the already dehydrated animal (DA) had little effect, the hex content remaining at 12.9, $0.3 > p > 0.2$ when paired with dehydrated rat (D) (Table II).

TABLE I. Comparison of Hexosamine Content of Renal Medulla-Cortex and Papillae.

Group	γ hex/mg dry wt		
	Medulla-cortex	Papillae	p value$_1$
1 (N) normals *ad lib* water	7.3	14.6	<.001
2 (D) normals dehydrated for 24-48 hr	6.8	12.4	<.001
p value$_2$	.05-.04	<.001	

p value$_1$—comparing values horizontally.
p value$_2$—comparing values vertically.

Peak levels of hexosamine after hydration were observed after one to two hours, gradually decreasing toward normal after 3 to 4 hours (Fig. 1). Maximum decrease in hex after administration of ADH was observed at 2 hours; values at one and four hours being within the normal range (Fig. 2). Normally hydrated rats showed no decrease in hex content in the first 4 hours of water deprivation.

Histologic sections revealed marked differences between the groups. During states of hydration the cells of the collecting tubules were large and abundant in cytoplasm. The interstitial tissue in the papillae stained with toluidine blue metachromatically and was positive with Hale's iron stain. During states of dehydration the cells of the collecting tubules were flattened and their nuclei protruded into the lumen. The interstitial tissue which previously stained metachromatically was pale.

Discussion. The results show significant differences in the renal papillae during states of varying diuresis. Dehydrated animals and

TABLE II. Comparison of Hexosamine Content of Renal Papillae During Various States of Diuresis and Antidiuresis.

Group	Time (hr) after last procedure	Mean γ hex/mg dry wt	Sample size	Standard error	P value	Paired sample
1 (N) normals, *ad lib* water		14.6	40	.27		
2 (D) normals, dehydrated	24–48	12.4	12	.20	<.001	1(N)
3 (NH) normals, hydrated	1 & 2	16.1	8	.52	.02–.01	1(N)
4 (NA) normals, given ADH	2	12.4	8	.56	.01–.001	1(N)
5 (DH) dehydrated, hydrated	1 & 2	15.2	12	.24	<.001	2(D)
6 (DA) dehydrated, given ADH	2	12.9	4	.33	.3–.2	2(D)
7 (DHA) hydrated, given ADH	2	12.1	4	.31	<.001	3(NH)

Dehydrated = deprivation of water for 24-48 hr.
Hydrated = water (5% of body wt) given by stomach tube.

animals to which ADH was administered, regardless of their previous state of hydration, showed low levels of hexosamine and by inference low levels of mucopolysaccharide in the renal papillae. In contrast diuretic animals showed high levels of hexosamine in the renal papillae. The histologic results of this study support the previous findings of Ginetzinsky, Ivanova, and Stolarczyk, but do not support the findings of Breddy. Thus the above findings may be in agreement with the theory of Ginetzinsky that ADH acts on the kidney by depolymerizing mucopolysaccharides in the intercellular cement of the collecting tubules and the ground substance of the interstitial tissue.

Since in states of anti-diuresis the hexosamine content is decreased it must be concluded that the mucopolysaccharide is not only depolymerized, but decreased in total content. Ivanova *et al* have presented evidence by histochemical techniques of depolymerization of the papillary mucopolysaccharides in 45 minutes after administration of ADH. Our results indicate that after ADH there is no discernible change in hexosamine content at the end of one hour, but a significant decrease occurs in 2 hours. These observations may be explained by postulating that depolymerization is followed by the mucopolysaccharide components leaving the papillae.

During states of diuresis the hexosamine content in the renal papillae is increased. These studies, performed one to four hours after administration of water, demonstrated maximum levels after one to two hours. Ivanova *et al* have found histochemical changes as early as 16 minutes after administration of water.

These results indicate there is a relationship between hydration, dehydration, and ADH administration and mucopolysaccharide content in the renal papillae.

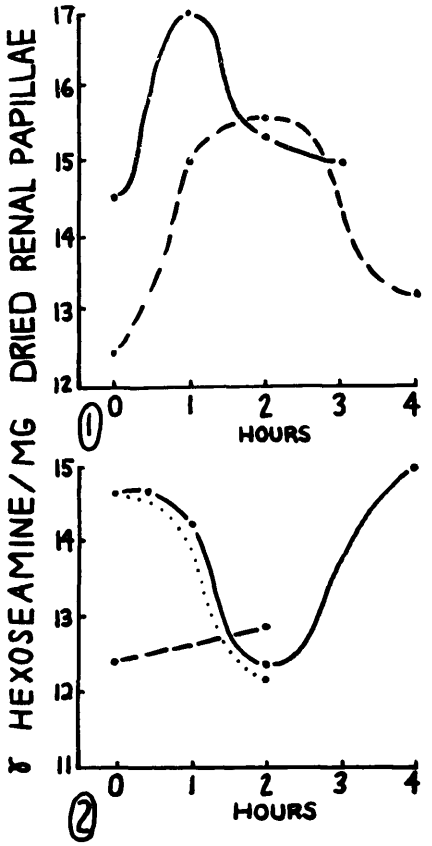

FIG. 1. Effect of hydration on normally hydrated ●——● and on dehydrated ●– – –● rat.
FIG. 2. Effect of ADH administration on normally hydrated ●——●, hydrated ● · · · ●, and dehydrated ●– – –● rat.

Summary. Hexosamine analysis reflecting the content of mucopolysaccharides of the renal papillae of rats in varying states of diuresis and anti-diuresis was carried out. States of anti-diuresis were associated with low levels of hexosamine, while states of diuresis were associated with high levels of hexosamine. Hexosamine contents were correlated with physiologic and histochemical data.

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Lens Cell Membrane Permeability and Cataract Formation.* (29141)

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Theories concerning the mechanism of cataract development following the production of experimental diabetes or the feeding of high galactose diets have been based on data obtained during the cataractogenic period(1-4) and on analyses of lenses with mature cataracts(5). There has been little correlation between morphological and physiological changes. It has been suggested that the vacuoles which appear early in the cataractogenic process are due to osmotic swelling(6) resulting from the high levels of intracellular polyols(7) which, in the lens, follow hyperglycemia. The later and sudden appearance of an opaque lens has not been correlated with a biochemical or physiological change. In this paper is presented evidence that the sudden appearance of cataract is associated with an equally sudden change in the permeability of the lens fiber or cell.

Methods. All experiments were done with diabetic or galactose fed Sprague-Dawley rats. Cataracts were determined with the naked eye by observing the lens daily and noting the day on which the lens became opaque.

The first experiment was run to determine the concentration of lens sorbitol, glucose, fructose, and adenosine triphosphate (ATP) and the values for the lens weight, and extracellular water, before and at the time of diabetic cataract formation. Thirty-eight day old rats were made diabetic by intravenous injection of 50 mg of alloxan monohydrate per kg of body weight. Only rats with average blood sugar over 400 mg percent were used. The median time for cataract formation was 76 days.

The second experiment was designed to provide information similar to that obtained in the first. In this case, however, the cataracts followed the feeding of a 35% galactose diet to rats with an initial age of 27 days. With this regimen, the median time for cataract formation is 18 days.

The lenses were removed and weighed as previously described(8).

Sorbitol was determined by the method of West and Rapoport(9) as modified by Faulkner(10); glucose by the oxidase method(11); fructose by the method of Roe(12); and

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