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BOVINE UROLITHIASIS IN ANDHRA PRADESH

By

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RAO, S. V. and A. UNMACK: *Bovine urolithiasis in Andhra Pradesh.* Acta vet. scand. 1973, *14,* 410—420. — Urinary calculi from three bulls, six bullocks and one male buffalo from the Guntur district were investigated. By chemical analysis only CO_3^{2} , Ca^{2+}, Mg^{2+}, and a trace of PO_4^{3-} were found. X-ray analysis showed that all samples consisted of magnesian calcite with an atomic percentage of magnesium between 14.5 and 21.9. The size of the crystallites varied approx. from 200 to 400 Å.

urolithiasis; magnesian calcite; calculi.

Bovine urolithiasis is endemic in three regions of Andhra Pradesh (South-East India): Guntur and Krishna districts of Coastal Andhra, Cuddapah, Kurnool and Anantapur districts of Rayalaseema, and Nalgonda and Mahaboobnagar districts of the Telangana regions.

In spite of the encouraging results obtained by simple surgical treatment of urethral obstruction, with or without rupture of the bladder (*Rao* 1972), urolithiasis still remains a problem due to recurrence after the operation because cystic calculi again migrate into the urethra. Their removal by laparocystotomy is not easy and the results under field conditions are discouraging.

It has been attempted to dissolve urinary calculi in situ by flushing with appropriate solvents (*Meyer* 1925, 1927, 1929, *Gehres & Raymond* 1951, *Mudvaney* 1959, 1960), but in order to choose the best solvent a correct knowledge of the composition of the calculi is essential. If calculi from the three regions are analysed and an exact knowledge of their composition is thus obtained, the possibility of preventing a recurrence after the operation by flushing can be considered. This idea prompted the first author to a study of the composition of the calculi.

In view of the extension of urolithiasis, the amount of analytical work done in India is relatively small. *Ranganathan* (1931a, b) from South India, *Kataria & Rao* (1969) from Central India, and *Sinha* (1952), *Mohanty* (1963), *Ranjhan et al.* (1965), and *Chakrabarti et al.* (1970) from North India published the compositions of urinary stones from cattle and buffaloes. Their results are given in Table 1. In the original papers the results were given on different scales — as percentage of oxides, carbonates, and the elements themselves. In order to facilitate comparison, all results have been recalculated and given as per cent Ca, Mg, etc.

The results given by *Ranganathan* (1931a), by *Sinha* and by *Mohanty* (the latter later with more details by *Ranjhan et al.*) show that their samples have been carbonate stones. Although carbonate is not explicitly mentioned, the same probably applies to *Kataria & Rao*'s silica-free stones and to some of *Chakrabarti et al.*'s samples. The stone taken from a calf, described in *Ranganathan*'s second paper (1931b), is of phosphatic type.

Table 1. Older Indian results.

Authors	% Ca	% Mg	% P	% N	% CO_2	% H_2O	% ox.ac.	ur.ac.
Ranganathan (1931a)[1]	31.46	2.92	0.39	0.37	39.07	3.10	0.7	0
Ranganathan (1931b)[2]	7.79	7.96	9.60	7.60	0	16.07		0
Sinha (1952)[3]	28.4	7.50	0.12				0	0
Mohanty (1963)[4]	12.135	2.322	0.096					
Ranjhan et al. (1965)[5]								
Kataria & Rao (1969)[6]	32.0	0.63	1.46					
Chakrabarti et al. (1970)[7]	26.9	5.2	1.85					

[1] Given as: 44.00 % CaO; 4.84 % MgO; 0.90 % P_2O_5; 0.6 % C_2O_3. Dried material.

[2] Given as: 10.90 % CaO; 12.05 % MgO; 22.00 % P_2O_5. Dried material.

[3] Given as: 71 % $CaCO_3$; 26 % $MgCO_3$; 3 % $Ca_3(PO_4)_2$. Probably undried material.

[4] and [5] same analyses. [4] gives only the average, [5] also individual values. Say: carbonate reaction "heavy". Probably undried material.

[6] 44 % of the samples investigated contained Si. The values given here are average values for six Si-free samples. Probably undried material.

[7] Average values for 24 samples of very varying composition. Probably undried.

The only results for cattle stones from Andhra Pradesh were published about 40 years ago (*Ranganathan,* loc. cit.). It was therefore considered desirable to analyse again calculi from the different regions in Andhra Pradesh using modern physical methods. Guntur District was taken first.

MATERIALS

Calculi were collected from three bulls, six bullocks and one male buffalo. As it was considered possible that the calculi might consist of hydrous silica and as it is known that the formation of this type of concrement depends on the content of silica in the fodder (*Whiting et al.* 1958, *Forman et al.* 1958, 1959, *Forman & Sauer* 1962, *Connell et al.* 1959), samples were also collected of the plants on which the cattle had been fed, as well as of the mud settling on the plants after floods, all from the same villages as the clinical cases. As it was found, however, that the calculi did not contain silica, but consisted of magnesian calcite, the fodder and the mud were not analysed.

Table 2. Origin and outer appearance of samples.

Animal				Description of samples		
sample no.	type	age (years)	weight (lbs.)	number	weight (g)	colour and special features
1	bullock	9	1500	21	2.80**	light grey to brownish grey; lustre
2	bullock	12	1000	5	3.33**	as 1, but less lustre
3	bullock	4	600	12*	2.14**	as 2
4	bullock	8	800	>50	3.49	yellowish; beautiful lustre
5	bull	3	700	18	1.19	light yellow; no lustre
6	bull	2½	500	>50	2.43	light yellow; lustre; small black spots
7	bullock	14	900	>50	4.36	as 6, but more contaminated
8	bullock	9½	1000	23*	0.81	greyish brown; no lustre
9	male buffalo	5	500	>50	2.13	light yellowish; lustre
10	bullock	8	950	25*	2.30**	as 9

* and some tiny fractions.

** weight of biggest stone: 0.72, 1.04, 1.15, and 0.62 g, respectively.

The information received together with the samples as to weight and age of the animals is given in Table 2 together with a short description of the samples.

Outwardly the samples were strikingly similar and evidently also similar to those examined by *Ranganathan* and by *Sinha* (both loc.cit.). The smaller calculi had the appearance of baroque pearls, including the pearly lustre. The bigger ones seemed to be conglomerates of several such small spheres, kept together and covered by a smooth layer through which the individual spheres were more or less visible. Some of the samples were contaminated with a black material not identified. The stones were hard. When crushed, the outer layer, visibly laminated, was often broken off, leaving a compact core. The weight of the calculi varied from less than 0.05 g to 1.15 g.

Representative specimens are shown in Fig. 1.

The chemical analysis and the X-ray investigation were carried out by the second author (A. Unmack). The results are given below.

CHEMICAL ANALYSIS

By qualitative analysis after the method described by *Tovborg Jensen & Thygesen* (1941), only CO_3^{2-}, Ca^{2+}, and Mg^{2+} were found. As PO_4^{3-} was not present in discernible amount, the calculi must be remarkably poor in phosphate compared with human urinary calculi (*Prien & Frondel* 1947) and with those of feedlot steers (*Cornelius & Bishop* 1961).

By application of a colorimetric micro method a trace of PO_4^{3-} (about 0.5 % (w/w)) was, however, found in all samples. The content of Ca^{2+} and Mg^{2+} was determined by atomic absorption. The water content was determined as the loss at 105 °C. This content (about 1.5 % (w/w)) was no bigger than what might be expected in a system of such high dispersion (see later).

From the analytical results it must be concluded that calcium and magnesium are present as carbonates. The results are given in Table 3. The sum of the contents of water, calcium carbonate and magnesium carbonate is never 100. The deficit (probably organic matter) is about 10 % in all cases. The content of organic matter was not determined.

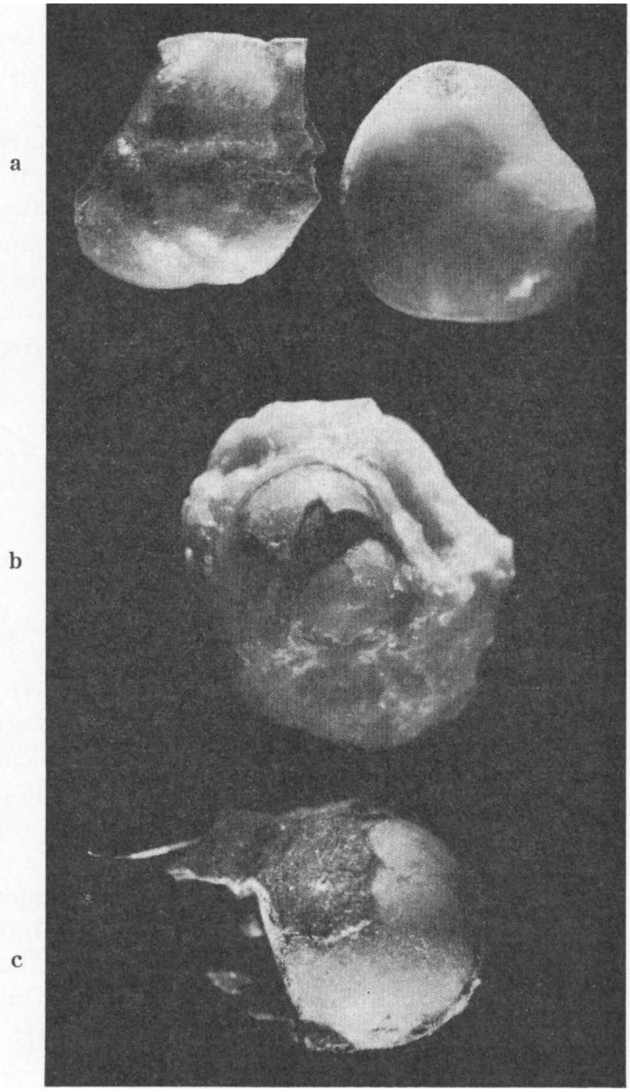

Figure 1. Urinary calculi. a: a lustrous pearl and a piece of shell; b: a partly crushed stone, showing the laminated shell and the inner core consisting of two spheres; c: a lustrous pearl contaminated with dark (unidentified) material. × 10.

Table 3. Chemical composition of the samples.

Sample No.	Percentage (w/w)					In 100 g of sample			Mol.% $MgCO_3$
	H_2O	$CaCO_3$	$MgCO_3$	PO_4^{3-}	total	mol. $CaCO_3$	mol. $MgCO_3$	Σmol.	
1	1.4	74.4	14.7	0.3	90.8	0.743	0.174	0.917	19.0
2	2.1	72.7	17.2	0.4	92.4	0.726	0.204	0.930	21.9
3	1.7	75.4	10.3	0.4	87.8	0.753	0.122	0.875	13.9
4	1.7	74.4	15.0	0.3	91.4	0.743	0.178	0.921	19.3
5	1.3	74.7	14.7	0.5	91.2	0.746	0.174	0.920	18.9
6	1.4	78.2	11.8	0.2	91.6	0.781	0.140	0.921	15.2
7	1.7	76.2	12.2	0.2	90.3	0.761	0.145	0.906	16.0
8	1.0	75.7	13.1	0.2	90.0	0.756	0.155	0.911	17.0
9	1.4	74.7	15.5	0.3	91.9	0.746	0.184	0.930	19.8
10	1.6	74.4	10.6	0.3	86.9	0.743	0.126	0.869	14.5

In all samples, reactions for oxalic acid, uric acid and ammonium salts were negative.

X-RAY INVESTIGATION

Chemical analysis has shown that the main constituent of the calculi is calcium carbonate, but of this compound three different crystal forms are known, each of them having its individual solubility. X-ray investigation was therefore undertaken to obtain information on the crystal structure. Powder diagrams were taken of all samples with Co-Kα-radiation (Fe filter) in 19 cm cameras of the Bradley type. In one case diagrams were taken of material from the inner core and the outer shell separately. No difference was seen. All diagrams corresponded to calcite, somewhat modified. All lines were considerably broadened and somewhat displaced towards higher diffraction angles.

The broadening may more or less be due to reduced particle size, but other possible causes exist, for instance an inhomogeneity of the material. If the crystallites to some degree have different composition (cf. p. 417, l. 15—17), the lattice constants will vary and the lines therefore be unsharp.

The displacement towards higher angles, corresponding to a contraction of the lattice, can be explained by a partial substitution in the lattice of the larger calcium ion by the smaller magnesium ion.

If it is assumed that the only reason for the line broadening is a small particle size, the average dimension of the crystallites can be approximately determined (see for instance *Peiser et al.*

1955, p. 413). As Bradley diagrams are not suited for this purpose, diffractometer curves were taken with Cu-Kα-radiation (Ni filter). By means of these curves the breadth of a certain peak at half peak height could be determined. From the difference in the value of this breadth for poorly and for well crystallized material (B and b, respectively) the average particle size may be calculated by means of the formula:

$$B - b = K\,\lambda/(t \cos \theta),$$

where λ is the wave length used, θ is the diffraction angle, and K is a constant approximately equal to 1. When B — b is given in radians, the formula gives t, the particle dimension, in Å.

The measurements gave the following approximate values:

samples Nos.	1	2	3	4	5	6	7	8	9	10
t (in Å)	450	200	300	350	350	400	400	250	400	300

The diffractometer curves were also used for a determination of the lattice spacing, d, of the strongest calcite line (d is given by the formula $d = \lambda/(2 \sin \theta)$).

In pure calcite d for this line is 3.035 Å. In magnesian calcites the spacing is smaller, as already mentioned. The decrease, Δd, is very nearly proportional to the magnesium content as seen from Fig. 2 where Δ d is plotted against mol.% $MgCO_3$ (x) deter-

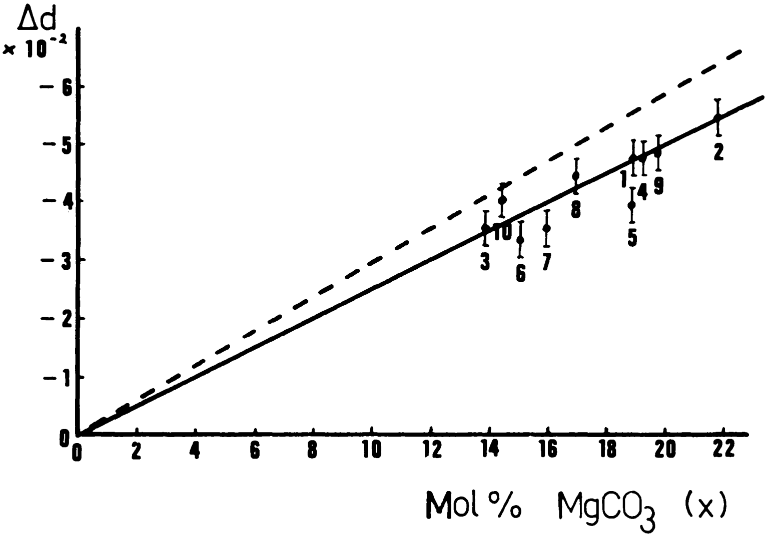

Figure 2. Experimental correlation between x and Δd for the 10 samples compared with Goldsmith's curve (------). The estimated uncertainty of Δd is indicated.

mined by atomic absorption. Because the material is poorly crystallized, the peak is low and broad, and the uncertainty in 2θ, and therefore also in Δ d is considerable.

The correlation between Δ d and x in magnesian calcites has been investigated by *Goldsmith et al.* (1955, 1961). They examined synthetic, homogeneous and well crystallized specimens, and their measurements are consequently more accurate than ours. They find significantly larger decrease in d with increasing magnesium content than we do. Considerations which we shall not discuss here make it reasonable that the decrease in d with increasing x will be smaller and more fluctuating in poorly crystallized and probably inhomogeneous material than in the perfectly crystalline, homogeneous material used by *Goldsmith et al.* (1955, 1961).

We know that our samples are poorly crystallized and it is more than likely that a sample formed over a longer period should not be homogeneous.

DISCUSSION

It might be of interest to compare the present results with those of the older Indian investigations in which carbonate stones were found, especially with those of *Ranganathan* (1931a), mostly from Andhra Pradesh. As already remarked all older results (given in Table 1) have been recalculated to a common scale (per cent Ca etc.). Even after this recalculation the values are not strictly comparable, because it has not always been stated whether the percentages are given in relation to the original sample or to the dried material. For carbonate stones the water content is always small, so for this type of stones the general

Table 4. Mol. per cent $MgCO_3$ in carbonate stones.

Authors	Number of samples	Mol. per cent $MgCO_3$ in carbonate		
		min.	max.	average
Ranganathan (1931a)	23	6.3	23.3	13.2
Sinha (1952)	unknown			30.3
Mohanty (1963) *Ranjhan et al.* (1965)	10	18.4	27.4	23.9
Kataria & Rao (1969)	6	0.4	5.5	3.1
Rao & Unmack (1973)	10	14.5	21.9	17.5

picture will not be much influenced by this uncertainty. Moreover, this uncertainty is of no importance when the values of mol.% $MgCO_3$ in the magnesian calcites are compared. As not only the average values, but also the lower and upper limits found are of interest, the mol.% $MgCO_3$ has been calculated for each individual sample (Table 4).

It is interesting to note that in the 40 years between the investigation of *Ranganathan* and the present one the character of the calculi found in cattle of Andhra Pradesh has not changed, and that the same type of concrements has been found in North and in Central India.

The remarkable constancy in constitution exhibited by carbonate calculi may possibly give some indication of the conditions which prevailed in the urine from which they were deposited. The authors are at present investigating the precipitation of magnesian calcites from aqueous solutions in the hope of finding the limits of composition of solutions resembling normal bovine urine between which highly dispersed magnesian calcites with about 14 to 22 mol.% $MgCO_3$, devoid of phosphate, will precipitate at body temperature. A solution of this problem would be of immense interest, but so far we have not been able to solve it. In our experiments phosphate free solutions invariably precipitated pure and well crystallized Mg-free calcite. When phosphate was added in concentrations likely to be present in bovine urine colloidal hydroxyapatite was formed.

The composition of the urine from the animals who had produced the samples was not known. In future it will be attempted to collect samples of urine simultaneously with the removal of the calculi.

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SAMMENDRAG

Urolithiasis hos kvæg i Andhra Pradesh.

Blæresten fra de tre tyre, seks stude og en bøffeltyr fra Guntur distriktet er blevet undersøgt. Ved kemisk analyse fandtes kun carbonat, calcium, magnesium og et spor af fosfat. Røntgenundersøgelse viste, at alle prøverne bestod af magnesiumholdig calcit med et magnesiumindhold mellem 14,5 og 21,9 atomprocent. Størrelsen af krystalitterne varierede fra ca. 200 til 400 Å.

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