TABLE II. Amounts of MPS Extracted from Urines of 3 Patients with Morquio-Ullrich Disease and Percentages of KS as Calculated from Content in Neutral Sugars of MPS.

	M.L.	B.R.	B.M.T.
Total MPS/liter of urine	22.1 mg	17.1 mg	30.5 mg
KS %	46.2	37.6	39.4

identical composition and contain approximately equimolar amounts of glucosamine, galactose and sulphate. These results and the infrared spectra (Fig. 1) indicate that MPS fraction studied is KS. Neither ChS B nor HMS were found in the urines of our patients. Amounts of MPS extracted from the urines and percentages of KS, as calculated from the content of neutral sugars of the MPS, are reported in Table II.

Conclusions. This study points to a disorder of the MPS metabolism in Morquio-Ullrich disease different from that found in Hurler's disease. Excretion of KS appears to be pathognomonic of Morquio-Ullrich disease although study of more cases of this and other osteochondrodystrophies is needed to corroborate our finding.

Summary. The MPS present in the urines of 3 patients with Morquio-Ullrich's disease have been studied. In all 3 cases a substantial amount of KS has been identified. The identification has been based on chemical, chromatographic and infrared data. This MPS has hitherto never been identified in urine, either normal or pathological. Neither ChS B or HMS was found in the urines of these children. The metabolic disorder of the MPS in Morquio-Ullrich's disease appears to be different from that observed in Hurler's disease.

1. Di Ferrante, N., Rich, C., *J. Lab. Clin. Med.*, 1956, v48, 491.
2. ———, *Clin. Chim. Acta*, 1956, v1, 519.
3. Di Ferrante, N., *J. Clin. Invest.*, 1957, v36, 1516.
4. Linker, A., Terry, K., Teller, W. M., *Fed. Proc.*, 1962, v21, 170.
5. Dorfman, A., Lorincz, A. E., *Proc. Nat. Acad. Sci.*, 1957, v43, 443.
6. Meyer, K., Grumbach, M. M., Linker, A., Hoffmann, P., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 275.
7. Meyer, K., Hoffmann, P., Linker, A., Grumbach, M. M., Sampson, P., *ibid.*, 1959, v102, 587.
8. Maroteaux, P., Lamy, M., *Rev. Franc. et Clin. Biol.*, 1961, v6, 481.
9. Zellwegger, H., Ponseti, I. V., Pedrini, V., Stamler, F. S., van Noorden, G. K., *J. Ped.*, 1961, v59, 549.
10. Sevag, M. G., *Bio. Chem. Zeit.*, 1934, v273, 419.
11. Dische, Z., *J. Biol. Chem.*, 1947, v167, 189.
12. Scott, J. A., Melvin, E. H., *Anal. Chem.*, 1953, v25, 165.
13. Schloss, B., *ibid.*, 1951, v23, 1321.
14. Dodgson, K. S., *Biochem. J.*, 1961, v78, 312.
15. Anastassiasis, P., Common, J., *Canad. J. Biochem. Physiol.*, 1958, v36, 413.
16. Chargaff, E., Levine, F., Green, C., *J. Biol. Chem.*, 1958, v175, 67.
17. Stoffin, P., Jeanloz, R. N., *Arch. Biochem. Biophys.*, 1954, v52, 373.
18. Partridge, S. M., *Nature*, 1949, v164, 443.

Received June 7, 1962. P.S.E.B.M., 1962, v110.

Morphology of the Collecting Tubules in Diuretic and Antidiuretic Rats. (27669)

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The mechanism of action of antidiuretic hormone (ADH) is still obscure. Ginetzinsky has proposed that the action of ADH is mediated through hyaluronidase(1). He showed that in rats during antidiuresis induced either by dehydration or by administration of exogenous vasopressin, the excreted urine contains a large amount of hyaluronidase. Similar results were found during osmotic diuresis. On the other hand, when water diuresis was in-

duced in rats, the excreted urine was virtually free of hyalurodinase. Microscopical examination of kidney slices stained with toluidine blue showed differences depending upon the type of diuresis. During water diuresis the cells of collecting tubules were large and rich in cytoplasm and a distinct metachromasia of the intracellular cement substance could be seen. By contrast, during antidiuresis and osmotic diuresis, the epithelium of collecting tubules was composed of flat cells, containing only a small amount of cytoplasm and no metachromasia of the cement substance could be seen. On the basis of these observations Ginetzinsky proposed that ADH causes an apocrine secretion of hyaluronidase by the cells of the collecting tubules. The released enzyme presumably hydrolyzes hyaluronic acid contained in the cement substance of tubular epithelium, thus rendering it permeable to water. The observations of Ginetzinsky on hyaluronidase excretion have been confirmed by Dicker and Eggleton(2) but not by Berlyne(3). The morphological findings have been disputed by Breddy *et al.*(4). It therefore seemed of value to report the results of our morphological studies which fully support the findings of Ginetzinsky.

Material and methods. Thirty white, male Wistar rats, weighing 250 to 300 g, fed a normal diet, were divided into 4 groups:

Group I (10 rats): 48 hours prior to sacrifice water and food were taken away. Two hours before the animals were killed 50 milliunits of pitressin tannate in oil were injected subcutaneously in each rat.

Group II (5 rats): 10 cc of 7% aqueous solution of urea were given by stomach tube to each rat. One and one-half hours later, when marked diuresis was established, the animals were sacrificed.

Group III (10 rats): 10 cc of distilled water, warmed to 38° C, were given by stomach tube to each rat. One and one-half to 2 hours later, when animals excreted markedly dilute urine, they were sacrificed.

Group IV (5 rats): Each animal received by stomach tube 1.5 cc of 48% ethyl alcohol. One and one-half to 2 hours later they were sacrificed.

All the animals were anesthetized with ether and both kidneys immediately removed. Transverse slices of the kidneys were fixed in Zenger's solution and then placed in paraffin. After paraffin was removed and tissue slices hydrated they were stained with toluidine blue in order to reveal gamma metachromasia (5). In examining the kidneys from all groups, care was taken to compare slices of medulla taken at similar distances from the papillary tip.

Results. Histological sections from kidneys of all rats in the same group presented a similar picture. Marked differences, on the other hand, were noted in the histology of kidneys from rats of different groups. These concerned the structure of the collecting tubules almost exclusively, though in some distal convoluted tubule changes similar (but much less marked) to those found in the collecting tubules were found. The morphological picture of the collecting tubules of rats of different experimental groups may be summarized as follows:

Group I (antidiuresis): The lumina of the collecting tubules appear large and cytoplasm of the lining cells is almost completely absent. The cell nuclei seem to lie free in the lumen of the tubules. Gamma metachromasia is seen only in the basal membrane of the epithelium (Fig. 1).

Group II (osmotic diuresis): The picture is similar to that of Group I, though more cellular cytoplasm is apparent. Neverthless, the amount of cytoplasm is small in comparison with the size of the nuclei. Gamma metachromasia can again be observed only in the basal membrane of the epithelium (Fig. 2).

Group III (water diuresis) and *Group IV* (alcohol diuresis): The microscopical picture is very similar in these 2 groups but differs completely from that found in Groups I and II. The lumen of the collecting tubules is narrow. The cells are rich in cytoplasm and their shape as well as intracellular granules can be distinctly seen. Gamma metachromasia can be seen not only in the basal membrane but also in the intraluminal margin and intercellular membrane (Fig. 3).

Discussion. The results show a marked difference in the morphology of the collecting tubules during antidiuresis and osmotic diuresis on one hand and during water and alcohol diuresis on the other. This agrees with the re-

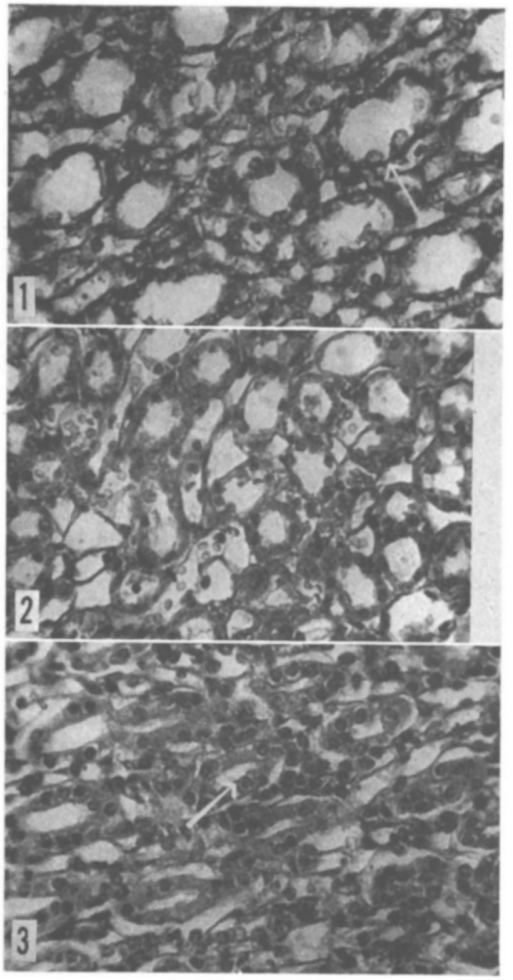

FIG. 1. Antidiuresis. Toluidine blue staining. Collecting tubules: large lumina, almost complete lack of cytoplasm of the cells. Gamma metachromasia of basal membrane (arrow).

FIG. 2. Osmotic diuresis. Toluidine blue staining. Collecting tubules similar to those in Fig. 1; large lumina, markedly flat tubular cells. Gamma metachromasia of basal membrane.

FIG. 3. Water diuresis. Toluidine blue staining. Collecting tubules: narrow lumina, large cells rich in cytoplasm. Gamma metachromasia of basal membrane, intraluminal margin and intercellular membrane (arrow). Picture similar to that found during alcohol diuresis. All figures ×325.

sults reported by Ginetzinsky(1) and does not support the findings of Breddy *et al.*(4). The latter authors suggested that a different morphological picture could be obtained if histological sections were taken at varying distances from the papillary tip. In the present studies care was taken to obtain all slices at a similar distance from the tip of the renal papilla, approximately halfway from the tip to the base.

The changes reported may not, of course, represent the morphological state of the collecting tubules *in vivo,* subject as they are to the influences of fixation, dehydration and staining. Hanssen has shown, however, in mice kidneys that early post mortem changes do not affect the collecting tubules appreciably (6). Although it seems reasonable to connect the morphological changes of the collecting tubules with changes in ADH activity(1) there is no direct evidence of such a relationship. It might be suggested that the observed changes of the collecting tubules are due simply to shrinking or swelling of the cells, this being related to changes in extracellular tonicity of the renal medulla. This would, however, not explain the changes noted in metachromasia.

Summary. Studies were carried out in rats during antidiuresis and during diuresis produced by urea, water and alcohol. Animals were killed and their kidneys removed for microscopical examination. During antidiuresis the appearance of the epithelium of the collecting tubules was very similar to that found during osmotic diuresis but completely different from that seen during water and alcohol diuresis. It is suggested that the changes found may be related to presence or absence of antidiuretic hormone.

1. Ginetzinsky, A. G., *Nature,* 1958, v182, 1218.

2. Dicker, S. E., Eggleton, M. G., *J. Physiol.,* 1961, v157, 351.

3. Berlyne, G. M., *Clin. Sci.,* 1960, v19, 619.

4. Breddy, P., Cooper, G. F., Boss, J. M. N., *Nature,* 1961, v192, 76.

5. Pearse, E., *Histochemistry,* Churchill Ltd., 1960, p835.

6. Hanssen, O. E., *Acta path. microbiol. Scand.,* 1960, v49, 299.

Received June 12, 1962. P.S.E.B.M., 1962, v110.