The elevation in total polysaccharide appeared to parallel the degree of gross pathology in the tuberculous animals. The group showing the greatest increase in polysaccharide (rabbits infected with bovine type tubercle bacilli) also demonstrated the most extensive tuberculosis.

Increase in alpha-2 globulin glycoprotein seen in all animals following tuberculosis has also been described by Seibert(10) in clinical tuberculosis. Seibert's study suggests a possible correlation of elevated alpha-2 glycoprotein and increased tissue destruction. Our findings did not reveal this relationship in experimental tuberculosis. The greatest increase, for example, occurred in the rat, which had no gross pathology. The rabbits infected with bovine type bacilli had gross involvement of lungs, liver, spleen and kidney; whereas the rabbits infected with human type tubercle bacilli had only minimal involvement of the lungs. Despite these marked differences in gross pathology, the increase in alpha-2 glycoprotein was essentially identical in both groups. The guinea pig, with more extensive tuberculosis than the human strain infected rabbits and less pathology than the bovine type infected rabbit, had a greater alpha-2 glycoprotein increase than either of the two groups of infected rabbits.

Summary. 1. Total serum protein, polysaccharide and paper electrophoresis of protein and glycoprotein were determined before and 4 weeks following, tuberculous infection of rats, rabbits and guinea pigs. Hyperproteinemia occurred in infected rabbits and guinea pigs. Elevation of alpha-1 globulin in the guinea pig and alpha-2 globulin in rabbits and rats was noted. Beta globulin decreased in infected rats and increased in guinea pigs, and bovine infected rabbits. Gamma globulin elevated in all species. 2. Total serum polysaccharide was elevated in all infected animals. Likewise, the alpha-2 glycoprotein was elevated in all species. Only the infected rabbit had elevated gamma globulin glycoprotein.

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Amino Acid and Carbohydrate Composition of the Mucoprotein Matrix in Various Calculi.* (23161)

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A technic for recovery of the organic matrix from calcigerous urinary calculi has been described(1). Preliminary studies of the amount, elemental composition and some histochemical characteristics were reported(1,2). This report is concerned with the carbohydrate and amino acid content of matrix recovered from calcium containing urinary and

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submaxillary gland calculi and from urinary calculi of essentially pure uric acid and cystine composition.

Methods. The matrix from calcigerous urinary and submaxillary calculi was recovered by decrystallization with ethylenediaminetetraacetate, dialysis, ultrafiltration and lyophilization as previously described(1). Uric acid and cystine calculi were decrystallized in 0.1 M 5,5-diethylbarbituric acid buffer (pH 8.6) containing 10% neutral formalin as a preservative. Decrystallization was adjudged complete when the supernatant solution gave a negative Folin's test for uric acid or cyanide test for cystine. The recovery of matrix from the buffer solution was in all other respects similar to the procedure for calcigerous stones. *Amino acid components.* Samples of the lyophilized matrix were hydrolyzed in 6 N HCl for 24 hours at 90°C, blown dry, and further desiccated *in vacuo* overnight. This residue was taken up in a disodium versenate (0.5% w/v)-*iso*propanol (10% v/v)-water solution and centrifuged. The supernatant solution was chromatographed in 2 dimensions in each of the following solvent systems: (a) 2,6-lutidine:water (65:35 v/v) and phenol:water (4:1 v/v); (b) *n*-butanol:acetic acid:water (4:1:5 v/v) and *n*-butanol:ethanol (95%):water (4:1:1 v/v); and (c) *n*-propanol:ethyl acetate:water (7:1:2 v/v) and *iso*-butyric acid:water (4:1 v/v). Whatman #1 paper was used. Solvent ascension was begun after a 24-hour equilibration period. A unidimensional set of known amino acids was included in each direction with each group of unknowns. After 40 to 50 hours of migration the chromatograms were developed by spraying with 0.25% ninhydrin in acetone. The spots were marked as they appeared and identified by comparison of the Rf values with those of the knowns. Microbiological assays for amino acids were made on two samples of pooled matrix by the method of Henderson and Snell(3). *Carbohydrate components.* The technics of Glegg and Eidinger(4) were used for a 48 hour resin hydrolysis of matrix samples and unidimensional chromatography of the sugars. A double or triple ascension of 40 cm was made in each of the following solvent systems: (a) 2,6-lutidine:water (65:35 v/v); (b) *n*-butanol:acetic acid:water (4:1:5 v/v); and (c) *n*-propanol:ethyl acetate:water (7:1:2 v/v). Known sugars were run side by side with the unknowns. Studies with the diphenylamine reagent were made after the method of Winzler(5) utilizing standards of both crystalline "sialic acid" derived from human meconium† and 2-deoxyribose-5-phosphate.‡ Naphthoresorcinol tests for hexuronic acids were made after hydrolysis either with resin(4) or by the DeFrates-Boyd procedure. Controls were material isolated from urine by the DiFerrante-Rich technic(6) and commercial chondroitin sulfate. All spectrophotometric measurements were made with the Beckman DU instrument. Tests for lipids were made by the method of Artom and Fishman(7).

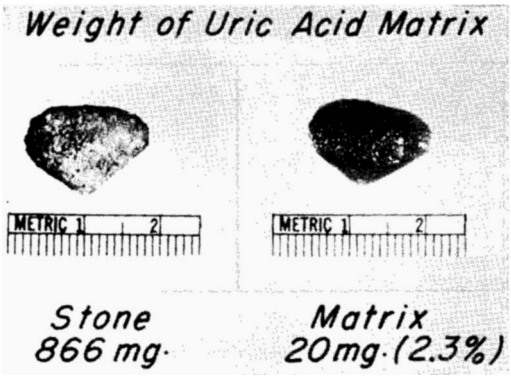

FIG. 1. Appearance and dry wt of typical uric acid stone before and after decrystallization.

Results. The organic matrix was distributed from the center to the surface of all calculi. In the majority of calculi the decrystallization left the matrix as a cast of the original stone. This was most apparent in the uric acid stones where the only visible evidence of decrystallization was a change in color from tan to greenish-brown (Fig. 1). In cystine stones the matrix usually collapsed into a disorganized sediment on decrystallization. The matrix accounted for 2.5% of the original dry weight of calcigerous stones, 2.3% of uric acid stones, and approximately

† Through the courtesy of Dr. Richard J. Winzler, Univ. of Illinois College of Medicine.

‡ Prepared by Dr. Sam. H. Love, Dept. of Microbiology, Bowman Gray School of Medicine.

TABLE I. Amino Acids Identified Chromatographically.

α-Alanine	Glycine
Glutamic acid	Threonine
Aspartic acid	Lysine
Serine	Proline
Leucine	(Tryptophane)*
Isoleucine	(Tyrosine)
Valine	(Methionine)
Phenylalanine	(Arginine)

* Amino acids in parentheses were faint and inconsistently seen.

9.0% of cystine stones.

The amino acids detectable by chromatography are presented in Table I in the descending order of visual intensity after ninhydrin development. Two or more unidentified ninhydrin-positive spots were consistently present in all matrix material. Their Rf values were not consistent with those of any commonly encountered amino acids. The severity of the hydrolysis should eliminate the possibility of any residual peptides in this material, but undoubtedly prevented the detection of certain compounds (*e.g.*, hexosamine). No differences were noted among the amino acid composition of any of the various matrix preparations regardless of the source or crystalline composition of the stones.

The results of microbiological assay of 17 detectable amino acids are given in Table II, with similar data for osseomucoid(8).

Chromatographically, 5 carbohydrates were clearly separated from the matrices of all calcigerous and cystine calculi, of both urinary and salivary origin. These migrated identically with the standards in each of 3 solvent systems and are the following, in the approximate order of their color intensity after treatment with aniline hydrogen oxalate: galactose, glucose, mannose, rhamnose, and fucose. In no instance has glucose been demonstrated in any pure uric acid calculus. This does not preclude its presence but does indicate that glucose is not present in uric acid matrix in the same concentration relative to other carbohydrates as it is in the other matrices.

Deoxypentose appears to be consistently present in small quantities in matrices from all calculi except that of cystine stones, which have not been thoroughly examined. This is based on the appearance of a pure blue color with the diphenylamine test for "sialic acid" (Fig. 2). This reaction is closely analogous to that described by Dische(9), and gives no color or different and weak colors, with other sugars so tested. Furthermore, the blue color developed in identically the same manner in both matrix and 2-deoxyribose solutions during the heating period of the test. The ultraviolet absorption curve gave no evidence of the presence of desoxyribonucleic acid in calculous matrix which gave the blue color reaction.

Quantitative assays of hexosamine and protein-bound hexose have given varying results with various pools and methods. The reason for these variations is being further studied.

Fig. 1 clearly demonstrates that "sialic acid" is not a component of matrix material. The tests for hexuronic acid were all negative although commercial chondroitin sulfate and material isolated from urine by the Di-Ferrante-Rich technic(6) gave positive results. There was no evidence of any cholesterol or lipides in a calcigerous matrix examined. A test for ketohexoses(10) was also negative in each of 3 calcigerous matrix pools.

Discussion. The finding of apparently the

TABLE II. Results of Microbiological Assay of Calcigerous Stone Matrix.

	Osseo-mucoid*	Stone matrix Range	Mean
Alanine	3.70	2.4 - 4.90	3.65
Glycine	2.65	2.1 - 3.26	2.82
Valine	4.50	1.8 - 2.12	2.11
Leucine	7.27	3.41 - 8.47	5.39
Isoleucine	3.65	2.23 - 4.00	2.88
Proline	4.24	1.10 - 2.11	1.53
Phenylalanine	2.86	2.11 - 2.46	2.29
Tyrosine	1.98	1.60 - 1.88	1.75
Tryptophan		.43 - .74	.53
Serine	3.61	2.70 - 5.36	4.07
Threonine	4.13	4.21 - 4.75	4.40
Cystine	1.13		
Methionine	1.09	.91 - .98	.95
Arginine	3.87	3.05 - 4.08	3.60
Histidine	2.65	1.19 - 1.52	1.30
Lysine	4.26	2.60 - 5.28	4.05
Aspartic	9.66	3.76 - 5.40	4.08
Glutamic	11.67	5.87 - 6.45	6.10
Total	72.92	41.47 -63.76	51.50

* From reference 8.

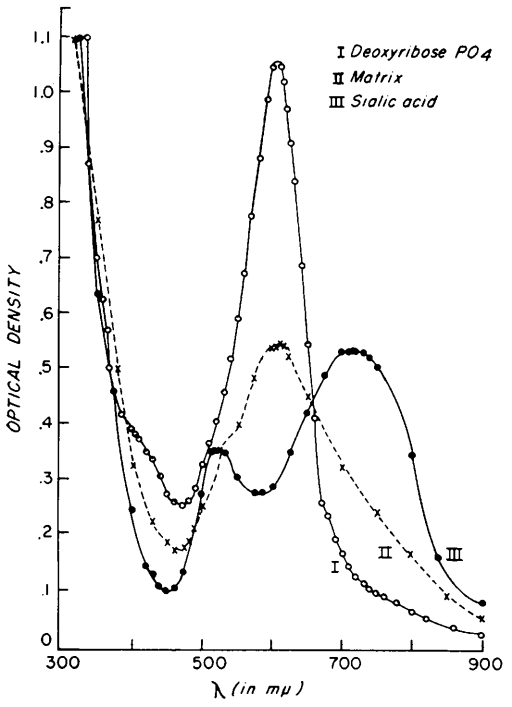

FIG. 2. Absorption curves obtained with 400 μg 2-deoxyribose-5-phosphate/ml (I), calcigerous stone matrix (II), and 100 μg crystalline sialic acid/ml (III) after reaction with diphenylamine. Each set on a blank without diphenylamine.

same spectra of amino acid and carbohydrate composition in the organic matrix of stones obtained from the ducts of the submaxillary gland and calcium containing stones in the urinary tract is of interest since the crystalline composition of stones from these sources is indistinguishable. Histochemical studies have previously indicated that matrices of both calcigerous urinary calculi and salivary gland calculi are PAS (periodic acid-Schiff leucofuchsin reaction) positive and also metachromatic with toluidine blue. Indeed, all urinary calculi are PAS positive, while metachromasia has not been observed in matrix from uric acid stones. It should be noted, however, that we have never seen a cystine calculus that did not show a faint opacification on clinical roentgenograms. Presumably this is due to a small amount of calcium bound in some way to the crystal surfaces. The absence of metachromasia in uric acid stone matrix may be related to the apparent absence of glucose. That is, the spatial configurations responsible for metachromasia in the mucoprotein may be related to the presence of the glucose molecule. There is no present evidence to indicate whether the glucose is related to the deposition of uric acid crystals or exclusion of calcium crystals from the matrix.

The demonstration that stone matrix is both PAS positive and metachromatic with toluidine blue has suggested the presence of both a neutral mucoprotein and an acid conjugated mucopolysaccharide. The fact that metachromasia is highly variable in many calculi and that it is necessary to study some serial sections of calcium containing stones before it is demonstrated seemed to bear out this supposition. If the metachromatic material is a mucopolysaccharide it is apparently lost in the dialysis procedures which are used to remove the decalcifying agents because we have found no hexuronic acid in the ultrafiltrate. Thus we have been able to obtain only a mucoprotein from stone matrix and it seems likely that the metachromasia is due to some specific configuration of a neutral mucoprotein which also gives a positive PAS reaction.

The occurrence of deoxypentose in this material is not proof that this material is an integral component of the carbohydrate moiety of the mucoprotein molecule. This sugar is presumed to be deoxyribose(11) since this is the only deoxypentose commonly encountered in human metabolism. Material isolated by the DiFerrante-Rich procedure (6) gives the same color reaction and is dialyzable (as is part, at least, of the hexuronic acid). The fact that it is present in matrices from both salivary gland and urinary calculi, is further indication that the matrix of these calcigerous stones is not a chance inclusion in a crystalline precipitate but a necessary part of calculus formation.

Summary. The amino acid and carbohydrate components of the organic matrix of stones from the submaxillary gland and from the urinary tract have been studied by paper chromatographic, microbiological and spectrophotometric technics. The chromatographic amino acid pattern was indistinguishable and

deoxypentose, hexosamine, and 5 other sugars were present in all of these matrices. The carbohydrate composition was similar in all stones with the exception of uric acid calculi, in which glucose appears to be absent.

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Metabolism of Steroids II. Half-Life of Various Steroids in Dogs.* (23162)

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Concentrations of free adrenal steroids in peripheral circulation are governed by a number of unknown factors, including rates of production by the adrenal cortex, of conjugation with other compounds, of excretion by the kidney and of *metabolism* in tissue. It has been established(1-3) that the disappearance of exogenous steroids from peripheral circulation is a first order reaction, *i.e.* rate of disappearance is proportional to plasma concentration. Accordingly, when logarithms of steroid concentration are plotted against time, a straight line results. From the equation for this regression line, the half-life of the steroid can be calculated. In human subjects the half-life of cortisol varies with age (4) and is influenced by certain diseases (1,2). Also, different steroids have different half-life values, but maintain a fairly consistent relationship to one another when studied in the same human subject(2,5). In the present study the rates of disappearance from the peripheral circulation of several different steroids have been measured in the dog.

Materials and methods. The animals used were trained, unanesthetized adult mongrel dogs, each weighing approximately 20 kg. Each animal was rested for at least one week and occasionally for several weeks between successive experiments. Thus, although each dog was used for many experiments during a 2-year period, and although blood loss per experiment approximated 200 ml, no animals developed anemia and the hematocrit values remained constant throughout an experiment. The steroids employed were cortisol (Compound F), cortisone (Compound E), corticosterone (Compound B), and Δ^1-cortisol (Δ^1-F). These compounds were prepared for intravenous administration by dissolving the free steroid in 50% ethanol.§ This solution was diluted in each case 15-20 fold with 5% dextrose in water and administered by intravenous drip during a 10 minute period. The amount of steroid given in each experiment was 2 mg/kg body weight. The moment the infusion was completed, was re-

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