aminoacetonitrile. 3. Diets high in casein, gelatin, vit. E, and other antioxidants did not protect the rats fed *Lathyrus odoratus* seeds from developing skeletal lesions. 4. Radioautographs of the skeleton after injection of S^{35} and studies on sulfur excretion of rats fed *Lathyrus odoratus* peas and rats fed a control diet showed that the metabolism of sulfur is probably not altered in lathyrism.

1. Wawzonek, S., Ponseti, I. V., Shepard, R. S., and Wiedenmann, L. G., *Science,* 1955, v121, 63.

2. Ponseti, I. V., and Shepard, R. S., *J. Bone and Joint Surg.,* 1954, v36A, 1031.

3. Bachhuber, T. E., Lalich, J. J., Angevine, D. M., Schilling, E. D., and Strong, F. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 294.

4. Dasler, W., *ibid.,* 1955, v88, 196.

5. Lee, J. G., *J. Nutrition,* 1950, v40, 587.

6. Bachhuber, T. E., and Lalich, J. J., *Science,* 1954, v120, 712.

7. Dasler, W., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 485.

8. Evans, T. C., and McGinn, W. E., Jr., *Cancer Research,* 1953, v13, 661.

9. Dziewiatkowski, D. D., *Metabolic Interrelations, 4th Conference,* J. Macy Foundation, New York, 1952.

10. Bauer, G. C. H., Carlsson, A., and Lindquist, B., *Acta Pathol. et Microbiol., Scandinavica,* 1955, v37, 407.

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Some Clinical Conditions Affecting Urinary Excretion of Non-Dialyzable Hexosamine.* (22481)

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In a preceding communication(1), the normal levels of adult human urinary excretion of non-dialyzable conjugated hexosamine were reported to be 32.4 ± 7.2 mg/day for males and 25.9 ± 5.8 mg/day for females. The hexosamine appeared to be principally glucosamine. Other recent publications(*e.g.,* 2-5) related to this subject indicate or suggest that more than one conjugate contributes to the total hexosamine present in the non-dialyzable fraction of both normal and pathological urine, part being present as a result of glomerular filtration and part originating from the urinary epithelium.

This communication reports the results of investigations of "bound" hexosamine excretion in certain clinical conditions. These determinations were undertaken in an effort to distinguish some aberrations affecting bound hexosamine excretion, which in turn might further elucidate its origin and nature.

Methods. The procedure was the same as that previously described(1). Twenty-four-hour urine specimens were accurately collected and preserved with phenylmercuric nitrate. As a check on the entire procedure, samples of urine from healthy individuals were included at intervals.

Results. The results are expressed as mg glucosamine per 24 hours. Initially, urines from patients with some selected conditions were examined. These preliminary experiments indicated an increase in bound hexosamine in several of these (Table I). Urolithiasis, atherosclerosis, and pregnancy were further studied.

Urolithiasis. Neither the chemical type of urolite, a low-calcium diet, nor the presence of infection (in each of these cases minimized by concurrent antibiotic or chemotherapy) appeared to influence the results. An elevated bound hexosamine excretion was found in all cases (Table II). This was particularly striking in cases with large or actively growing stones.

Three male patients were included who had

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TABLE I. Bound Hexosamine Excretion Found for Some Conditions.

Diagnosis	mg hexosamine/day
Males	
Idiopathic myocarditis	48.7
Pernicious anemia	53.8
Pulmonary emphysema	29.5
Total colectomy, ilio-proctostomy, ulcerative colitis (on cortisone)	19.1
Controlled diabetes mellitus	48.0
Bilateral cutaneous ureterostomy	13.0
Chronic osteomyelitis	31.9, 26.0
Bilateral nephrocalcinosis	48.7
Females	
Post-infection cystitis	45.7
Polycystic kidney disease	19.0, 24.0, 39.5, 29.2
Hyperthyroidism	47.7
Hypothyroidism	29.3
Adrenal hyperplasia (on cortisone)	32.0
Glomerulonephritis	13.3
Controlled diabetes mellitus	25.3
Subclinical diabetes insipidus	30.1
Rheumatoid arthritis (on ACTH)	32.1
" " (no medication)	30.3

normal urinary tracts at the time of the urine collection, but with a past history of formation of a 2 mm, or smaller, stone. These patients had subnormal levels of bound hexosamine: five samples averaged 22.7 mg per day.

Two days on a high (800 mg/day) calcium diet did not elevate the bound hexosamine level of a normal male, 28.1 mg being found.

Atherosclerosis. This group, comprised mostly of males (44-55 years old), had typical clinical evidence of fulminating atherosclerotic disease, as reflected symptomatically in electrocardiograms, and by serum lipoprotein and serum cholesterol levels. A significant increase in bound hexosamine excretion was invariably present (Table II).

Pregnancy. Specimens were obtained from 4 multiparas and 5 primaparas during pregnancy and in 2 subjects also subsequent to parturition. An augmented appearance of bound hexosamine regularly occurred during the last 5 months of pregnancy, with a return to the normal value in about 2 months (Table III).

Although 2 of the patients with renal calculi listed in Table II had previous unilateral nephrectomies, they still gave values no different from the others, suggesting that the urothelium of the collecting system is not a major contributor of bound hexosamine in this condition (but *cf.* ureterostomy patient, Table I).

Chemical studies. Since any determination of hexosamine in biological material is liable to error from many sources, comparisons have been made using other technics of hexosamine measurement. Parallel samples of dialyzed, hydrolyzed urine were assayed by the usual procedure(1), by Prodi's(6) modification of it, or by the method of Dische and Borenfreund(7). The method of Prodi (in 10 normal and clinical samples) was less precise, but gave values ranging within 20 mg per day of the present method, while that of Dische and Borenfreund gave much higher values, having no apparent constant relationship to those obtained by our technic. The latter results probably reflect an interference(8) by the hexoses present(4).

Hexosamine was estimated by the Prodi technic, along with samples including 1 ml of 3.2% $Na_2B_4O_7 \cdot 10H_2O$, according to Tracey (9). The volatile fraction from the Elson and Morgan reaction is affected in the same way by borate as is the total reaction product, *i.e.*, glucosamine had its color intensity reduced about 75% by borate treatment while that of galactosamine was reduced only about 50%. Samples of dialyzed, hydrolyzed normal urine so treated exhibited an average

TABLE II. Bound, Non-Dialyzable Hexosamine Excretion by Normal Individuals, and Patients with Atherosclerosis or Urolithiasis.

Disease	No. subjects ♂	No. subjects ♀	No. specimens ♂	No. specimens ♀	Hexosamine/day (mg avg ± S.D.) ♂	Hexosamine/day (mg avg ± S.D.) ♀	Probability of significance ♂	Probability of significance ♀
None*	14	13	30	18	32.4 ± 7.2	25.9 ± 5.8		
Urolithiasis	15	16	20	27	50.0 ± 11.9	53.8 ± 22.5	$P < .001$	$P < .001$
Atherosclerosis	7	1	16	1	46.4 ± 9.4	56.0	"	

* From reference (1).

TABLE III. Prenatal and Postpartum Values for Non-Dialyzable Hexosamine for 9 Subjects.

Weeks pregnant	8	12	16	20	24	28	32	36
mg hexosamine	25.0		26.9					
	32.3	19.0	26.5	39.9	43.9	47.3	43.0	36.0
	29.7		25.0	56.8	51.4	52.2	54.6	36.8
	25.2		28.6	51.0		52.8	56.5	62.5
							192.5*	
Weeks post-part.	1	2	8	9				
mg hexosamine	107.8	43.9	28.9	49.6				
			28.4	25.3				
				31.6				

* Proteinuric.

color reduction of 73% after borate treatment, confirming both the specificity of the Prodi procedure and the predominance of glucosamine over galactosamine.

Discussion. The finding of an increased hexosamine excretion by patients with urinary calculi is in accord with Boyce *et al.*(2), and others(10,11), who find a similar increase in hexosamine-containing biocolloids in the urine and urinary tracts of such patients.

The increased mucinous secretion by the epithelium of the urinary tract in pregnancy is accompanied by an increase in urinary mucoprotein(12), which is evidently reflected in bound hexosamine measurement. Since these specimens were not obtained by catheterization, the vaginal epithelium could also have contributed. The rise in the hexosamine-containing plasma proteins during pregnancy (13) could be connected with these observations.

Summary. 1. The quantity of non-dialyzable hexosamine in human urine increases significantly and consistently during the last half of pregnancy, and in patients with renal calculi or atherosclerosis. 2. The results indicate that this hexosamine may have a variety of origins.

1. King, Jr., J. S., and Hyder, N., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 342.

2. Boyce, W. H., Garvey, F. K., and Norfleet, Jr, C. M., *Am. J. Med.*, 1955, v19, 87.

3. Craddock, J. G., and Kerby, G. P., *J. Lab. Clin. Med.*, 1955, v46, 193.

4. Hamerman, D., Hatch, F. T., Reife, A., and Bartz, K. W., *ibid.*, 1955, v46, 848.

5. Chatagnon, C., and Chatagnon, P., *Ann. biol. clin.* (Paris), 1955, v13, 49.

6. Prodi, G., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 605.

7. Dische, Z., and Borenfreund, E., *J. Biol. Chem.*, 1950, v184, 517.

8. McCrea, J. F., *Biochem. J.*, 1951, v48, xlix.

9. Tracey, M. V., *Biochim. et Bioph. Acta*, 1955, v17, 159.

10. Baker, R., and Sison, F., *J. Urol.*, 1954, v72, 1032.

11. Baratta, P. F., Mari, E., Rizzati, E., and Angeli, G., *Giorn. biochim.*, 1951, v1, 101.

12. Boyce, W. H., personal communication.

13. Mack, H. C., *The Plasma Proteins in Pregnancy, A Clinical Interpretation*, Chas. C. Thomas Co., Springfield, 1955.

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