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Infrared spectroscopy was used to determine the composition of 120 urinary calculi from Macedonia (Yugoslavia). Of the compounds usually mentioned as constituents of urinary calculi only the following were found in the collection under consideration: calcium oxalates (mono- and dihydrate), apatites (carbonato-apatite and, probably, octacalcium phosphate), magnesium ammonium phosphate hexahydrate (struvite), uric acid and its dihydrate, ammonium urate and cystine. Calcium oxalates are by far the most common of the stone-forming compounds (found in more than three fourths of the investigated calculi). Apatites are found in 44.2% of the stones and the occurrence of other constiuents is far less frequent (uric acid dihydrate, ammonium urate and cystine are found only occasionally). Apatites can combine with almost all the other constituents, the mixtures with oxalates being unseparable and with a higher concentration of phosphate near the centre of the urolith. On the other hand, uric acid and urates, cystine and, almost always, struvite form clearly separated regions or layers. Almost all compounds, except apatites, are well crystalized.

INTRODUCTION

Despite the fact that the composition of urinary calculi has been extensively studied by different techniques (Prien and Frondel, 1947; Balkrishna Rao et al., 1964; Flerovskii, 1965; Morris and Beeler, 1967; Otto und Ihmann, 1967), the great variety of the results obtained seems to jutsify further examination of this interesting problem. It is to be hoped that a comparative study of the results obtained in various regions of the world where the way of living, nutritional habits etc. are different could throw some more light on the problem of the causes of urolithiasis and, hence, the possibility of its prevention and treatment. Such a study is, however, beyond the scope of the present work in which we merely report the results of our infrared analysis of a collection of 120 urinary calculi from Macedonia (Yugoslavia). The infrared technique was chosen because of its simplicity and the possibility of identification of individual chemical compounds as such, rather than of ions, as is the case when the classical chemical analysis is used. Our interest in the infrared spectroscopy of calcium phosphates (Petrov et al., 1967; Šoptrajanov and Petrov, 1967; Petrov et al., 1968) was a further reason to undertake the present study.

MATERIALS AND METHODS

The calculi used for the study were kindly supplied by the staff of medical clinics in Skopje (Macedonia) for which we express our deepest gratitude.

The composition of the calculi was studied by comparing the infrared spectra, recorded mainly on KBr pressed discs (Ford and Wilkinson, 1954), of urolithic material and of stone-forming compounds, either pure or in mixture with one another. This method is described in detail by Stojanova et al. (1969) and further reference should be made therein.

The smaller stones were analysed as a whole, whereas the bigger ones were carefully cut as close to the centre as possible and samples (weighing approximately 1 mg) were taken from the surface and from the centre. When layers or parts of apparently different constitution were visible, samples were taken from each of them.

The infrared spectra were recorded on a Perkin-Elmer Model 521 Infrared Spectrophotometer.

RESULTS AND DISCUSSION

A total of 120 urinary calculi, collected during a period of over a year were analysed. The origin of uroliths was as follows: 75 were from males, 33 from females and 12 from patients for whom there were no data about sex. Altogether over 200 infrared spectra were recorded.

All the spectra examined could be interpreted on the basis of the spectra of the folowing stone-forming compounds:

— calcium oxalate monohydrate (whewellite), $CaC_2O_4 \cdot H_2O$
— calcium oxalate dihydrate (weddellite), $CaC_2O_4 \cdot 2H_2O$
— apatites (carbonato-apatite, $Ca_{10}(PO_4)_6CO_3$ and, quite probably, octacalcium phosphate, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$
— magnesium ammonium phosphate hexahydrate (struvite), $MgNH_4PO_4 \cdot 6H_2O$
— uric acid, $C_5N_4H_4O_3$
— uric acid dihydrate, $C_5N_4H_4O_3 \cdot 2H_2O$
— ammonium urate, $C_5N_4H_3O_3NH_4$
— cystine, $C_6H_{12}N_2O_4S_2$.

The frequency of the appearance of these compounds in the investigated uroliths is given in Table I.

In both Table I and Table II the number and percent values are given separately for stones from male, female and unknown patients and also for the total number of 120 investigated uroliths.

The first thing suggested by Table I is the fact that calcium oxalates are by far the most common of urinary calculi constituents: they are found

Table I. — Frequency of appearance of individual constituents in the investigated calculi

Constituent	Male Number	Male %	Female Number	Female %	Unknown Number	Unknown %	Total Number	Total %
Whewellite	42	56.0	21	63.6	5	41.7	68	56.7
Weddellite	44	58.7	7	21.2	3	25.0	54	45.0
Ca-oxalates (whewellite and weddellite taken together)	62	82.7	24	72.7	7	58.3	93	77.5
Apatites	28	37.3	16	48.5	9	75.0	53	44.2
Struvite	8	10.7	3	9.1	6	50.0	17	14.2
Uric acid	7	9.3	5	15.2	1	8.3	13	10.8
Uric acid dihydrate	—	—	1	3.0	—	—	1	0.8
Amonium urate	3	4.0	—	—	1	8.3	4	3.3
Cystine	1	1.3	1	3.0	—	—	2	1.7

in almost 80% of the investigated calculi (over 80% if only the stones from male patients are considered). Of the two oxalates, whewellite and weddellite are approximately equally often found in the calculi from male patients, whereas those from female patients show a much higher rate of incidence of whewellite. Apatites are encountered in somewhat less than half of the investigated uroliths (relatively more often in female patients), whilst struvite and uric acid are far less frequent. Ammonium urate, cystine and the dihydrate of uric acid are found only occasionally.

Another way of looking at the composition of calculi is to examine *all* components taking part in the formation of *one* calculus, irrespective of whether the components are intimately mixed or form separate layers or regions in the calculi. The results, summarized in such a way, are presented in Table II.

Even a casual look at Table II reveals a significantly larger number of calculi from male than from female patients. We are presently unable to tell whether urolithiasis is absolutely more common in men than in women or this finding is purely accidental and due to the insufficient number of investigated stones. Only an analysis of a larger collection, assembled during a longer period of time, can give a definite answer to this question.

Another interesting fact, suggested by Table II, is the relatively higher percent of apatites and the lower percent of weddellite in the calculi from female with respect to those from male patients. In fact, pure apatite (carbonato-apatite) was found only in female and pure weddelite only in male patients.

Calcium oxalates and apatites can be found in any possible combination between themselves and approximately 70% of the analysed stones consist of these compounds, without other constituents. If the calculi containing struvite are added, i. e. if only the salts of oxalic and phosphoric acids are considered, the value rises to 84%. On the other hand, the number of urate stones (i. e. those containing uric acid, its dihydrate and ammonium urate) is much smaller: 17 uroliths or 14%. Thus, we found somewhat less calcium and magnesium salt of oxalic and phosphoric acids and more than twice uric acid and its salts than did Prien and Froundel (1947) and, on the other hand, somewhat more of the former and much less of the latter

Table II. — Composition of the investigated calculi

Composition	Male Number	Male %	Female Number	Female %	Unknown Number	Unknown %	Total Number	Total %
Whewellite	9	12.0	9	27.3	2	16.7	20	16.7
Weddellite	11	14.7	—	—	—	—	11	9.2
Whewellite Weddellite	15	20.0	1	3.0	—	—	16	13.3
Whewellite Apatite	3	4.0	4	12.1	1	8.3	8	6.7
Weddellite Apatite	9	12.0	3	9.1	2	16.7	14	11.7
Whewellite Weddellite Apatite	9	12.0	3	9.1	1	8.3	13	10.8
Apatite	—	—	3	9.1	—	—	3	2.5
Apatite Struvite	5	6.7	2	6.1	4	33.3	11	9.2
Whewellite Apatite Struvite	1	1.3	1	3.0	1	8.3	3	2.5
Struvite	2	2.7	—	—	—	—	2	1.7
Struvite Ammonium urate Uric acid	—	—	—	—	1	8.3	1	0.8
Apatite Ammonium urate	1	1.3	—	—	—	—	1	0.8
Whewellite Ammonium urate	2	2.7	—	—	—	—	2	1.7
Whewellite Uric acid	3	4.0	3	9.1	—	—	6	5.0
Uric acid	4	5.3	2	6.1	—	—	6	5.0
Uric acid dihydrate Struvite	—	—	1	3.0	—	—	1	0.8
Cystine	1	1.3	1	3.0	—	—	2	1.7
Total	75	100.0	33	100.0	12	99.9	120	100.0

than did Otto and Ihmann (1967). The number of cases in which cystine is found is always extremely small, so that the percent values given by various authors do not really bear any absolute significance.

It is also characteristic that apatites can combine with practically all encountered stone-forming compounds. In fact, only cystine and uric acid and its dihydrate were not found in the same stone with apatite. Whewellite was not found to combine, besides with these compounds, with struvite either.

Probably more significant than the fact of two or more constituents being present in one stone is their distribution through it and the formation of intimate mixtures or, alternatively, of separate layers or regions. It was found that in mixtures of apatite and the two oxalates, usually inseparable mixtures are encountered with the concentration of phosphate higher near the center than on the surface. Of the two oxalates, weddelite, as a rule, was found more often at the surface than inside the uroliths. On the other hand, uric acid, its dihydrate, cystine and, almost always, struvite formed layers or regions clearly separated from the other components.

Another fact worth mentioning is the crystallinity of the constituents of uroliths. It is well-known that a higher degree of crystallinity of a substance results in a sharpening of infrared absorption bands with frequent splitting of the latter due to the coupling of oscillations if more than one identical structural unit is present in the unit cell. On the other hand, substances with a low degree of crystallinity show broad and ill-defined bands as a consequence of the random orientation of structural units in the crystal lattice. Comparison of the spectra of uroliths with those of synthetic or commercial compounds showed that the constituents of calculi are, as a rule, better crystallized than the compounds serving as standards. Only the apatites are almost always amorphous (or very nearly so) which makes the indentification of individual members of this class of compounds extremely difficult not only by infrared methods, but also by other techniques (Haugh et al., 1966). Nevertheless, the existence of characteristic carbonate bands (at 1470 and 1423 cm^{-1}) shows that among substances with apatitic structure, carbonato-apatite is by far the commonest one. In cases in which this is not so, octacalcium phosphate, rather than hydroxyapatite, seems to take part in the formation of uroliths (Petrov et al., 1969).

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ИСПИТУВАЊЕ НА 120 УРИНАРНИ КАЛКУЛУСИ ОД МАКЕДОНИЈА (ЈУГОСЛАВИЈА) СО ПОМОШ НА ИНФРАЦРВЕНАТА СПЕКТРОСКОПИЈА

Корнелија СТОЈАНОВА, Иван ПЕТРОВ и Бојан ШОПТРАЈАНОВ

Извод

Инфрацрвената спектроскопија беше употребена за утврдување на составот на 120 уринарни калкулуси од Македонија. Од соединенијата што обично се споменуваат како составни делови на уролитите, во нашата колекција беа најдени само следниве: калциумови оксалати (моно- и дихидрат), апатити (карбонатен апатит и, веројатно, октакалциум фосфат), магнезиум амониум фосфат хексахидрат (струвит), мочна киселина и нејзиниот дихидрат, амониум урат и цистин. Калциумовите оксалати се далеку најчестите конституенти (ги има во повеќе од три четвртини од испитаните уролити). Апатити се најдени во 44,2% од калкулусите, а појавата на другите конституенти е знатно поретка (дихидратот на мочната киселина, амониум уратот и цистинот се најдени само во неколку случаи).

Апатитите доаѓаат заедно со скоро сите други конституенти. Смешите со оксалати не може да се разделат, а концентрацијата на фосфатот е обично поголема близу до центарот на уролитот. Од друга страна, мочната киселина, цистинот и, скоро секогаш, струвитот образуваат јасно оддeлени области или слоеви.

Скоро сите конституенти, освен апатитите, се убаво кристализирани.