A

CLINICAL STUDY

OF

CANINE UROLITHIASIS

by

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FOREWORD

The purpose of this study on some clinical aspects of Canine Urolithiasis was to provide essential background data for future investigations on this disease intended to be undertaken in the Royal (Dick) School of Veterinary Studies. Knowledge of the incidence of the disease in the clinic population, the incidence of the different chemical types of uroliths, and the incidence of other aspects of this disorder as it occurs in this area was therefore required, since only when these facts are available can efficient planning of future investigations into the more important aspects of this anomaly be effected. By necessity, therefore, the present investigation has in part, taken the form of a survey of these aspects in a number of clinical cases of the disease - the results of this survey are embodied in the first part of this thesis.

Although Urolithiasis has been known to be a serious condition in dogs for many years, the suspected aetiologic factors associated with urolithogenesis have been little studied and consequently the position of their individual contributions to the overall disease-complex remains obscure. In an endeavour to clarify the position of some of these causative factors, their correlation with the different chemical types of uroliths and occasionally the inter-relationships between one factor and another, were studied and the results of this have been recorded *inter alia* in part two.

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The remaining section of this thesis deals with the analysis of uroliths obtained from the patients studied. It is immediately apparent in a condition of this kind where we are probably dealing with, not one disease, not a series of similar diseases, but a number of seemingly unrelated diseases which may have little in common apart from the resultant calculus formation, that in order to differentiate between the possible conditions involved, an accurate method of analysis of the uroliths was required. It being assumed that the different chemical types of urolith form as a result of different disorders. In this study, a combination of quantitative micro-chemical and X-ray diffraction crystallographic analysis was selected to fulfil these requirements.

PART ONE

INCIDENCE

PART ONE

INCIDENCE

1. INCIDENCE OF UROLITHIASIS IN THE CANINE POPULATION.

Review of the Literature.

Several Authors (Krabbe, 1949; Krook and Arwedsson, 1956; Uller, 1959; and White, Treacher and Porter, 1961) have recorded the incidence of clinical urolithiasis in the dog (Table 1). Krabbe (1949) found that 72 cases presented for consultation during a 15 year period at the Royal Veterinary and Agricultural College in Copenhagen constituted 0.6% of the total clinic population. The same incidence of 0.6% was reported by Krook and Arwedsson (1956) with 84 cases observed in 'hospitalised' patients during an 18 year period in Stockholm. In 17,308 small animal cases presented over a 30 year period at the Veterinary High School in Vienna, Uller (1959) reported 554 cases of canine urolithiasis, which indicates a much higher incidence (3.2%). but since the total population included both cats and dogs the precise incidence could not be assessed. White, Treacher and Porter (1961) calculated the percentage incidence of the disease in the population of animals presented at the Beaumont Animals Hospital of the Royal Veterinary College, London, during a 6 year period, to be 2.0%; 223 clinical cases were observed in an estimated population of 11,049 dogs.

Materials/

Materials and Methods. The present study concerns dogs which were presented at the small animal clinic of the Royal (Dick) School of Veterinary Studies during the 2 years 1964-65. In the first year 6,518 dogs were examined, in the second 5,734. However, a proportion of dogs in the first year also attended as revisits during the second year. To estimate this proportion a random sample of 10% of the 5,734 dogs presented in the second year was examined and it was found that 39% of them had attended during the previous year. The estimated total of individual dogs seen in the 2 years was therefore 10,016.

Diagnosis of urolithiasis was by radiographic examination of the entire urinary tract of animals showing urinary tract disorders, viz. all urinary obstructive conditions and most of the irritative cases when characterised by haematuria and/or frequency. In obstructive and irritative conditions of unknown aetiology, examination for the presence of the more radiolucent stones by cystography was also undertaken.

The sex, ages and breeds of all except 323 animals were available in the clinic records.

Result: 41 clinical cases of urolithiasis were observed in the estimated population of 10,016. The percentage incidence of the disease was thus 0.41.

Discussion.

The present percentage incidence is therefore lower than in any previously/

previously reported clinical series. It is roughly 0.2% below the percentage recorded by Krabbe (1949) and by Krook and Arwedsson (1956); it is approximately 1.6% below the value recorded in London by White, Treacher and Porter (1961) and 2.8% below that reported by Uller (1959) in Vienna.

This disparity from the previously reported incidences of the condition may be due to true regional differences such as occur in man (Gershoff, 1964) or it may merely be an apparent difference due to the sampling method. Sampling variations however seem unlikely to account for the entire gross differences recorded, and in this connection it may be stated that the number of clinical cases presented at the Edinburgh Clinic during each of the previous five years has remained approximately constant as has also the total yearly number of dogs presented. It seems likely therefore that the incidence recorded over the period under review represents closely the true incidence for dogs presented at the small animal clinic of the Royal (Dick) School of Veterinary Studies in Edinburgh.

Regional variations in popularity of the breeds more prone to urinary lithiasis may influence the percentage incidence; this may in part explain some of the discrepancies. However, values of incidence derived from the population of dogs presented at a clinic cannot be taken as that occuring in the population at large because the sample is not a random one. The extent of this bias may well vary from clinic to clinic and so account for some of the variations in incidence reported.

Incidence/

Incidence figures from data derived from pathological material are also difficult to assess. Krook and Arwedsson (1956) recorded 161 cases which represented 1.9% of the total number of dogs autopsied and considered that this material gave a more representative picture of the distribution in the sexes, breeds and age groups. This claim however can hardly be supported unless the animals which were examined post mortem were a representative selection from the dog population. The earlier report by Klemmer (1895) of the Dresden Pathological Institute gives the incidence of urolithiasis in 3,301 dogs examined post mortem as 0.38% a figure that closely corresponds with the incidence recorded in this investigation.

TABLE 1

INCIDENCE OF CANINE UROLITHIASIS

(A) Clinical Series

Author	Area	Total Population of dogs	No. of Cases	Percentage Incidence
Krabbe (1949)	Copenhagen	11,291	72	0.6
Krook and Ardwedsson (1956)	Stockholm	-	84	0.6
Uller (1959)	Vienna	17,308	554	3.2
White, Treacher and Porter (1961)	London	11,049	223	2.0
This STUDY	Edinburgh	10,016	41	0.41

(B) Pathological Material

Author	Area	Total number of post-mortems	No. of Cases	Percentage Incidence
Krook and Ardwedsson (1956)	Stockholm	-	161	1.9
Klemmer (1895)	Dresden	3,301	-	0.38

2. INCIDENCE OF THE CHEMICALLY DISTINCT TYPES OF CALCULI.

Review of the Literature.

Chemical analysis of a series of canine urinary calculi, was first undertaken by Lassaigne in 1823 who recorded the following incidence of chemical types from a series of museum specimens at the Royal Veterinary College at Alfort. "Uric Acid 58%: Ammonia 30.8%: Phosphate of Lime 10.1%: Oxalate of Lime 1.1%". Lassaigne also reported the occurrence of cystine in a canine vesical calculus.

A few years later Furstenberg (1844) from Germany reported that the composition of uroliths from carnivores varied in the following order of decreasing frequency: "Ammonio-phosphate of line, phosphate of lime, Carbonate of lime, Silicic acid, Oxalate of lime and Oxide of iron". Triple Phosphate was also known to occur in canine uroliths at this time (Gamgee, 1862).

During the present century a number of authors have recorded the incidence of the chemically distinct types of stones occurring in the canine urinary tract (Hobday, 1922; Klarenbeek, Langer and Raabe, 1935; White, 1944; Krabbe, 1949; Uller, 1959; Schlaaff, 1961; White, Treacher and Porter, 1961; White, 1963; Bruyere, 1963; Verstraete, van der Stock and Mattheeuws, 1964; and White,1966). Table 2. The reports of White in 1963 and 1966 being extensions to the initial series of White, Treacher and Porter (1961).

All the above authors, with the exception of Verstraete van der Stock and Mattheeuws (1964), have apparently used qualitative micro-chemical methods of analysis to determine the chemical type of the majority of the calculi.

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TABLE 2.

PERCENTAGE OCCURRENCE OF THE DIFFERENT CHEMICAL TYPES OF URINARY CALCULI

Author	No. of Calculi Analysed	Phosphate %	Oxalate %	Cystine %	Urate or Uric Acid %	Miscellaneous %
Hobday, 1922	41	85.3	7.3	2.4	-	4.8
Klarenbeek, Langer and Raabe, 1935	28	64.4	28.6	3.5	3.5	-
White, 1944	103	64.0	10.5	18.0	7.5	-
Krabbe, 1949	32	75.0	18.8	3.1	3.1	-
Uller, 1959	163	65.0	20.9	6.8	7.3	-
Schlaaff, 1961	45	73.3	22.2	-	2.2	2.2
White, Treacher and Porter, 1961	122	61.5	15.5	11.5	11.5	-
White, 1963	265	64.5	13.9	15.1	6.5	-
White, 1966	350	60.0	15.0	20.0	5.0	-
Bruyere, 1963	34	91.2	2.9	5.9	-	-
Verstraete, van der Stock and Mattheeuws, 1964	26	69.2	26.9	3.9	-	-

It is very difficult to assess the effects of sampling in these reports since many of the authors appear to have selected and analysed small samples of larger series presented at their clinics, whilst others have included stones sent to their laboratories by practising veterinary surgeons. It seems probable that in both methods of sampling some selection of the larger, more spectacular and unusual stones may be present.

Phosphate Calculi proved to be the commonest chemical type in all the series. With the exceptions of Bruyere (1963) and White (1944, 1963 and 1966), there is general agreement that the second most common type of stone contained calcium oxalate as its major constituent. Cystine stones were the second most common type in all of White's three reports and in Bruyere's series.

In all these reports urate stones comprised less than 12% of the total number of cases.

Brodey (1955) and Krook and Arwedsson (1956) also carried out chemical analysis on series of stones, but these have not been included in Table 2 as, Brodey's series contained no oxalate or cystine stones and, according to Porter(1963), the method of analysis used in this investigation may have been inaccurate; Krook and Arwedsson recorded only the percentage of the ions, and from these figures it was not possible to accurately determine the percentage of the different chemical types of stones present.

Calculi composed of xanthine (Milks, 1935; Krook and Arwedsson, 1956); guanine (Hobday, 1922); urostealith (Milks, 1935); Muller and Glass/

Glass, 1911; Hobday, 1922); bilirubin (Wilkinson, 1962) and tetracycline (Mulvaney, Beck and Qureshi, 1965), have all been reported but the incidence of these substances must be extremely low. Indeed Bloom (1954) was of the opinion that calculi consisting solely or principally of calcium carbonate, xanthine, indican, fibrin or fatty substances, either do not occur or are extremely rare in the dog.

<u>Material, Methods and Results</u>: The patients examined consisted of 41 presented consecutively at the Small Animal Clinic of the Royal (Dick) School of Veterinary Studies and 14 cases referred for study by local veterinary surgeons. The latter cases also represented a consecutive sample of their urolithiasis patients. The calculi analysed were taken from the first episode encountered in each animal during this two year period of study. Calculi removed at subsequent episodes were not included in the incidence records.

The stones were washed free of blood and other organic matter and allowed to dry at room temperature for a period exceeding one month before analytical tests were carried out.

Routine qualitative chemical tests (Varley 1954) were used to assess the approximate nature of the stones. These tests were then supplemented in most instances by quantitative micro-chemical analysis. To confirm the results of the chemical analyses a selected 35 stones were analysed by X-ray diffraction crystallography. The selection was made so that specimens from all the chemical stone types encountered were represented.

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The method and results of the quantitative micro-chemical analysis and the crystallographic analysis by X-ray diffraction are described and discussed in a later chapter.

The analyses showed that the uroliths could be divided into 5 chemical types on the basis of their chief chemical constituent. The chemical types of calculi and the number of patients with each type were: Triple Phosphate, 19: Calcium Oxalate, 18: Cystine 13: Calcium Phosphate, 4: Urate, 1. (Table 3).

TABLE 3.

INCIDENCE OF THE DIFFERENT CHEMICAL TYPES OF URINARY CALCULI FOUND IN THE PRESENT SURVEY

Main Chemical Constituent	No. of Cases	Percentage of Total
Triple Phosphate	19	34.5
Calcium Oxalate	18	32.7
Calcium Phosphate	4	7.3
Cystine	13	23.7
Urate	1	1.8
Total	55	100.0

The distribution of the chemical types of stone in this series is not in accord with any previous reports. To assess whether this difference was due to chance a X^2 statistical analysis was used to compare the distribution in the present series with the distribution in a summary of the 9 recorded series shown in Table 2. An X^2 value of 18.10/

18.10 was found and indicated that the difference was statistically significant ($P < 0.01$).

Discussion: The more recent reports from most authors show that the chemical analysis of series of canine uroliths have conformed to a general pattern. The percentage of phosphate cases constituting more than the total percentage of the other chemical stone types combined. Oxalate stones and cystine stones averaged approximately 16% and 13% respectively while urate stones were less than 12% of the total.

In the present study of 55 patients suffering from Urolithiasis where the calculi were examined using accurate micro-chemical and, in 35 crystallographic techniques of detection, the distribution of chemical types of stone was found to be at variance with earlier reports. Statistical analysis indicates that this difference is unlikely to be due to chance.

In the series of calculi examined in this survey an almost equal incidence of triple phosphate stones (34.5%) and calcium oxalate stones (32.7%) was recorded. In some instances previous authors have not differentiated between calcium phosphate and triple phosphate stones. In this review even if these two types of calculi were grouped together they would still not exceed 50% of all chemical types. A closely comparable ratio maintains if both the calcium containing types (calcium oxalate and calcium phosphate) are grouped together. In this survey the calcium containing calculi were more commonly encountered than magnesium ammonium phosphate stones.

The incidence of cystine stones recorded in this survey is slightly higher than in previous records, but corresponds closely with the incidence/

incidence reported by White (1966). These two reports confirm the opinion of White (1944) and White, Treacher and Porter (1961) that this type of stone is a common cause of urolithiasis in male dogs in the United Kingdom.

Only one urate stone was found in this survey.

The difference in incidence of the chemically distinct types of stone between this and previous series may well be due to several factors. In some previous series, the possibility exists that some selection of calculi may have taken place, resulting in an abnormally high incidence of the more spectacular and unusually shaped stones (usually phosphates). Some of the difference may also have been due to the difference in methods of stone analysis. In many previous series only qualitative micro-chemical tests were used. The sensitivity of these tests varies for different components. For example, tests for phosphate are sensitive and will readily detect minute amounts of this ion in the stones. Routine tests for oxalate, on the other hand, are less readily interpreted and unless confirmatory tests are carried out in every case inaccuracies may ensue. It seems reasonable to assume that true area variations may also exist and account for some differences. These variations may be influenced by several factors. Since some breeds appear to be prone to certain chemical types of stone (e.g. urate stones in Dalmations) variation in breed popularity between the areas may be responsible for some differences. Similarly since it is generally accepted that sex may influence the chemical type of stone formed/

formed (Brodey, 1955; White, 1963 and 1966) the ratio of the sexes in the population at risk may have a bearing on the variation of incidence of the different chemical stone types. In Stockholm, Krook and Arwedsson (1956) report a male to female ratio of 98:90 which is considerably different from the male to female ratio of 3:2 in patients attending the Small Animal Clinic of the Royal (Dick) School of Veterinary Studies in Edinburgh.

3. AGE INCIDENCE.

Review of the Literature.

That the incidence of urinary calculi could be associated with age was recognised as long ago as 1906 by Hobday who suggested that it was more common in the adult animal. Since then the effect of age on the incidence of urolithiasis has been reported by White, (1944); Brodey, (1955); Krook and Arwedsson, (1956); De Souza, (1957); Uller, (1959); and White, Treacher and Porter, (1961). In the series recorded by White (1944) the ages of 40 dogs were known; 25 of these animals were in the 4 - 8 year age group, while the range varied from 2 - 18 years. Brodey (1955) recorded a similar age incidence, with 32 of his 52 cases occurring in the 3-8 year age group, while the overall incidence varied from 4 months to 14 years. Uller (1959) also recorded that the frequency of the condition increased up to 8 years of age and he considered that the apparent decline in incidence after this age was due to a general reduction in the number of aged dogs. In a series of 67 cases reported by De Souza (1957), 49 were found to occur in dogs more than 5 years of age, however, he gave no indication of the age distribution of the clinic population. Krook and Arwedsson (1956), and White, Treacher and Porter (1961) compared age incidences of urolithiasis with the average age of the total clinic population and the former authors found a statistically significant difference. The average age of the entire population in Krook and Arwedsson's series was 4.40 ± 0.05 years while the average age/

age of 191 cases of urolithiasis was 6.74 ± 0.27 years indicating that the disease was more prevalent in the higher age groups. Both clinical patients and animals examined at post mortem were used in calculations. White, Treacher and Porter (1961) found in their London series that the average age of calculi cases presented was 6.5 years as compared with an average clinic population age of 4.7 years. In the Liverpool series of the above authors the average age for urolithiasis cases was 5.9 years. From these records White, Treacher and Porter (1961) considered that urinary calculi were most common between the ages of 4 and 10 years with a peak incidence of 6 to 7 years.

Thus there appears to be general agreement in the literature that the incidence of the disease increases with age up to 7 or 8 years.

With regard to the occurrence of calculi in puppies, from the series of Brodey (1955), De Souza (1957) and White, Treacher and Porter (1961) in which a total of 464 cases of urolithiasis were reported only 7 (1.5%) were found in animals less than 1 year of age. It would seem, therefore, that the condition in puppies is rare.

Little attention has been paid to the effect of age on the occurrence of the different chemical types of stone. Treacher (1962) however, found the peak incidence of cystine stone disease to be in a younger age group (2 - 6 years) than phosphate stones, but his figures were not statistically significant. Furthermore, he did not record whether or not his cases were initial episodes of the condition. In Porter's 1963 series of urate stones, the age incidence did not appear to/

to differ appreciably from the incidence of all calculous disorders; the disease occurring between 1 and 11 years with an average age incidence of 5.3 years.

Materials, Methods and Results: The ages of all 55 dogs studied in this investigation were recorded as was any earlier clinical episodes of the disease. Careful history of the date of the initial episode and the duration of signs associated with it was obtained from the owner or the clinic records. The ages recorded for the affected animals therefore indicate the onset of signs of the initial episode. These ages varied greatly; the youngest animal encountered was eighteen weeks of age and the oldest twelve years. (Table 4 and Fig. 1).

TABLE 4.

AGE INCIDENCE OF 55 CASES OF UROLITHIASIS

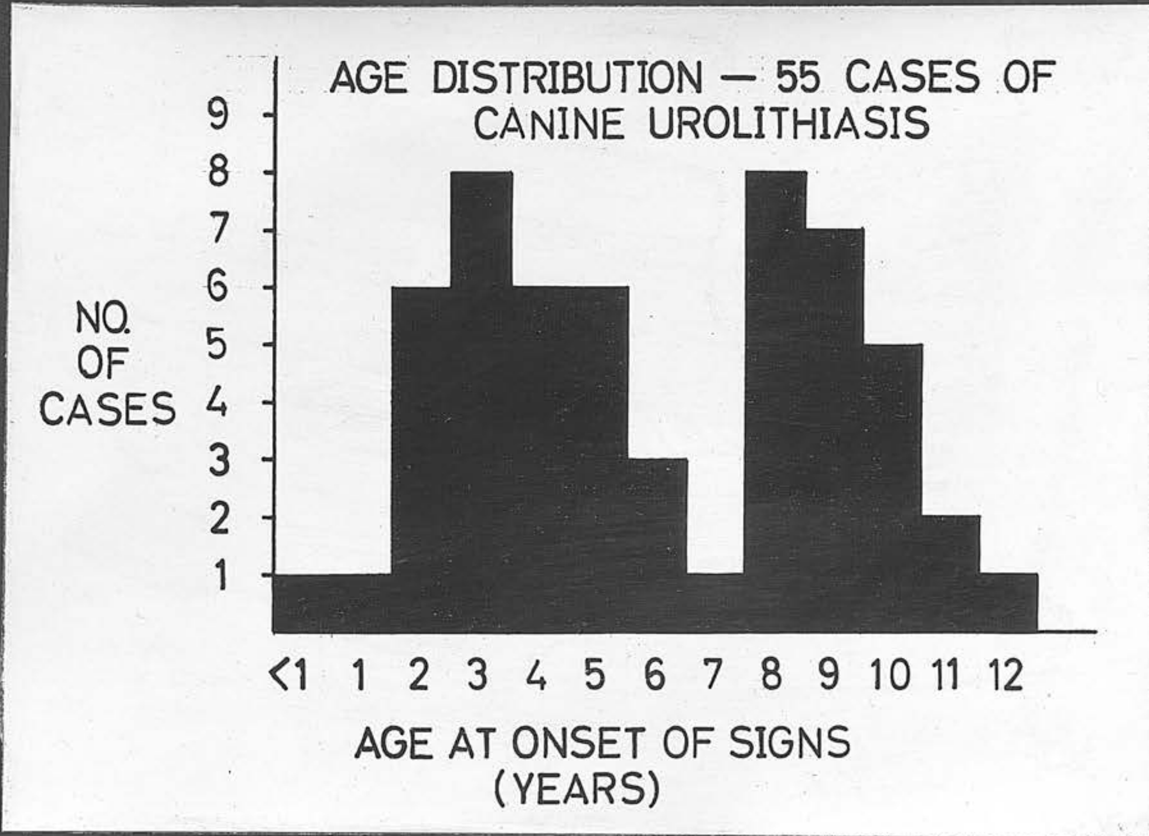
Age	No. of Cases	Age	No. of Cases
$<$ 1 year	1	8 years	8
1 "	1	9 "	7
2 years	6	10 "	5
3 "	8	11 "	2
4 "	6	12 "	1
5 "	6	12 plus	nil
6 "	3		
7 "	1		

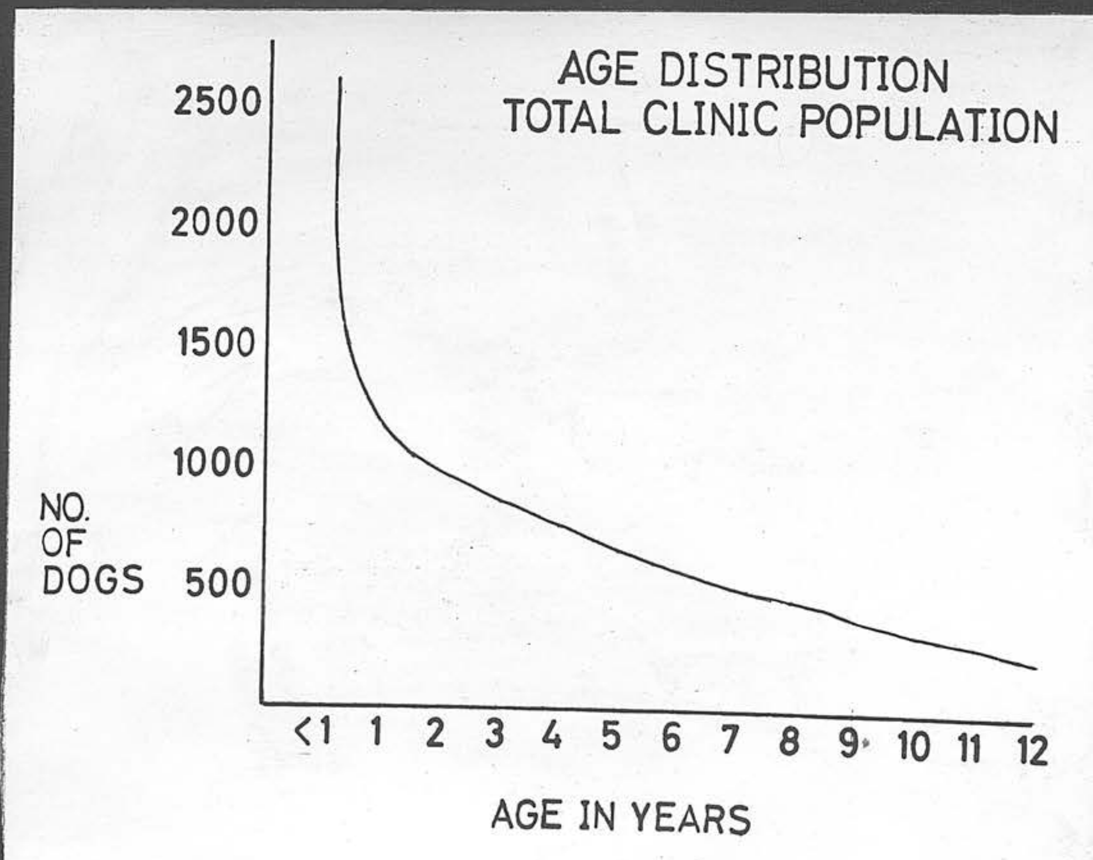
The mean age of these dogs was 6.0 years. Peak incidences occurred at 2 - 5 years and 8 - 10 years.

The/

Fig. 1. Age Distribution of 55 Cases of Urolithiasis.

Fig. 2. Age Distribution of the Total Clinic Population.


AGE DISTRIBUTION — 55 CASES OF CANINE UROLITHIASIS
NO. OF CASES
9
8
7
6
5
4
3
2
1
<1 1 2 3 4 5 6 7 8 9 10 11 12
AGE AT ONSET OF SIGNS (YEARS)


AGE DISTRIBUTION TOTAL CLINIC POPULATION
NO. OF DOGS
2500
2000
1500
1000
500
<1 1 2 3 4 5 6 7 8 9 10 11 12
AGE IN YEARS

The age distribution of the total clinic population is shown in Table 5 and Fig. 2.

TABLE 5

AGE DISTRIBUTION OF THE TOTAL CLINIC POPULATION

Age	No. of Individual Dogs presented	Age	No. of Individual dogs presented
< 1 year	2,618	7 years	459
1 "	1,099	8 "	405
2 years	971	9 "	391
3 "	866	10 "	329
4 "	748	11 "	228
5 "	592	12 "	202
6 "	533	12 plus	252

Total 9,693

Age of 323 cases not known.

Animals less than one year old were those most commonly presented, with progressively diminishing numbers seen in each of the higher age groups. The mean age of the estimated total population was 4.26 years.

Influence of age on the onset of clinical signs of urolithiasis.

For statistical analysis, affected animals in the clinic population were divided into two groups; those less than 6 years of age and those 6 years or older and this ratio was found to be 21:20. The total clinic population was also divided in a similar manner and 71.1% were less than 6 years of age and 28.9% were 6 years or older. Table 6.

TABLE 6/

TABLE 6

AGE INCIDENCE OF THE ONSET OF CLINICAL SIGNS OF UROLITHIASIS IN THE CLINIC POPULATION

Age	No. of Urolithiasis cases	Distribution in Total Clinic population %
<6 years	21	71.1
>6 "	20	28.9
Total	41*	100.0

* The urolithiasis patients (14) presented for study by local veterinary surgeons were not included in this total as the age distribution of the population from whence they originated was not known.

The application of the X^2 test to these figures resulted in an X^2 value of 8.0 which indicated that the incidence of urolithiasis is statistically significantly higher ($P < 0.01$) in animals 6 years of age and older.

Relationship of age and the different chemical types of stone.

The relationship between age and the 55 cases of urolithiasis divided into groups on the basis of the chief chemical constituent is shown in Table 7.

TABLE 7/

TABLE 7

RELATIONSHIP BETWEEN AGE AND CHEMICAL TYPE OF STONE

Age (years)	Triple Phosphate	Oxalate	Cystine	Calcium Phosphate	Urate	Total
2	2	0	0	0	0	2
2+3	6	1	5	1	1	14
4+5	2	4	6	0	0	12
6+7	1	2	0	1	0	4
8+9	5	6	2	2	0	15
10+11	3	4	0	0	0	7
12+	0	1	0	0	0	1
Total	19	18	13	4	1	55

For statistical analysis, the affected animals were divided into 2 age groups as previously and an X^2 test applied to the relationship of age and the chemical types of stone. An X^2 value of 11.9 resulted from this analysis which was significant at the 2.5% level; indicating that the effect of age was not equally distributed throughout the different chemical types of stones. Oxalate stones occurred more frequently in dogs in the higher age group and cystine stones more frequently in young dogs.

In order to assess whether this apparent increased incidence of cystine stones in younger animals and oxalate stones in older animals was a true increased susceptibility of animals of these ages and not just a reflection of the number of animals at risk in each age group, a statistical analysis was applied to the ages of urolithiasis cases presented at the clinic and the age distribution (divided into two groups of less than six years of age and six

years/

years of age and older) of the clinic population. In the case of oxalate stones an X^2 value of 8.37 indicated that older dogs are significantly ($P < 0.01$) more prone to oxalate stone disease than younger animals. On the other hand the apparent increased susceptibility of young animals to cystine stone disease was not statistically significant. Thus it is possible that the greater number of young animals with cystine stone disease may possibly be a reflection of the greater number at risk. The difference in distribution of triple phosphate cases between the two age groups was not statistically significant.

Discussion.

The age distribution of urolithiasis patients in the series is similar to that described by Krook and Arwedsson (1956) and White, Treacher and Porter (1961). The criterion for age in these previous reports is not clear, as it has not been stated whether the ages recorded are limited to initial episodes.

The age distribution of the onset of signs of the initial episode of the 55 cases shown in Fig. 1 exhibits a tendency towards a bimodal form. A similar but less obvious decrease in incidence at approximately 7 years of age is present in the histogram recorded by White, Treacher and Porter (1961). It seems possible therefore that peak incidences at 2 - 5 years and at 8 - 10 years may reflect a true bimodal distribution. This may well be due to the higher incidence of oxalate stones in older animals and the possible increased incidence of cystine stones in younger animals. Further records will doubtless confirm or reject this possibility.

The/

The significantly higher incidence of urolithiasis in older animals supports the findings of Krook and Arwedsson (1956). In the past it has been generally assumed that this effect was due to increased incidence of triple phosphate stones in the older dogs. While this may well be so, at least in this survey, the major factor responsible for the increased incidence of urolithiasis in older dogs was due to the unequal distribution of oxalate stones in the different age groups. The fact that older dogs are more susceptable to oxalate stones does not appear to have been recorded previously.

The age incidence of cystine stone disease is similar to that recorded by Treacher (1962) and on the evidence of both surveys it seems likely that cystine stone disease occurs most commonly in young adult animals.

4. SEX INCIDENCE.

Review of the Literature.

Recent reports on the effect of sex on the incidence of clinical urolithiasis show considerable variations (Table 8). Several authors (Klarenbeek, Langer and Raabe, 1935; Krook and Arwedsson, 1956; De Souza, 1957 and Uller, 1959) have reported an increased incidence in the male, with a male to female ratio varying from 3 : 1 to 7 : 1. By comparison, only minor differences in sex incidence are reported by White (1944), Brodey (1955), White, Treacher and Porter (1961) and in White's (1963) expanded series. In this latter report the females outnumbered males.

Krook and Arwedsson (1956) concluded from a statistical comparison of clinical and pathological cases that urolithiasis was not influenced by sex but was clinically more manifest in the male.

In recent years the association between sex and the different chemical stone types has been recognised. In Brodey's (1955) series 42 cases of phosphate lithiasis were encountered; 25 of these occurred in females and 17 in males. White, Treacher and Porter (1961) recorded a female to male ratio of 3 : 1 in phosphate stone cases and in 1966 White stated that 4 out of every 5 urolithiasis cases in the bitch were due to this chemical type of stone. With regard to the remaining chemical varieties (oxalate, cystine and urate) White, Treacher and Porter (1961) found them to occur almost/

TABLE 8

SEX INCIDENCE OF CANINE UROLITHIASIS

Author	No. of Affected Males	No. of Affected Females	Total
Klarenbeek, Langer & Raabe 1935	42	9	51
White 1945	40	38	78
Brodey 1955	27	25	52
Krook & Arwedsson 1956	63	9	72
De Souza 1957	50	17	67
Uller 1959	447	127	574
White, Treacher & Porter 1961			
London Series	127	94	221
Liverpool Series	(60)	(55)	(115)
White 1963 Expanded Liverpool Series	120	133	253
TOTAL	916	452	1,368
RATIO	2	1	

almost invariably in the male. In a total of more than 300 cases White (1966) recorded that all the cystine and urate stones seen occurred in male dogs and almost all the oxalate stones also occurred in dogs of this sex.

From these reports it would seem that triple phosphate stones are the usual type found in females and that other varieties either do not occur or are very rare in this sex. In males oxalate and cystine stones seem to be much more common than phosphates.

Material, Methods and Results: The sex of all 55 cases of urolithiasis was recorded. Seventeen were females and 38 males. Of the 17 females, 15 were found to have triple phosphate stones present; in one female oxalate calculi occurred and one had a calcium phosphate stone. Table 9.

TABLE 9.

RELATIONSHIP OF SEX AND THE DIFFERENT CHEMICAL TYPES OF STONE

Chief Constituent of Stone	Number of Males affected	Number of Females affected.	Total
Triple Phosphate	4	15	19
Calcium Oxalate	17	1	18
Cystine	13	0	13
Calcium Phosphate	3	1	4
Urate	1	0	1
Total	38	17	55

The/

The Influence of Sex on Urolithiasis.

The urolithiasis patients in the clinic population were classified according to sex and compared with the sex ratio for all dogs presented. (Table 10).

The total estimated clinic population of 10,016 consisted of approximately 5,576 males, 3,937 females and 323 animals, the sex of which was not recorded; the percentage ratio of males to females was therefore 59.4 to 40.6

TABLE 10.

RELATIONSHIP OF SEX TO UROLITHIASIS.

	Males	Females	Total
Urolithiasis Patients	30	11	41*
Percentage of Total Population	59.4	40.6	100

* The 14 urolithiasis patients presented for study by local Veterinary Surgeons were not included in this total as the sex distribution of the population from whence they originated was not known.

An X^2 analysis of these figures resulted in an X^2 value of 3.18 which indicated that the difference in incidence between affected males and females in the population was not statistically significant.

The/

The Influence of Sex on the different chemical types of stone.

The affected sexes in the clinic population were divided into types according to their chief chemical constituent and compared with the percentage distribution of the sexes in the total clinic population using the X^2 analysis. (Table 11).

TABLE 11.

THE INFLUENCE OF SEX ON THE DIFFERENT CHEMICAL TYPES OF STONE

Chief Chemical Constituent	Males	Females	Total
Triple Phosphate	4	9	13
Calcium Oxalate	14	1	15
Cystine	9	0	9
Calcium Phosphate	3	1	4
Total	30	11	41*
% Distribution of the sexes in the clinic population	59.4	40.6	100

* The 14 urolithiasis patients presented for study by local Veterinary Surgeons were not included for the reason stated above.

The resultant X^2 values were found to be:-

Triple Phosphate Cases 3.26
Calcium Oxalate Cases 5.84
Cystine Cases 4.69
Calcium Phosphate Cases 0.37

These/

These figures suggest that sex probably influences the incidence of both cystine stone disease and oxalate stone formation ($P<0.05$).

In human urolithiasis the onset of stone formation occurs slightly later in males than in females, (Sutherland, 1954; Williams, 1963). In this series of dogs however, the average age of onset (males 6.6 years: females 6.1 years) did not appear to be significantly different.

Discussion.

In cases of urolithiasis presented at this clinic a ratio of approximately three males to one female was recorded. The total clinic population, however, also contained a preponderance of males; the ratio being approximately 1.5:1. The application of the X^2 statistical analysis to these figures revealed that the increased male incidence of urolithiasis in the population was not statistically significant.

A summary of 8 previously recorded series in the literature revealed that approximately twice as many males were affected than females, but in most of these series the ratio of males to females in the population at risk was not recorded. In two series the clinic or area population was discussed in relation to the sex incidence of the clinical condition. In one, a statistically higher incidence occurred in the male (Krook and Arwedsson, 1956) and in the other (London series of White, Treacher and Porter, 1961) no statistical analytical method was used. However, by applying a X^2/

X^2 test to the figures presented in this article, the difference in sex incidence recorded was not statistically significant.

The results of this series do not support the extreme sex variation in Krook and Arwedsson's 1956 series (male to female ratio of 7 : 1) although a slightly increased male incidence is recorded. In this investigation where 15 out of 19 cases of triple phosphate calculi occurred in the bitch and all 13 cases of cystine calculi occurred in the male, there is further evidence to suggest that triple phosphate stones most commonly occur in the female and that cystine stones are largely, if not entirely, confined to the male. Statistical analysis on the cases observed in this survey has shown that oxalate stones also occur significantly more often in the male dog ($P < 0.05$).

In the clinic population, in which a preponderance of males was present, triple phosphate cases were distributed in the female to male ratio of 9:4. An X^2 statistical analysis on this material was not statistically significant at the 5% level but did indicate that a possible tendency for sex to influence the occurrence of this stone type may exist.

The method by which such an influence could be exerted is not known but since triple phosphate stones are thought to be associated with urinary tract infection (Brodey, 1955) and since it seems likely that the short urethramay allow easier access to the bladder by bacteria; leading to an increased incidence in urinary infections in females, as occurs in humans(Carroll, 1963) Smellie et al, 1964; Sutherland, 1965) the higher incidence of triple phosphate calculi in this sex may be due to these factors.

White/

White, Treacher and Porter (1961) also postulated this theory and suggested that a further possible factor was that phosphate stones were the only ones to commonly reach a large size and that since "stones up to 1 cm. in diameter are readily passed through the female urethra", most of the other chemical types of stones would be too small to cause an obstruction, and presumably would be passed without causing clincal signs.

It is of interest to note, however, that in the human patient where, in both sexes, calculi most commonly form in the upper urinary tract, a region in which there is no apparent anatomical sex difference, oxalate and urate stones still occur more often in the male. It seems possible therefore that the simple and widely held concept that the difference in sex incidence of the chemical types of stone is due to a combination of anatomical urethral differences, and to an increased susceptibility of the female to infection, may not be entirely correct.

5. BREED INCIDENCE.

Review of the Literature.

The realisation that a breed incidence may occur in urolithiasis was slow to be appreciated. However in 1934 McCunn reported that he had never seen calculi in a Greyhound. The following year Klarenbeek et al. (1935) in a series of 58 cases recorded a remarkably high incidence in Pekingese. 10% of all stones in the series occurred in this breed in spite of the fact that the breed was apparently not popular in the area. A few years later Keeler (1940) found kidney stones in 7 out of 9 interrelated adult Dalmatians at autopsy and suggested that there was an inherent tendency to renal stones in this breed. Krabbe (1949) reported that all of 30 cases of cystolithiasis occurred in small dogs, mostly terriers and Pekingese, but that these breeds did not constitute more than half the total clinic population. Dachshunds, Pekingese and Poodles were the breeds most commonly affected in the series of Krook and Arwedsson (1956). These authors classified their breeds into chondrodystrophoid and non-chondrodystrophoid types and by statistical analysis concluded that the former showed a predisposition to the condition.

In a number of recently recorded series (Brodey, 1955; Uller, 1959; White, Treacher and Porter, 1961; White, 1963 and 1966), the breeds of the majority of the affected animals were noted. In Brodey's series the mongrel and the Cocker Spaniel were most commonly affected but no indication/

indication of the proportion of these breeds in the total population was recorded, consequently it is not possible to assess whether or not a predisposition was present.

Uller (1959) also failed to record the popularity of the different breeds of dogs in his area when recording that small terriers and Dachshunds were most commonly affected. Uller found that almost twice as many cases (306) of urolithiasis occurred in the smaller breeds than in the larger dogs (115).

An incidence of 5.13 - 6.66% of Dachshunds, Cairn Terriers, Welsh Corgis and Scottish Terriers presented at the Beaumont Animals' Hospitals was reported by White, Treacher and Porter (1961). Other breeds of above average incidence were Poodles, Labrador-Retrievers, Cocker Spaniels and Pekingese. The affected breeds in the Liverpool series of the same authors corresponded closely to those recorded in London with the exceptions that the Labrador Retriever had a much lower incidence.

The expanded Liverpool series of 265 cases recorded by White, (1963) did not reveal any significant differences in Breed Incidence from the previously reported figures of White, Treacher and Porter (1961).

Apart from the Dalmatian's predisposition to urate calculi (Keeler, 1940; White, Treacher and Porter, 1961; White, 1963), little is known of the susceptibility of the breeds to the different chemical types of stones.

It is apparent from the recent literature, however, (Brodey, 1964) that the Dachshund has become regarded as being prone to cystine/

cystine stone disease. This view is based on two reports; one from Treacher (1962) who found a slightly higher incidence of this disease in Dachshunds, but whose figures were not statistically significant and the second from Uller (1959) who found 9 of 11 cystine stones in his series occurred in Dachshunds. Uller, however, did not indicate the popularity of the breed in his area nor did he indicate whether the Dachshunds were related as such figures could have resulted from a single family of breeding cystinuric Dachshunds.

Another possible predisposition to a single chemical type of stone is presented in White's (1963) series in which approximately one quarter of 131 phosphate stones were found in Welsh Corgis. It seems unlikely that the Corgi would represent a quarter of the total population; so it seems possible that an increased susceptibility to phosphate lithiasis may have been present in this breed.

From a study of the literature some degree of resistance to urolithiasis would appear to be present in Alsatians, Airdale Terriers and Boxers (Krook and Arwedsson 1956; White, Treacher and Porter 1961) and White in his 1963 and 1966 series also found the condition to be less common in Alsatians and Boxers together with Poodles, Labrador Retrievers and Mongrels.

Material, Methods and Results. The breed in the 55 cases is shown in Table 12. The relationship between the breed and the chemical types of calculi is recorded in Table 13.

In calculating breed incidence the 14 referred cases were rejected and only the 41 cases presented at the Clinic, in which the total population could be estimated, were used. The distribution of/

TABLE 12

BREED INCIDENCE IN 55 CASES OF UROLITHIASIS

Breed	Number of Cases	
Cairn Terrier	11	One case occurred in each of the following breeds:- Keeshound, Dandie Dinmont, Basset Hound, Pug, Dalmatian, Yorkshire Terrier, Springer Spaniel, Scottish Terrier, Pekinese, Boxer, Cocker Spaniel, Labrador, Retriever.
Miniature Poodle	7	
Corgi	6	
Mongrels	5	
Dachshund	4	
Border Terrier	3	
Border Collie	3	
Miniature Pinscher	2	
West Highland White Terrier	2	

TABLE 13

RELATIONSHIP BETWEEN BREEDS AND THE CHEMICALLY DISTINCT STONE TYPES

Chemical Stone Type	Number of Cases	Breeds
Triple Phosphate	19	Welsh Corgi 5: Cairn Terrier 4: Dachshund 3: Miniature Poodle 2: West Highland White Terrier, Cocker Spaniel, Mongrel, Scottish Terrier, Border Collie, all one each.
Calcium Oxalate	18	Cairn Terrier 4: Border Terrier 3: Mongrel 3: Border Collie, Boxer, Dachshund, Dandie Dinmont, Miniature Pinscher, Pekinese, Pug and Yorkshire Terrier, all one each.
Cystine	13	Miniature Poodle 4: Cairn Terrier 3: Basset Hound, Border collie, Labrador, Retriever, Mongrel, Welsh Corgi, and West Highland White Terrier, all one each
Calcium Phosphate	4	Miniature Pinscher, Miniature Poodle, Keeshound and Springer Spaniel, all one each
Urate	1	Dalmatian

of calculous cases in the breeds and the estimated number of each breed is shown in Table 14.

The most commonly affected breed in this investigation was the Cairn Terrier in which 11 cases were encountered, 10 of these were from the clinic population.

In Table 14, little or no significance of value can be attributed to the incidence of calculi in the less popular breeds. With such small numbers at risk, the absence of the disease in a breed or its occasional occurrence may well be a disproportionate representation of its true suceptibility.

Statistical analysis (X^2 test) was applied to the incidence in some of the more popular breeds and it was found that statistically higher incidences occurred in the Cairn Terrier ($P < 0.01$), Border Terrier ($P < 0.01$), Dachshund ($P < 0.01$) and Corgi ($P < 0.05$). A lower incidence to the disease was observed only in the mongrel ($P < 0.05$).

The incidence in the Miniature Poodle, Border Collie, Greyhound, Alsation and Labrador Retriever, all popular breeds, did not differ significantly in a statistical sense from their representation in the total population. No urolithiasis cases were recorded in the Greyhounds, Alsatians or Labradors seen at the clinic. Thus the fact that the incidence of the disease in these breeds did not differ significantly from the incidence in the total population may well be attributed to the lack of numbers presented.

The relationship between breed and chemical type of stone was tested statistically in the following cases:-

(a)/

TABLE 14

BREED INCIDENCE OF UROLITHIASIS IN THE CLINIC POPULATION

Breed	Number of Calculous Cases	Estimated Number of Dogs Presented	Breed % of Total Population	% Incidence
Keeshound	1	12	0.12	8.3
Dandie Dinmont	1	13	0.13	7.7
Miniature Pinscher	2	22	0.22	6.1
Basset Hound	1	17	0.17	5.9
Border Terrier	3	134	1.3	2.2
Cairn Terrier	10	447	4.47	2.0
Dachshund	4	230	2.3	1.7
Corgi	4	263	2.6	1.5
Yorkshire Terrier	1	90	0.90	1.1
Springer Spaniel	1	93	0.93	1.1
Scottish Terrier	1	98	0.98	1.0
Miniature Poodle	5	996	9.96	0.5
Pekinese	1	190	1.9	0.5
Border Collie	3	804	8.0	0.4
Boxer	1	302	3.0	0.3
Cocker Spaniel	1	411	4.1	0.2
Mongrels	1	2,268	22.7	0.1
Labrador	0	679	6.79	0
Alsatian	0	618	6.18	0
Greyhound	0	340	3.40	0
Shetland Sheep Dog	0	173	1.73	0
Wire Haired Fox Terrier	0	161	1.61	0
Other Breeds	0	1,655	16.55	0
TOTAL	41	10,016	100.00	100

(a) Triple Phosphate stones in the Corgi, Cairn Terrier and Dachshund.

(b) Oxalate stones in the Cairn Terrier.

(c) Cystine stones in the Miniature Poodle and the Cairn Terrier.

These were selected as 3 or more cases of urolithiasis due to a similar chemical stone type occurred in each of these breeds and each breed was represented by more than 1.5% of the total population.

The Corgi, Cairn Terrier and Dachshund had a statistically higher incidence with respect to Triple Phosphate Stones ($P < 0.01$ in each case) while the Cairn Terrier showed increased susceptibility to cystine and oxalate types of calculi ($P < 0.01$ in each case). The incidence of cystine stones in Miniature Poodles was not significantly different from the incidence of these stones in the total population.

As the application of statistical analysis to such small numbers of affected cases may give misleading results, the significance of these results is conjecteral and should not be interpreted as establishing a definite increased susceptibility or resistance, rather they are intended to indicate a possible tendency towards resistance or susceptibility of the breeds concerned with the chemical types of stones involved.

Discussion.

The breed incidence recorded in this investigation is similar in many respects to that reported by White, Treacher and Porter (1961). In both studies Cairn Terriers, Dachshunds, and Corgis

were /

were more commonly affected and the incidence in mongrels was less than the population average. The low incidence in Alsatians and Labrador Retrievers also conforms with the opinion of White (1966) that calculi are relatively less common in these breeds.

With regard to the susceptibility of breeds, to the different chemical stone types, some differences are apparent. Uller, (1959) and Brodey, (1964) consider that Dachshunds are more prone to cystine stone disease, yet no cases of cystine calculi were found in this breed in the present investigation. This result does not preclude the possibility of there being an increased incidence in this breed, but it does emphasise the need for further evidence before such a susceptibility can be established. Similarly, the possible increased incidence of Triple Phosphate, cystine and oxalate stones in Cairn Terriers recorded in this survey is not supported by the report of White, (1963) and again further evidence from a larger series is required before any special susceptibility can be assumed.

The possible increased incidence of triple phosphate stones in Corgis and Dachshunds does, however, support White's (1963) figures and it seems likely that an increased incidence of this type of calculi may well occur in these breeds.

6. INCIDENCE OF CALCULI IN THE DIFFERENT ANATOMICAL POSITIONS OF THE URINARY TRACT

Review of the Literature.

From the recordings of Blaine (1832) and Morton (1844) it was apparent that, in the dog, calculi occurred more frequently in the bladder than in the kidney. The frequent occurrence of urethral calculi in the male dog was later reported by Dollar (1895); Moller and Dollar (1903); and Hobday (1906); and this became generally accepted as the commonest site of urinary calculi (Stainton, 1922).

In 1949 Krabbe described a series of 72 urolithiasis cases and also found the male urethra to be the most commonly affected site. White (1944), Brodey (1955), White, Treacher and Porter (1961), and White (1963), however, recorded a higher incidence of calculi in the bladder. (Table 15).

TABLE 15.

INCIDENCE OF CALCULI IN THE DIFFERENT ANATOMICAL POSITIONS OF THE LOWER URINARY TRACT

Authors	Urethral Stones only	Urethral and Vesical Stones	Vesical Stones only
White, 1944	31	2	65
Brodey, 1955	7	14	26
White, Treacher and Porter, 1961 London Series Liverpool Series	52 (36)	40 (9)	119 (69)
White, 1963 Expanded Liverpool Series	68	21	158
TOTAL	158	77	368

With/

With the exception of Brodey (1955) these latter authors recorded a larger number of cases of urethral involvement alone than concurrently occurring urethral and vesical stones in affected male animals. Since in White's report many of the calculi were sent from Veterinary Surgeons practising throughout the United Kingdom it can be assumed that all patients were not examined radiographically and it seems likely that some cases recorded as urethral calculi may have also had concurrently occurring vesical stones. The majority of bitches affected in these previous reports had vesical stones; in fact only two reports of urethral stones in this sex (Grey, 1897 and Krabbe, 1949) could be found in the literature.

It is apparent that renal lithiasis is rare in the dog (Morton, 1844; Milks, 1935; Stephenson, 1939; Brodey, 1955; and Medway, Archibald and Bishop, 1955) and it also seems that when they do occur at this site they rarely cause serious clinical signs (Klarenbeek et al., 1935; Krabbe, 1949; Brodey, 1955; and Boddie, 1962). This last feature may in part account for the comparative rarity of the clinical condition. White (1966) reported that only 5% of canine urothiasis patients had stones in the upper urinary tract. From the reports of White (1944), Krabbe (1949), Brodey (1955), White, Treacher and Porter (1961) and White (1963) it seems that the occurrence of renal calculi is usually associated with stones in other parts of the urinary system. (Table 16).

The/

The occurrence of ureteral stones in dogs appears to be extremely rare. Occasionally stones lodged in this site have been observed in experimental cases of urolithiasis (Stainton, 1922; and Rosenow and Meisser, 1923). In a series of 72 urolithiasis patients, Krabbe (1949) reported 2 cases of ureteral stone found at autopsy; Brodey (1955) found one case in a series of 52 patients but White (1944 and 1963) in a combined total of more than 350 cases of urolithiasis recorded no case of ureteral calculus. In cases of renal stones, suspected ureteral lithiasis has been reported by Silva (1933), McCarthy and Perry (1962), Pearson, Gibbs and Hillson (1965), and Theran, Henry and Thornton (1965).

TABLE 16.

RELATIONSHIP OF RENAL STONES WITH STONES IN OTHER PORTIONS OF THE URINARY TRACT

Authors	Renal Stones only	Renal Stones with Stones in other Portions of the Urinary Tract.
White, 1944	0	3
Krabbe, 1949	1	8
Brodey, 1955	2	3
White, Treacher and Porter, 1961 London Series Liverpool Series	 7 (1)	 5 (2)
White, 1963 Expanded Liverpool Series	6	4
TOTAL	16	23

The/

The relationship between the main chemical constituents of calculi and the site in which they were found was studied by White, Teacher and Porter (1961). These authors found that approximately half the total number of urethral obstructions were due to oxalate or cystine stones and that 65 of 80 cases with vesical calculi contained phosphate as their major constituent.

Material, Methods and Results.

In order to detect all the anatomical sites of lodgement of urinary calculi in each animal the entire urinary system of all patients in this investigation was examined radiographically. On occasions when calculi of poor radio-density were removed from the urethra, pneumocystography was performed to assist in the detection of calculi in the bladder.

The positions of lodgement were recorded in only the first episode observed in each animal during the two year period. The reason for limiting the records to this first episode was that, since removal of all calculi from all the urinary tract was not always achieved, recurrence of the condition may have been due to the original calculi.

Under these conditions 23 patients were found to have concurrently occurring urethral and vesical stones; 19 had vesical stones only; 10 cases urethral stones only, and in 3 patients kidney stones were present. (Table 17).

TABLE 17/

TABLE 17.

INCIDENCE OF CALCULI IN THE DIFFERENT ANATOMICAL POSITIONS OF THE URINARY TRACT

	Position	Number of Cases
Lower Urinary Tract Involvement only.	Urethra only	10
	Urethral and Vesical stone	23
	Vesical stones only	19
Upper Urinary Tract Involvement.	Bilateral renal stones	2
	Unilateral renal and Vesical Stone	1

When the distribution of stones in the lower urinary tract of this series was compared by X^2 analysis with the distribution in previous series (shown in Table 15) a statistically significant difference was found ($P < 0.01$).

When the distribution of the anatomical sites involved is examined against the chemical types of calculi, it is evident that certain types have a predisposition for certain sites. (Table 18)

TABLE 18/

TABLE 18

RELATIONSHIP BETWEEN THE POSITION OF LODGEMENT OF CALCULI AND THE CHEMICALLY DISTINCT STONE TYPES

Chemical Type	Urethral Stones	Urethral+ Vesical Stones	Vesical Stones	Renal+ Vesical Stones	Renal Stones	Total
Triple Phosphate	-	3	16	-	-	19
Calcium Oxalate	6	9	2	1	-	18
Cystine	3	10	-	-	-	13
Calcium Phosphate	-	1	1	-	2	4
Ammonium Urate	1	-	-	-	-	1
TOTAL	10	23	19	1	2	55

For example in 19 cases of triple phosphate stones 16 occurred only in the bladder and in only three cases was there urethral involvement. On the other hand in 31 cases of oxalate and cystine stones urethral involvement was noted in all except 2 and in the majority (19) of these both urethral and vesical stones were found. Since so few calcium phosphate and ammonium urate stones were recorded in this series, little comment of value can be made on their distribution in the urinary tract. It is of particular interest that in 2 of the 4 patients with calcium phosphate calculi, bilateral renal stones were present and both patients were found to be suffering from primary hyperparathyroidism.

With/

With regard to the relationship between sex and the position of lodgement of uroliths, it was found that all affected females had vesical lithiasis, although one bitch also had unilateral renal stones present (Table 19).

TABLE 19

RELATIONSHIP BETWEEN THE POSITION OF LODGEMENT OF CALCULI AND THE SEX OF THE ANIMAL

Position of Lodgement	No. of Males	No. of Females	Total
Urethral stones only	10	0	10
Urethral and Vesical stones	23	0	23
Vesical stones only	3	16	19
Bilateral Renal stones	2	0	2
Unilateral renal and Vesical stones	0	1	1
TOTAL	38	17	55

In the 38 male dogs, 33 had stones lodged in the urethra; twenty three of these animals also had vesical stones present. Of the remaining 5 cases, 2 had bilateral renal stones and 3 had vesical stones.

In only 15 (27%) of the 55 cases were single stones present. In the remaining 40 cases either multiple stones were present in one anatomical site, or more than one site was affected. (Table 20).

TABLE 20

THE NUMBER OF CALCULI PRESENT IN THE AFFECTED ANATOMICAL SITES

Position of Lodgement of stones		No. of Calculi present			Total
		Single	2-3	More than 3	
Urethral only		4	2	4	10
Urethral & Vesical	Urethral site	3	5	15	23
	Vesical site	2	-	21	23
Vesical only		6	2	11	19
Renal stones	Bilateral renal	1	-	1	2
	Unilateral renal and Vesical	-	-	1	1

Urethral obstruction in male dogs occurred in one or more of 5 different anatomical parts of the urethra. (Table 21).

TABLE 21

POSITION OF URETHRAL OBSTRUCTIONS IN MALE DOGS

	No. in which only one Site was involved.	No. in which one Site was involved.	Total
Prostatic Urethra	2	-	2
Ischial Urethra	2	3	5
Perineal and Scrotal Urethra	1	2	3
Immediately Posterior to the os penis	11	2	13
In Groove of os penis	12	3	15

In/

In 28 of the 33 affected animals only one urethral site was obstructed; the remainder having stones in at least two urethral positions. The most common site of obstruction was within the groove of the os penis (15 cases). Stones also lodged immediately posterior to the os penis (13 cases), at the ischial arch (5 cases) in the Perineal or Scrotal portions of the urethra (3 cases), and in the prostatic urethra (2 cases).

Discussion.

Appraisal of the literature of canine urolithiasis reveals that whilst urethral stones in the male dog and vesical stones in both sexes are relatively common, stones in the kidney are less frequently seen and calculi in the male or female ureter or the female urethra are extremely rare. The data recorded in this investigation conforms with this distribution of stones in the different anatomical sites. In general, however, previous authors have found more cases of vesical stones than urethral stones, and in most series, the urethral stones are more commonly found un-accompanied by stones in the more anterior portions of the urinary tract. In this series urethral stones have been encountered more often than vesical stones and more than two thirds of the cases with urethral obstructions have had stones present in other parts of the urinary system.

Comparison by X^2 analysis between the incidence of lodgement of calculi in the different anatomical sites in this series with a summary of previously recorded series indicates that the difference in distribution of stones is unlikely to be due to chance. Two factors seem likely to be responsible for this disparity - one is the effect/

effect of sampling differences between the present and previous series; the other being the fact that in the present survey radiographic detection techniques were applied to the entire urinary tract in all cases where calculi in any one portion of the tract were suspected. As this routine critical examination was probably not carried out in every instance in the earlier series, calculi may well have been present but not revealed. The main sampling differences between this investigation and those reported by others can be summarised as follows:

(a) This series consisted of a non-selected sample of urolithiasis patients whereas in the reports of others some selection may have taken place.

(b) In this investigation the position of the uroliths was assessed and recorded only at the first episode observed in each of the affected animals, by so doing the possible bias of false episodes (i.e., those due to incomplete removal of calculi at previous episodes) was avoided.

The position of lodgement of the different chemical types of stones approximates that recorded by White, Treacher and Porter (1961) and White (1963). In the present series, triple phosphate calculi constituted 84% of the cases in which bladder stones only were present and cystine and oxalate stones caused 85% of the urethral obstructions. If these figures truely represent the incidence of lodgement of calculi in the different anatomical positions of the urinary/

urinary tract in the dog, it would appear that the widely held view that triple phosphate stones are a common cause of urethral obstruction as well as vesical lithiasis, is grossly inaccurate as only 10% of urethral obstructions were due to stones of this type.

With regard to the position of lodgement of calculi within the urethra in the male animal, the generally held opinion that the commonest site of stones is immediately behind the os penis may also be fallacious. In this series, obstructions by calculi within the groove of the os penis were slightly more frequent than those resulting from stones lodged immediately posterior to this bone.

TABLE 22

RELATIONSHIP BETWEEN POSITION OF LODGEMENT OF CALCULI, SEX OF THE AFFECTED ANIMALS, AND THE CHEMICAL TYPE OF STONES

Chemical Type of Stone	Bladder		Bladder and Urethra		Urethra		Bilateral Renal		Unilateral Renal and Vesical		Total
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
Triple Phosphate	1	15	3	-	-	-	-	-	-	-	19
Calcium Oxalate	2	-	9	-	6	-	-	-	-	1	18
Cystine	-	-	10	-	3	-	-	-	-	-	13
Calcium Phosphate	-	1	1	-	-	-	2	-	-	-	4
Ammonium Urate	-	-	-	-	1	-	-	-	-	-	1
Total	3	16	23	-	10	-	2	-	-	1	55

TABLE 23

TABULATED SUMMARY OF THE INCIDENCE OF UROLITHIASIS

CASE NO.	AGE AT ONSET OF SIGNS (YEARS)	SEX	BREED	MAIN CONSTITUENT OF STONE	POSITION OF LODGEMENT
1*	2	Female	West Highland White Terrier	Triple Phosphate	Two calculi present in the bladder
2	8	Male	Cocker Spaniel	Triple Phosphate	Multiple calculi in Bladder. Three present at ischial arch and two in the groove of the os penis
3	2	Female	Miniature Poodle	Triple Phosphate	Single calculus present in the bladder
4	9	Female	Dachshund	Triple Phosphate	Multiple calculi present in the bladder
5*	8	Female	Welsh Corgi	Triple Phosphate	Single calculus present in the bladder
6*	5	Female	Mongrel	Triple Phosphate	Multiple calculi present in the bladder
7	$4\frac{1}{2}$m.	Male	Cairn Terrier	Triple Phosphate	Single calculus in bladder. Two at ischial arch and one in groove of os penis
8	3	Female	Cairn Terrier	Triple Phosphate	Multiple calculi present in the bladder
9	3	Female	Welsh Corgi	Triple Phosphate	Multiple calculi present in the bladder
10*	8	Female	Welsh Corgi	Triple Phosphate	Multiple calculi present in the bladder

CASE NO.	AGE AT ONSET OF SIGNS (YEARS)	SEX	BREED	MAIN CONSTITUENT OF STONE	POSITION OF LODGEMENT
11	11	Female	Cairn Terrier	Triple Phosphate	Single calculus present in the bladder
12	3	Female	Scottish Terrier	Triple Phosphate	Single calculus present in the bladder
13	10	Male	Border Collie	Triple Phosphate	Multiple calculi in the bladder and immediately behind the os penis
14*	2	Female	Miniature Poodle	Triple Phosphate	Multiple calculi present in the bladder
15	10	Female	Cairn Terrier	Triple Phosphate	Two calculi present in the bladder
16	6	Female	Welsh Corgi	Triple Phosphate	Multiple calculi present in the bladder
17	4	Female	Dachshund	Triple Phosphate	Multiple calculi present in the bladder
18	8	Female	Dachshund	Triple Phosphate	Multiple calculi present in the bladder
19	1	Male	Welsh Corgi	Triple Phosphate	Single calculus present in the bladder
20	8	Male	Border Terrier	Calcium Oxalate	Multiple calculi in the bladder. Three present immediately behind the os penis
21	5	Male	Boxer	Calcium Oxalate	Multiple calculi present in the bladder
22	10	Male	Dandie Dinmont	Calcium Oxalate	Multiple calculi present in the bladder

CASE NO.	AGE AT ONSET OF SIGNS (YEARS)	SEX	BREED	MAIN CONSTITUENT OF STONE	POSITION OF LODGEMENT
23	5	Male	Cairn Terrier	Calcium Oxalate	Multiple calculi present immediately behind the os penis
24*	10	Male	Mongrel	Calcium Oxalate	Multiple calculi present in the bladder and 2 present within the groove of the os penis
25	4	Male	Dachshund	Calcium Oxalate	Multiple calculi present in the bladder and within the groove of the os penis
26	9	Male	Mongrel	Calcium Oxalate	Multiple calculi present in the bladder and within the groove of the os penis
27	8	Male	Border Terrier	Calcium Oxalate	Single calculus present in the bladder and within the groove of the os penis
28	2	Male	Cairn Terrier	Calcium Oxalate	Single calculus in Scrotal area of urethra. Two calculi present immediately behind os penis
29	6	Male	Border Terrier	Calcium Oxalate	Three calculi present immediately behind the os penis
30*	12	Male	Mongrel	Calcium Oxalate	Multiple calculi in bladder. Two present immediately behind os penis
31	9	Male	Miniature Pinscher	Calcium Oxalate	Multiple calculi present in bladder and in groove of os penis.
32	7	Male	Cairn Terrier	Calcium Oxalate	Multiple calculi present at ischial arch

CASE NO.	AGE AT ONSET OF SIGNS (YEARS)	SEX	BREED	MAIN CONSTITUENT OF STONE	POSITION OF LODGEMENT
33	11	Male	Pekingese	Calcium Oxalate	Three calculi present immediately behind os penis
34*	10	Male	Pug	Calcium Oxalate	Multiple calculi present in bladder and within groove of os penis
35	8	Male	Border Collie	Calcium Oxalate	Single calculus present within the groove of the os penis
36	9	Female	Cairn Terrier	Calcium Oxalate	Multiple calculi present in the bladder
37	4	Male	Yorkshire Terrier	Calcium Oxalate	Multiple calculi present in the bladder and in the groove of the os penis
38	6	Male	Miniature Pinscher	Calcium Phosphate	Multiple calculi present in the bladder and within the groove of the os penis
39	3	Female	Miniature Poodle	Calcium Phosphate	Single calculus present in the bladder
40	9	Male	Keeshond	Calcium Phosphate	Multiple Bilateral renal calculi
41	8	Male	Springer Spaniel	Calcium Phosphate	Single Bilateral renal calculi
42*	9	Male	West Highland White Terrier	Cystine	Multiple calculi within the groove of the os penis
43	9	Male	Border Collie	Cystine	Multiple calculi present in the bladder and two present immediately behind the os penis

CASE NO.	AGE AT ONSET OF SIGNS (YEARS)	SEX	BREED	MAIN CONSTITUENT OF STONE	POSITION OF LODGEMENT
44*	5	Male	Labrador Retriever	Cystine	Multiple calculi present in the bladder and immediately behind the os penis
45*	4	Male	Mongrel	Cystine	Single calculus present in Scrotal area of the os penis
46	3	Male	Miniature Poodle	Cystine	Multiple calculi present in the bladder and at the ischial arch
47	5	Male	Miniature Poodle	Cystine	Multiple calculi present in the bladder within the groove of the os penis
48	3	Male	Miniature Poodle	Cystine	Multiple bladder calculi and multiple calculi within the groove of the os penis. Two present in Scrotal area
49	3	Male	Basset Hound	Cystine	Multiple calculi present in the bladder and single calculus within os penis
50	4	Male	Cairn Terrier	Cystine	Multiple calculi present immediately behind the os penis
51	2	Male	Cairn Terrier	Cystine	Multiple calculi present in the bladder and one present in the pelvic urethra
52	5	Male	Miniature Poodle	Cystine	Multiple calculi present in the bladder and immediately behind the os penis

CASE NO.	AGE AT ONSET OF SIGNS (YEARS)	SEX	BREED	MAIN CONSTITUENT OF STONE	POSITION OF LODGEMENT
53	4	Male	Cairn Terrier	Cystine	Multiple calculi present in the bladder and a single calculus present in the prostatic urethra
54	3	Male	Welsh Corgi	Cystine	Multiple calculi present in the bladder and within the groove of the os penis
55*	2	Male	Dalmatian	Urate	One calculus present within the groove of the os penis

* Cases presented by local Veterinarians

PART TWO

AETIOLOGIC FACTORS

1. INFECTION.

Review of the Literature.

Some veterinary authors (Krabbe, 1949; Joest, 1924; and Bloom, 1954) have divided calculi in the canine urinary tract into inflammatory and non-inflammatory types and they suggest that infection plays an important part in the aetiology of the former. These inflammatory calculi which form in the presence of infected urine appear to be chiefly composed of phosphate compounds (Milks, 1935; Krabbe, 1949; and Brodey, 1955).

Experimental evidence of the influence of infection on urolithogenesis.

Experimental evidence of the influence of infection on the production of calculi in the urinary tract of the dog was first reported by Rosenow and Meisser (1923) who produced renal and vesical calculi by creating a focus of infection in the teeth of these animals with streptococci obtained from the urine of human patients suffering from urolithiasis. On the basis of these results, Rosenow and Meisser suggested that such a focus of infection, when caused by certain specific bacteria which have an elective affinity for the urinary tract, may lead to urinary calculi formation. Control animals in this experiment were kept under identical conditions and had an incidence of urinary calculi of 11% which is considerably higher than reported incidences in the canine population at large, so it is possible that some other factor (or factors) apart from the procedure used, was responsible for the results. Hryntschak (1935) using the same technique as Rosenow and Meisser infected the dental pulp of 8 dogs with staphylococci and streptococci and/

and in no case did urinary calculus formation occur.

Randall (1937), however, was successful in producing macroscopic sand or gravel in two dogs by traumatising the wall of the kidney pelvis and introducing bacteria from a recurrent case of human urolithiasis. Collins (1948) also working with dogs found that calculi formed on foreign bodies inserted into the kidney pelvis when partial ureteral obstruction and renal infection were present. This relationship between infection and obstruction in the formation of calculi had also been observed in the experimental work of Hryntschak (1935) and Suby and Suby (1947).

Braude, Shapiro and Siemienski (1959), Shapiro, Braude and Siemienski (1959), and Vivaldi, Zangwill, Cotran and Kass (1960) recorded the production of magnesium ammonium phosphate calculi in rats by experimental establishment of renal infections using urea-splitting (Proteus) organisms.

Interesting experiments by King and Boyce (1963) showed that mixed calcium phosphate and magnesium ammonium phosphate type calculi could be produced in vitro when calcium was added to an acid urine that contained "an optimal bacterial flora". No calculi formed under similar conditions in sterile urine.

Clinical evidence of the influence of infection on urolithiasis.

In man there is abundant evidence to incriminate infection

as/

as an aetiological factor in urinary lithiasis (Hellström 1956). However, apart from occasional case reports and the series recorded by Brodey (1955), little has been written on the association of infection with urinary calculi in dogs. In Brodey's investigation 35 cases of urinary infection were reported in 47 patients tested. With only one exception these 35 infected cases had phosphate calculi. Brodey performed urease tests on 31 of the 35 positive cultures and of these 25 were urease positive. Eleven of these patients had mixed infections and these were usually associated with severe urinary conditions which had extended over a period of one year or more. The organisms isolated in animals with urolithiasis were: Micrococcus pyogenes var aureus; haemolytic, coagulase-positive Micrococcus pyogenes var albus; non-haemolytic, coagulase-negative Micrococcus pyogenes var albus; Proteus and Clostridia. In 27 of the 35 positive cultures Brodey found that micrococci were the predominant organism. Three of the 4 cases in which Proteus sp. were present suffered from recurrent lithiasis. From the information recorded in Brodey's series it would seem that urinary infection is a common finding in patients with urolithiasis and that the stones formed in such cases are composed predominantly of phosphates.

Materials and Methods.

Bacteriological examinations were carried out on all 55 urolithiasis cases, when the patient was first presented. Post-treatment bacteriological examinations were also performed in 38 of the 55

cases/

cases. In 42 of the original 55 examinations quantitative bacteriology was also carried out. The majority (37) of these were examined by a serial dilution technique; 3 were examined by the use of the triphenyl tetrazolium chloride test and the remaining 2 by a gram. smear of the fresh uncentrifuged urine.

Method of Urine Collection for Bacteriological Examination.

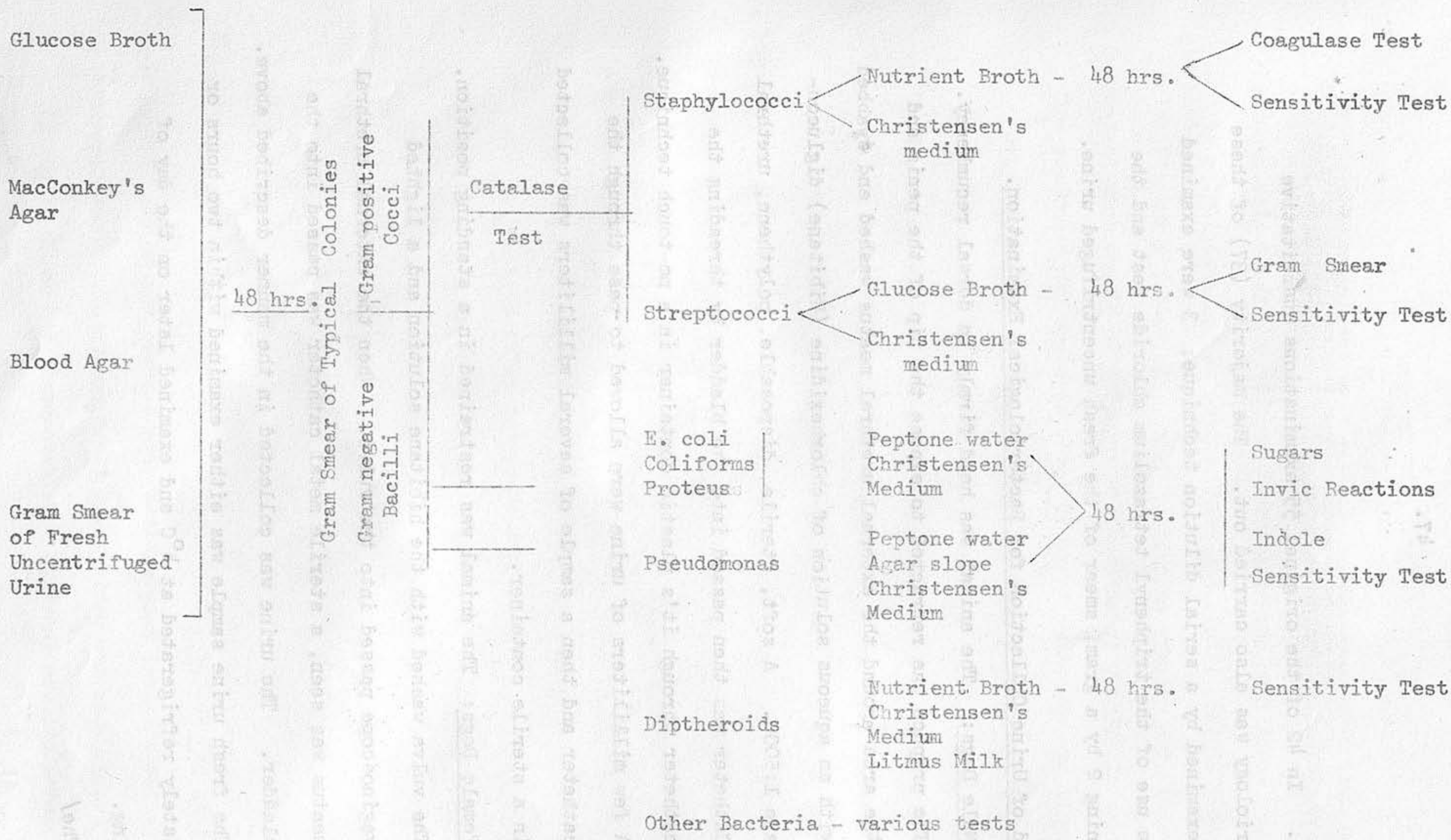
(a) Male Dogs: The animal was held firmly in dorsal recumbency. The prepuce was retracted to expose the tip of the penis and the area around the external urethral meatus washed and swabbed with an aqueous solution of chlorhexidine (Hibitane) digluconate 1:5000. A soft, sterile, disposable, polythene, urethral Catheter was then passed into the bladder by threading the catheter through it's plastic container in a no-touch technique. A few milliliters of urine were allowed to pass through the catheter and then a sample of several milliliters was collected in a sterile container.

(b) Female Dogs: The animal was restrained in a standing position. The vulva washed with the hibitane solution and a lighted vaginoscope passed into the vulva. When the external urethral meatus was seen, a sterile metal catheter was passed into the bladder. The urine was collected in the manner described above.

The fresh urine sample was either examined within two hours or immediately refrigerated at $4^{\circ}C$ and examined later on the day of sampling.

The/

ROUTINE BACTERIAL IDENTIFICATION AND SENSITIVITY TESTING



The following Bacteriological techniques were used:

1. Routine Bacteriological Identification and Sensitivity Testing.

2. Quantitative Bacteriology.

1. Routine Bacteriological Identification.

As already stated bacteriological examination was performed on all 55 cases of lithiasis.

Uncentrifuged urine was placed onto Horse Blood Agar, MacConkey's Agar and Glucose Broth in the usual manner; a loop-full of urine was also placed onto a glass-slide for gram smear examination. The cultures were incubated at 37°C for 48 hours and partly identified by observation of the resultant cultural features and by examination of a gram-stained sample of a representative colony.

The identification procedures used were carried out as described in Mackie and McCartney's Handbook of Bacteriology (1960).

Colonies of Gram-positive cocci were placed onto an Agar Slope and Christensen's medium. After 48 hours incubation at 37°C a catalase activity test was performed on the resultant Agar-slop colonies. When (a) Positive (signifying the presence of Staphylococci) an original colony was transferred to nutrient Broth and after a similar incubation period a coagulase test (using 1:10 Rabbit plasma) was performed. The remaining portion of the Nutrient Broth culture was flooded over two horse blood Agar plates and sensitivity tests carried out with "Multodisks".* When (b) Negative (signifying the presence of Streptococci) a typical original blood agar/

* Oxoid Division of Oxo Ltd. Code No. 30-1H and 11-15F

agar colony was placed into glucose broth and after 48 hours incubation examined by gram smear and flooded over two horse blood agar plates for sensitivity testing with "Multodisks".

Original colonies of gram-negative organisms were placed into Peptone water and Christensen's medium. If the presence of Pseudomonas organisms was suspected a sample colony was spread over an agar slope for the detection of pigment production. The peptone water was incubated for 48 hours and the resulting culture subjected to the following examinations:

(a) Sugar fermentation tests using - Glucose, Lactose, Maltose, Dulcite, Mannite, Inositol and Sucrose.

(b) Invic Reactions, which included the citrate utilization test, Voges-Proskanuer reaction (Barrett's modification) and Methyl-red reaction.

(c) Indole Production test.

(d) Sensitivity testing using "Multodisks" on horse blood Agar.

When Diphtheroids were suspected a colony was inoculated into Litmus-milk media and incubated for three days.

2. <u>Quantitative Bacteriology.</u>

It is generally recognised that in man the presence of micro-organisms in urine samples is not sufficient evidence on which to base a diagnosis of urinary infection. The distinction between contamination and infection is necessary, and is usually achieved by/

by the use of bacterial counting techniques.

Although such techniques are standard procedures in human urology their use in veterinary practice has been very limited. In this study, urolithiasis patients, normal dogs (i.e., dogs with no signs of urinary dysfunction and with a normal urinary sediment) and dogs with miscellaneous urinary tract disorders were examined by this technique. Altogether three different quantitative tests were used, these were:

(a) Serial dilution (colony counting) technique;

(b) Triphenyl Tetrazolium Chloride Test;

(c) Gram-smear examination of the fresh uncentrifuged Urine.

(a) Serial Dilution (colony-counting) Technique.

Method:

Using sterile graduated pipettes, 99 ml. of sterile physiological saline was placed into a sterile bottle containing glass beads; 9 ml. of the same solution were placed in 2 similar bottles. One ml. of the well-shaken urine sample was then added to the 99 ml. bottle and the contents shaken vigorously. In a similar manner 1 cc of this mixture was then transferred to a bottle (marked 10^3) containing 9 ml. of sterile physiological saline and again the contents shaken vigorously. The procedure was repeated, transferring 1 ml. of this latter mixture to the third bottle (Marked 10^4). A separate sterile graduated pipette was used for each dilution.

From/

From the bottle labelled 10^4, 0.1 ml. was removed and placed on 2 MacConkey's Agar plates and 2 Nutrient Agar Plates. The urine was spread evenly over the surface of the plates using a freshly made Pasteur pipette with an L shaped narrow end. Using a fresh sterile pipette 0.1 ml. was transferred from the bottle marked 10^3 to each of 4 similar plates and spread as described above. Similarly 0.1 ml. of the undiluted original urine sample was placed and spread evenly on plates of both MacConkey's and Nutrient Agar. In order to dry the surface of the plates they were then placed in an incubator at 37^{o} for 10 minutes with the lids slightly opened. The lids were then placed in the usual position and the cultures incubated for 48 hours.

After incubation a smear was made from a typical colony (if more than one colony type was present a smear was taken from a typical colony of each type), Gram stained and compared with the bacteria observed in the original sample.

The Agar plates were freshly prepared on the day of use. The 100 ml. pipette used in the first dilution was tin foil wrapped and sterilized in an autoclave, the wrapping being removed immediately before use; the other pipettes were bulk sterilized by autoclaving in copper cylinders.

Analysis of Results.

Each colony which developed in the Agar plate onto which the greatest dilution was placed represented 100,000 organisms per millilitre in the original sample (Fig. 3). When colony numbers were approximately equal on all four plates at this dilution the average/

average number present, per plate, was multiplied by 100,000. Whe colonies were found to be growing on only one of the media used or when one media contained a greater number of colonies than the other, the average of the two plates of this media was multiplied by 100,000.

A similar method of colony counting was undertaken for the lesser dilutions. The average number of colonies per plate on the intermediate dilution representing 10,000 organisms per ml. of urine and on the undiluted urine plates, representing 10 organisms per ml. of urine.

(b) Triphenyltetrazolium Chloride Test.

This test was performed on a number of occasions but only on 3 samples to which other quantitative bacteriological tests had not been applied. The test is based on the reduction of soluble triphenyltetrazolium to triphenylformazan which forms a red precipitate. This reduction is achieved by products of active respiratory processes of rapidly dividing bacteria. By the use of a suitable concentration of the reagent in urine the presence of bacteria in numbers greater than 100,000 micro-organisms per millilitre of urine can be recognised. The reagent is available in test tubes* to which 2 ml. of urine are added. The tubes are then incubated for 4 hours. The presence of a red precipitate denotes that more than 100,000 bacteria are present in each millilitre of urine.

(c) Gram Smear Examination of the Fresh Uncentrifuged Urine.

This was carried out using Jensen's Modification of the gram

staining /

* Pfizer Ltd.

staining method described in Mackie and McCartney's "Handbook of Bacteriology" 1960. At least 50 oil immersion fields being studied in each smear.

Results.

Bacterial examination in 55 cases of Urolithiasis.

Initial Examination.

Bacterial infection was found to be present in 22 of the 55 cases. Five of the 55 cases, however, had received anti-bacterial therapy prior to sampling. In 2 of these 5, gram positive organisms were seen on gram smear examination of the fresh uncentrifuged urine sample and were thus probably infected although the cultures were negative. Seventeen of the 22 infected cases contained

were negative. Seventeen of the 22 infected cases contained

staphylococci (Table 24). In 13 of these cases *Staph. var albus* was present either alone or with other organisms. In one case *Staph. pyogenes var aureus* was found and in three cases *Staph. epidermis* was isolated. Streptococci were cultured on five occasions. Four of these were *Strept. faecalis* and the other a β haemolytic Streptococcus. *E. coli* were also isolated from 5 dogs.

In 12 of the 22 infected urolithiasis cases more than 1 colony type of bacteria was present.

Post Treatment Examination.

(a) Initially infected cases.

In the 22 animals with urolithiasis and concurrent infection at the initial examination, 18 were re-examined following treatment. Seven of these were examined in the immediate post-treatment month; 7 were examined during this period and at a later date; 4 were examined during a period greater than one month after treatment.

Of/

TABLE 24

RELATIONSHIP OF INFECTION TO THE CHEMICALLY DISTINCT TYPES OF CALCULI

	Triple Phosphate Cases	Calcium Oxalate Cases	Cystine Cases	Calcium Phosphate Cases	Urate Cases	Total
Staphylococci	8	3	-	1	-	12
Staphylococci with other bacteria	5	-	-	-	-	5
Bacteria other than Staphylococci	2	3	-	-	-	5
Probably infected*	2	-	-	-	-	2
Unknown**	1	1	-	1	-	3
No organisms isolated	1	11	13	2	1	28
TOTAL	19	18	13	4	1	55

* Recently treated with antibiotics and although no organisms were cultured, examination of a gram-smear of fresh uncentrifuged urine revealed bacteria (gram-positive cocci) to be present in more than one field.

** Recently treated with antibiotics, no organisms isolated and no bacteria seen on examination of a gram-smear of fresh uncentrifuged urine.

TABLE 25

PRE AND POST-TREATMENT BACTERIOLOGY ON UROLITHIASIS CASES

Case No.	Bacteria Isolated On Initial Examination and number present/ml. urine		Bacteria Isolated within 1 month following treatment		Bacteria Isolated more than 1 month Following Treatment		REMARKS
			Period after treatment (Days)	Number and Type of Bacteria Isolated	Period after Treatment (Months)	Number and Type of Bacteria Isolated	
1	No organisms isolated	T	2	20/ml	$1\frac{1}{2}$ $4\frac{1}{2}$	S. Faecalis 78,000/ml 0/ml	Antibiotics given prior to initial sampling.
2	Staph. pyogenes var. Albus	R E A		Not carried out	7 15	3 colony-types Mixed Coliforms 16.3×10^6/ml S. faecalis and E.coli 40.8×10^6/ml.	Radiographic Signs of reforming calcu at 15 months post-treatment.
3	No organisms isolated	T M E N T		Not carried out		Not carried out	Antibiotics given prior to initial sampling
4	Staph. pyogenes var. albus. 9.8×10^6/ml Str. faecalis 28×10^6/ml		30	Str. faecalis 125,000/ml	4	Gram Smear contained Gram-Positive cocci. 0/ml.	No radiographic signs of reforming calculi 4 months following treatmen
5	Staph. pyogenes var. albus. (2 colony types) 10.6×10^6/ml		21	E. Coli more than 50×10^6/ml.		Not carried out	No treatment was given between sampling

Case No.	Bacteria Isolated on Initial Examination and number present/ml. urine		Bacteria Isolated within 1 month following treatment: Period after Treatment (Days)	Bacteria Isolated within 1 month following treatment: Number and Type of Bacteria Isolated	Bacteria Isolated more than 1 month Following Treatment: Period after Treatment (Months)	Bacteria Isolated more than 1 month Following Treatment: Number and Type of Bacteria Isolated	REMARKS
6	Str. faecalis 50×10^6/ml Staph. pyogenes var albus 17.5×10^6/ml	TREATMENT	7	Staph. pyogenes var albus. 350,000 ml. Str. faecalis 42.1×10^6/ml.		Not carried out	Died whelping, 2 months after trea[tment]. No clinical signs of urinary dysfunction.
7	Staph. pyogenes var. albus.			Not carried out	14	10/ml.	No radiological signs of reformin[g] calculi at 14 months post-operation.
8	No organisms Isolated			Not carried out	$1\frac{1}{2}$	30/ml.	Antibiotics given prior to initial sampling. No radiological sign of reforming calculi 6 weeks post operative.
9	Staph. pyogenes var. albus. (2 colony types)		7	Staph. pyogenes var albus (urease negative) Strep. faecalis	4 12	10/ml. 10/ml.	No radiological signs of calculi 1 year after treatment.
10	Staph. pyogenes var albus (2 colony types) 13.5×10^6/ml.		10	No Bacteria isolated	9	No Bacteria Isolated	No radiological signs of reforming calculi 9 months post-treatment.

11	Staph. pyogenes var. albus. (2 colony types) 18.9 x 10^6/ml.		4	30/ml		Not carried out	Died 6 months post-treatment with pyometra. N calculi seen on post-mortem
12	B. Haemolytic Streptococci more than 50 x 10^6/ml. Staph. epidermis 0.35 x 10^6			Not carried out	2½	0/ml.	No radiological signs of reformir calculi 6 months post-treatment
13	Staph. Epidermis More than 50 x 10^6/ml.		3	0/ml.	6	Staph. pyogenes var. albus 50 x 10^6/ml.	Radiographic sign of reforming calculi 6 months after treatment.
14	E. coli - more than 50 x 10^6/ml. Staph.pyogenes var. aureus. 0.65 x 10^6/ml.	TREATMENT	14	15/ml.		Not carried out	
15	Staph. pyogenes var. albus E. coli more than 50 x 10^6/ml.		14	30/ml			No clinical signs of reforming calculi 6 months post-treatment
16	P. mirabilis more than 50 x 10^6/ml		14	350/ml.			
17	Staph.pyogenes var. albus. More than 50 x 10^6/ml.		14	0/ml.			
18	Str. faecalis & E. coli more than 50 x 10^6/ml						Destroyed with acute syndrome associated with Intervertebral Disc Prolapse. Not treated for urolithiasis.

Case No.	Bacteria Isolated on Initial Examination and number present/ml.urine	Bacteria Isolated within 1 month following treatment		Bacteria Isolated more than 1 month Following Treatment		REMARKS
		Period after Treatment (Days)	Number and Type of Bacteria Isolated	Period after Treatment (Months)	Number and Type of Bacteria Isolated	
19	No organisms isolated 0/ml.		Not carried out	$6\frac{1}{2}$ 8	10/ml 10/Ml	Radiographic signs of calculi re-forming 8 months post-treatment
20	Staph. pyogenes var. albus 0.4×10^6/ml.		Not carried out		Not carried out	Died 2 months after treatment
21	10/ml	2	10/ml			Treated with anti-biotics prior to initial sample.
22	Str. faecalis 2.5×10^6/ml. E. coli 50×10^6/ml.		Not carried out		Not carried out	Some organisms isolated on 2 occasions in previous 12 months when calculi were known to be developing. Destroyed without treatment.
23	40/ml.		Not carried out		Not carried out	
24	0/ml	5	No organisms isolated		Not carried	
25	10/ml	7	0/ml		Not carried out	
26	E. coli more than 50×10^6/ml.	3 21	0/ml 0/ml.	4	10/ml	Radiographic signs of re-forming calculi 4 months post treatment.

27	0/ml			Not carried out	1½	No organisms isolated	
28	No organisms isolated		21	0/ml.	6	0/ml.	No radiological signs of re-form- calculi 6 months post-treatment
29	0/ml		4	No organisms isolated	2	0/ml	
30	Coliforms (2 colony types) 4.77 x 10^6/ml.		2	No organisms isolated	1	30/ml.	No radiological signs of re-form- ing calculi at 1 month post-treatme
31	No organisms isolated			Not carried out	12	430/ml.	Radiographic signs of re-forming calculi 12 months post-treatment.
32	No organisms isolated	T R E A T M E N T		Not carried out		Not carried out	
33	<u>Staph. pyogenes var. albus</u>			Not carried out	18	<u>P. mirabilis</u>	No radiographic signs of re-forming 18 months after treatment.
34	0/ml.			Not carried out	1½	0/ml.	Radiographic signs of re-forming calculi 6 weeks after treatment.
35	20/ml.		5	0/ml.	3½	0/ml.	Radiographic signs of re-forming calculi 10 months after treatment
36	<u>Staph. pyogenes var. albus</u> 43.5 x 10^6/ml.			Not carried out		Not carried out	Unilateral vesico- urethral reflux and calculi in pelvis of ipsilateral kidney

Case No.	Bacteria Isolated on Initial Examination and number present/ml. urine		Bacteria Isolated within 1 month following treatment		Bacteria Isolated more than 1 month Following Treatment		REMARKS
			Period after Treatment (Days)	Number and Type of Bacteria Isolated	Period after Treatment (Months)	Number and Type of Bacteria Isolated	
37	No organisms isolated	TREATMENT		Not carried out	6 7	30/ml 200/ml.	Radiographic signs of re-forming calculi 7 months post-treatment.
38	No organisms isolated		30	0/ml.	2	0/ml.	Radiographic signs of re-forming calculi together with clinical signs 7 months post-treatment.
39	Staph. epidermis 0.4 x 10^6/ml.		2	0/ml.	2	20/ml.	No radiographic signs of re-forming calculi 11 months after treatment.
40	0/ml.			Not carried out		Not carried out	Destroyed at initia episode.
41	0/ml.			Not carried out		Not carried out	Destroyed at initia episode.
42	No organisms isolated		2	No organisms isolated		Not carried out	
43	42,000/ml.			Not carried out		Not carried out	Died at initial episode.
44	No organisms isolated			Not carried out		Not carried out	Destroyed at initia episode
45	10/ml.			Not carried out		Not carried out	
46	20/ml.			Not carried out	12	10/ml.	No radiographic sig of re-forming calculi 12 months post treatment.

47	0/ml.			Not carried out		Not carried out	Died at initial episode.
48	No organisms isolated			Not carried out	2 12 16	40/ml. 0/ml. 40/ml.	Repeat clinical ep: sode 12 months pos' treatment. Radio- graphic evidence o: reforming calculi months after repea episode.
49	10/ml.		4	20/ml.	6	20/ml.	No radiographic signs of re-formin calculi 6 months after treatment.
50	No organisms isolated	T R E A T M E N T		Not carried out		Not carried out	
51	No organisms isolated			Not carried out		Not carried out	
52	30/ml.			Not carried out		Not carried out	Died at initial episode.
53	10/ml.		30	No bacteria isolated		Not carried out	
54	No organisms isolated			Not carried out	6 7 11 12 $12\frac{1}{2}$ 13 19	Proteus organisms isolated Following 10 days' anti- biotic treatment and sample taken 2 days later. No organisms isolated. P. mirabilis 35 x 10^6/ml. P. mirabilis P. mirabilis 50 x 10^6/ml. P. mirabilis more than 50 x 10^6/ml. P. mirabilis	No radiographic si of re-forming cal- culi 19 months pos treatment. Persis ent infection with P. mirabilis from 6 months following treatment. Infec- tion intractable wi both long term ant bacterial and auto- genous vaccine therapy.
55	0/ml.			Not carried out		Not carried out	Died at initial episode.

Of the 14 animals examined in the immediate post-treatment period, 4 (29%) had organisms still present in the urine and in 2 of these a different bacterial genera from that present prior to treatment was isolated. Of the 4 animals with organisms still present following treatment only two were examined at a later date; one was found to be no longer infected, and in the other no organisms were cultured but were observed in a gram smear of fresh uncentrifuged urine.

In the 11 cases examined at periods over 1 month bacteria were still present in 3 (27%). In these 3 dogs different bacterial genera from that present prior to treatment were found. (Table 25).

(b) Initially uninfected cases.

Of the 33 cases of urolithiasis not infected prior to treatment 20 were re-examined bacteriologically following treatment; 5 being examined in the month immediately following treatment; 6 during this period and at a later date and 9 during the period of more than 1 month, after treatment.

No bacteria were isolated in the 11 examined in the month following treatment and only 1 (7%) of the 15 examined at longer intervals was infected.

Urease Activity.

Tests for the presence of urea- splitting ability were carried out on the organisms isolated. In 17 of the 22 infected dogs a urea-splitting/

TABLE 26

URFASE ACTIVITY IN INFECTED CASES OF UROLITHIASIS

Case No.	Organisms Isolated	Urease Activity	Chemical Stone Type.
2.	Staph. pyogenes var albus.	+	Triple Phosphate.
4.	Staph. pyogenes var albus. Strep. faecalis	+ -	Triple Phosphate.
5.	Staph. pyogenes var albus. (2 colony types)	+ (both)	Triple Phosphate.
6.	Staph. pyogenes var albus. Strep. faecalis	+ -	Triple Phosphate.
7.	Staph. pyogenes var albus.	+	Triple Phosphate.
9.	Staph. pyogenes var albus. (2 colony types)	+ (both)	Triple Phosphate.
10.	Staph. pyogenes var albus. (2 colony types)	+ (both)	Triple Phosphate.
11.	Staph. pyogenes var albus. (2 colony types)	+ (both)	Triple Phosphate.
12.	β Haemolytic Streptococci. Staph. epidermis	- +	Triple Phosphate.
13.	Staph. epidermis	+	Triple Phosphate.
14.	E. coli Staph. pyogenes var aureus	- +	Triple Phosphate.
15.	Staph. pyogenes var albus. E. coli	+ -	Triple Phosphate.
16.	Proteus mirabilis	+	Triple Phosphate.
17.	Staph. pyogenes var albus.	+	Triple Phosphate.
18.	Strep. faecalis E. coli	- -	Triple Phosphate.
20.	Staph. pyogenes var albus.	+	Calcium Oxalate.
22.	Strep. faecalis E. coli	- -	Calcium Oxalate.
26.	E. coli	-	Calcium Oxalate.
30.	Coliforms (2 colony types)	-	Calcium Oxalate.
33.	Staph. pyogenes var albus.	+	Calcium Oxalate.
36.	Staph. pyogenes var albus.	-	Calcium Oxalate.
39.	Staph. epidermis	+	Calcium Phosphate.

splitting organism was present. All Staphylococci associated with triple phosphate stones were urease positive. All Streptococci and E. coli were urease negative. (Table 26).

Results of Quantitative Bacteriology.

(a) Bacterial counts in 16 normal dogs.

Fourteen of the 16 animals had less than 50 organisms per ml. of urine present. (Table 27 and Fig. 4). Bacterial contamination of the counting plates during the examination occasionally resulted in up to 5 colonies per plate, thus in the 14 animals involved it is possible that no organisms were present in their urine. In the remaining 2 dogs 1 had 1,070 organisms per ml. and the other 20,000 organisms per ml. It seems likely therefore that uninfected urine samples collected and examined in this manner would have less than 100,000 organisms per ml. of urine.

(b) Bacterial Counts in 21 dogs with Urological Disorders unassociated with Urolithiasis.

Eleven of the 21 animals examined had more than one million organisms present per ml. urine. The remaining 10 animals had less than 50,000 organisms per ml. (Table 28 and Fig. 5). Thus it would seem that a bimodal distribution of cases is present and that 100,000 organisms per ml. is a suitable arbitrary figure for the differentiation of contaminated from infected urine under these circumstances.

(c) Bacterial Counts in 37 Urolithiasis Patients.

The distribution of colony Counts for cases of urolithiasis is shown in Table 29 and Fig. 6. Of the 37 cases, 18 had less than 100 organisms/

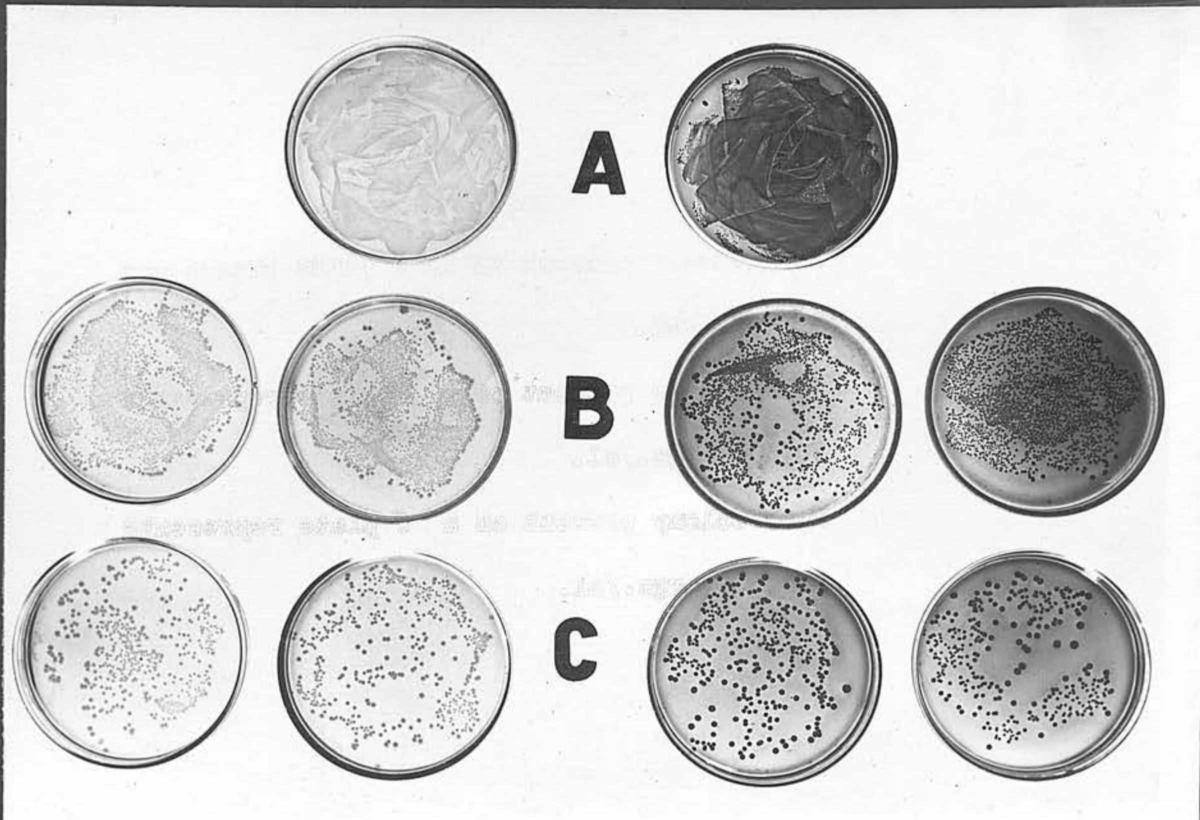
Fig. 3. Agar Plates used in Colony Counting Urine from a Patient with Urinary Infection.

Each Colony present on an A plate represents 10 orgs./ml.

Each Colony present on a B plate represents 10,000 orgs./ml.

Each Colony present on a C plate represents 100,000 orgs./ml.

Fig. 4. Distribution of Urine Colony Counts in 16-Normal Dogs.


A
B
C

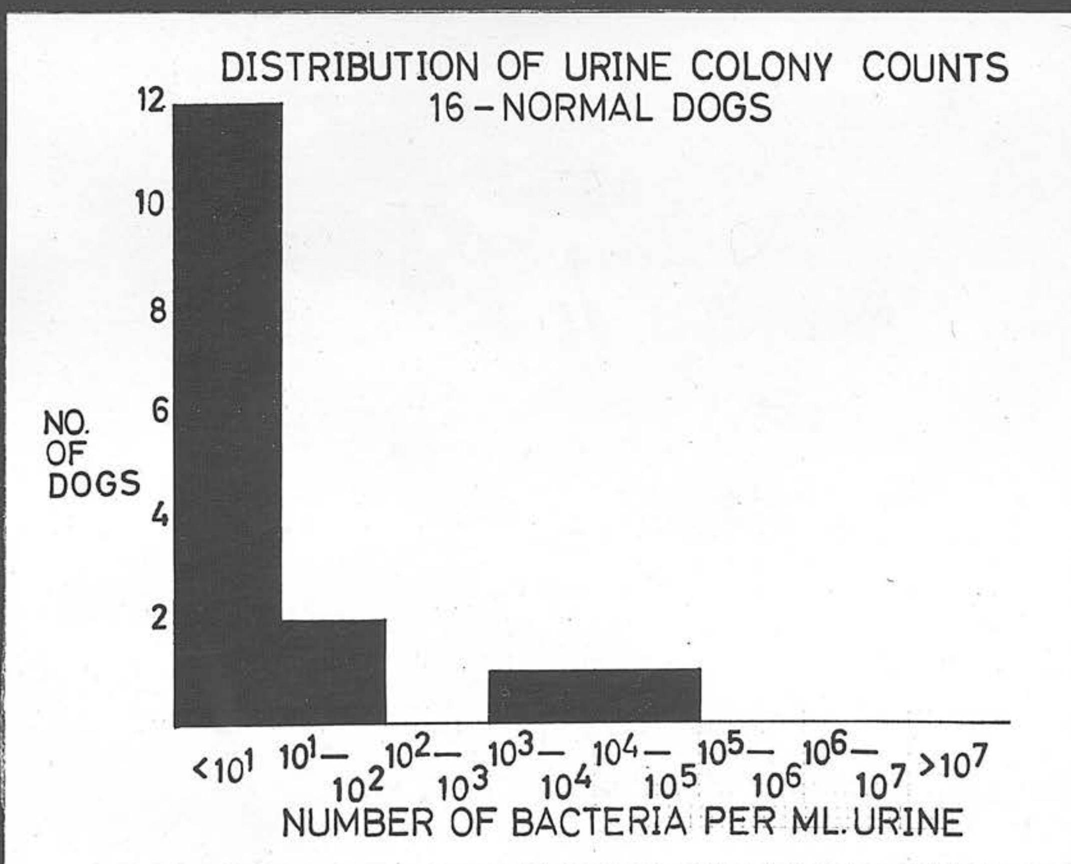
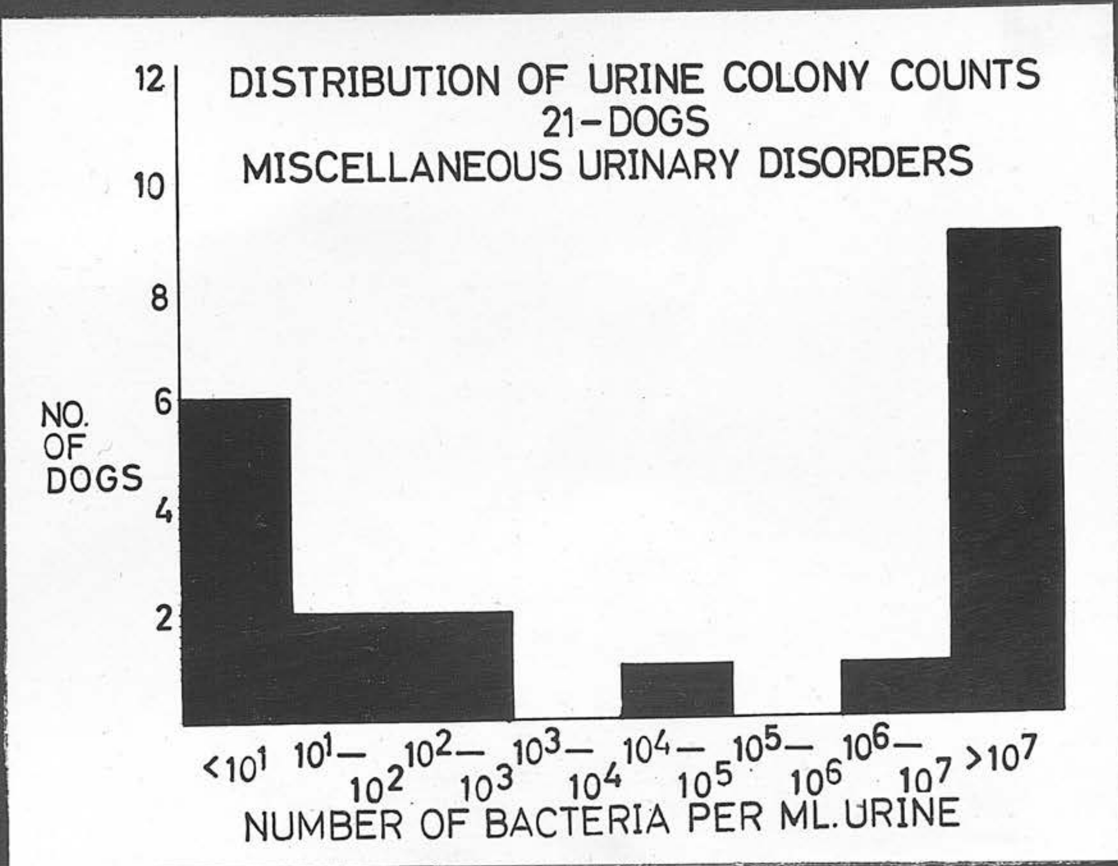

DISTRIBUTION OF URINE COLONY COUNTS
16 - NORMAL DOGS
12
10
8
6
4
2
NO. OF DOGS
<10^1
10^1—10^2
10^2—10^3
10^3—10^4
10^4—10^5
10^5—10^6
10^6—10^7
>10^7
NUMBER OF BACTERIA PER ML. URINE

Fig. 5. Distribution of Urine Colony Counts in 21-Dogs with Miscellaneous Urinary Disorders.

Fig. 6. Distribution of Urine Colony Counts in 37 Dogs with Urolithiasis.


DISTRIBUTION OF URINE COLONY COUNTS
21-DOGS
MISCELLANEOUS URINARY DISORDERS
NO. OF DOGS
12
10
8
6
4
2
<10¹
10¹—10²
10²—10³
10³—10⁴
10⁴—10⁵
10⁵—10⁶
10⁶—10⁷
>10⁷
NUMBER OF BACTERIA PER ML. URINE

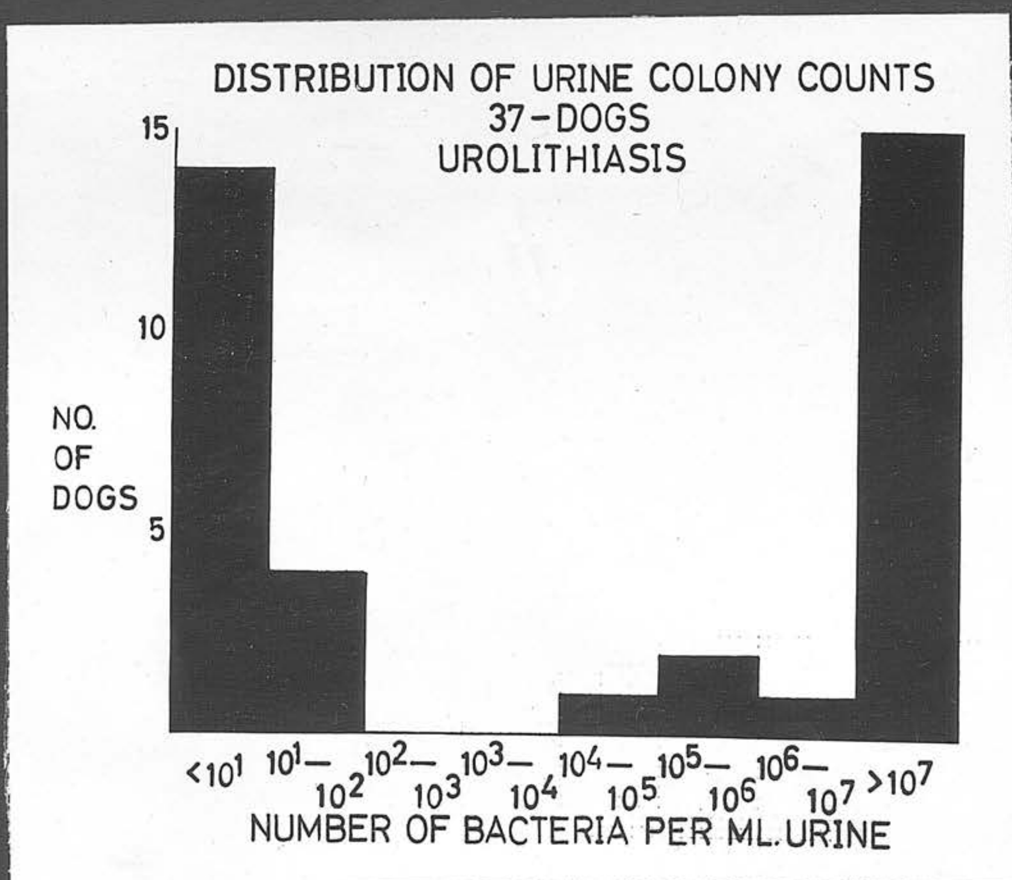

DISTRIBUTION OF URINE COLONY COUNTS
37-DOGS
UROLITHIASIS
NO. OF DOGS
15
10
5
<10¹
10¹—10²
10²—10³
10³—10⁴
10⁴—10⁵
10⁵—10⁶
10⁶—10⁷
>10⁷
NUMBER OF BACTERIA PER ML. URINE

Fig. 7. Oil Immersion Field of a Gram-Smear of Infected Urine in a Case of Urolithiasis. Showing short chains of Cocci.

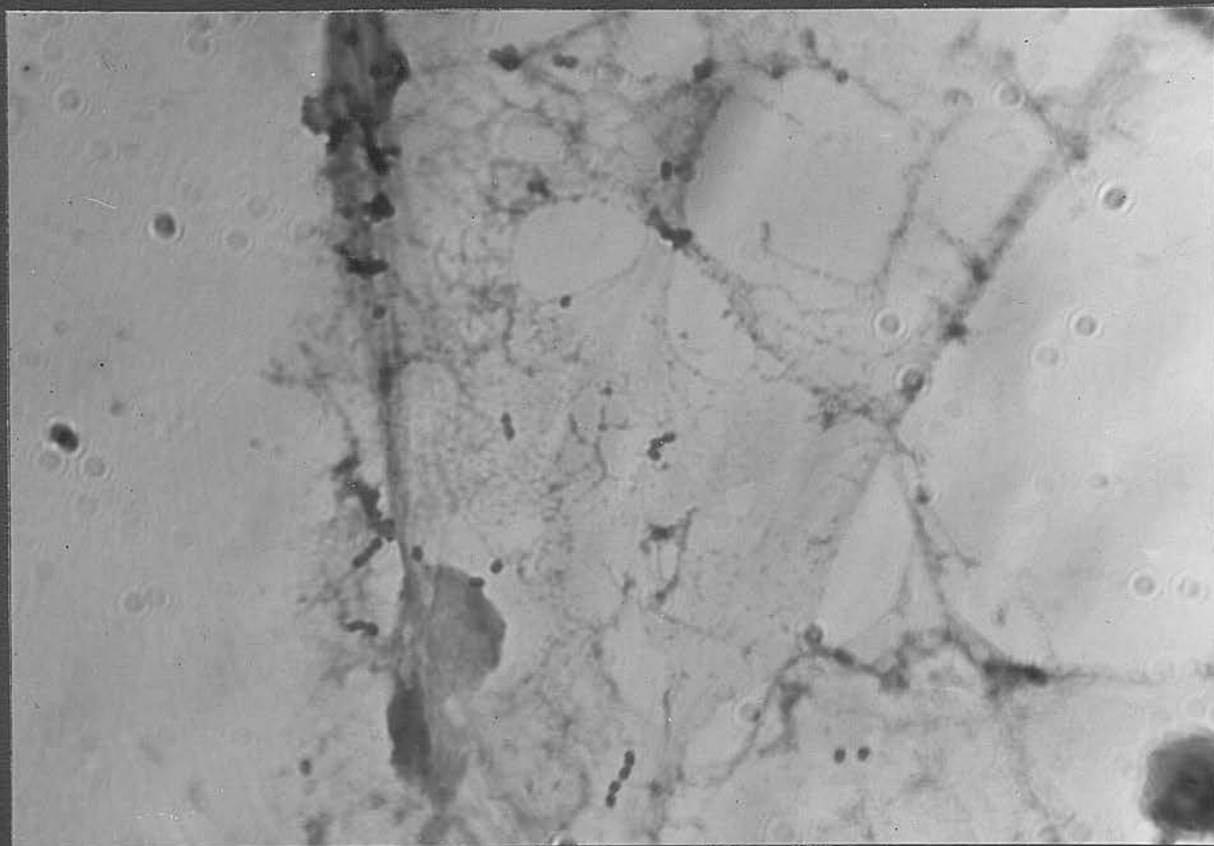


TABLE 27

BACTERIAL COLONY COUNTS FROM 16 "NORMAL" DOGS

Breed	Age	Sex	Quantitative Bacteriology Organisms/ml. Urine.
Staffordshire Bull Terrier	7 yrs.	Female	0
Mongrel	7 mths.	Male	0
Mongrel	6 yrs.	Male	0
Shetland Collie	1 yr.	Male	0
Boxer	2 yrs.	Male	30
Greyhound	5 yrs.	Male	0
Doberman Pinscher	1 yr.	Female	10
Border Collie	10 yrs.	Female	0
Greyhound	7 yrs.	Male	0
Alsatian	6 yrs.	Female	10
Boxer	7 yrs.	Male	10
Mongrel	10 yrs.	Female	0
Great Dane	11 yrs.	Female	1,070
West Highland White Terrier	11 yrs.	Female	20,000
Greyhound	8 yrs.	Male	40
Beagle	8 yrs.	Male	10

TABLE 28

BACTERIAL COLONY COUNTS FROM 21 DOGS WITH UROLOGICAL DISORDERS UNASSOCIATED WITH UROLITHIASIS

Breed	Age	Sex	Quantitative Bacteriology Orgs./ml. urine	Organisms Isolated
Dachshund	8 yr.	Male	45	nil
Cocker Spaniel	12 yr.	Male	0	nil
Dachshund	9 yr.	Female	39.6×10^6	P. mirabilis.
Welsh Corgi	7 yr.	Male	170	nil
Welsh Corgi	5 yr.	Male	$>50 \times 10^6$	P. mirabilis.
Boxer	7 yr.	Female	37.8×10^6	Proteus sp. Str. faecalis.
Border Collie	11 yr.	Female	50.0×10^6	E. coli.
Alsatian	4 yr.	Female	31.5×10^6	E. coli.
Border Collie	7 yr.	Male	0	nil
Mongrel	5 yr.	Female	$>50.0 \times 10^6$	P. mirabilis.
St. Bernard	9 yr.	Female	580	nil
Welsh Corgi	4 mths.	Male	0	nil
Welsh Corgi	-	-	10	nil
Welsh Corgi	4 yr.	Female	4.4×10^6	P. mirabilis.
Mongrel	10 mths.	Female	40×10^6	E. coli.
Yorkshire Terrier	3 yr.	Male	0	nil
Shetland Collie	7 yr.	Male	0	nil
Welsh Corgi	6 yr.	Female	1.75×10^6 $>50 \times 10^6$	St. pyogenes Str. faecalis
Mongrel	8 yr.	Male	22,500	nil
Mongrel	9 yr.	Male	90	nil
Labrador Retriever	10 yr.	Female	$>50 \times 10^6$	P. mirabilis.

TABLE 29

BACTERIAL COLONY COUNTS FROM 37 DOGS WITH UROLITHIASIS

Case No.	Breed	Age	Sex	Quantitative Bacteriology Orgs./ml. Urine	Organisms Isolated
4.	Dachshund	9 yr.	Female	9.8×10^6 $28. \times 10^6$	Staph. pyogenes var albus Str. faecalis
5.	Welsh Corgi	8 yr.	Female	10.6×10^6	Staph. pyogenes var albus (2 colony types)
6.	Mongrel	7 yr.	Female	$50. \times 10^6$ 17.5×10^6	Str. faecalis Staph. pyogenes var albus
10.	Welsh Corgi	8 yr.	Female	13.5×10^6	Staph. pyogenes var albus (2 colony types)
11.	Cairn Terrier	11 yr.	Female	18.9×10^6	Staph. pyogenes var albus
12.	Scottish Terrier	4 yr.	Female	$>50. \times 10^6$ 0.35×10^6	β Haemolytic Strept. Staph. epidermis.
13.	Border Collie	10 yr.	Male	$>50. \times 10^6$	Staph. epidermis.
14.	Miniature Poodle	2 yr.	Female	$>50. \times 10^6$ 0.65×10^6	E. coli. Staph. pyogenes var aureus
15.	Cairn Terrier	10 yr.	Female	$>50. \times 10^6$	Staph. pyogenes var albus E. coli.
16.	Welsh Corgi	6 yr.	Female	$>50. \times 10^6$	P. mirabilis.
17.	Dachshund	4 yr.	Female	$>50. \times 10^6$	Staph. pyogenes var albus
18.	Dachshund	8 yr.	Female	$>50. \times 10^6$	Str. faecalis and E. coli.
19.	Welsh Corgi	1 yr.	Male	0	nil.
20.	Border Terrier	14 yr.	Male	0.4×10^6	Staph. pyogenes var albus
21.	Boxer	5 yr.	Male	10	nil.
22.	Dandie Dinmont	12 yr.	Male	2.5×10^6 $50. \times 10^6$	Str. faecalis E. coli.
23.	Cairn Terrier	5 yr.	Male	40	nil.
24.	Mongrel	10 yr.	Male	0	nil.
25.	Dachshund	4 yr.	Male	10	nil.
26.	Mongrel	11 yr.	Male	$>50. \times 10^6$	E. coli.

Case No.	Breed	Age	Sex	Quantitative Bacteriology	
				Orgs./ml. Urine	Organisms Isolated
27.	Border Terrier	8 yr.	Male	0	nil
29.	Border Terrier	6 yr.	Male	0	nil
30.	Mongrel	12 yr.	Male	4.77×10^6	Coliforms (2 colony types).
34.	Pug.	10 yr.	Male	0	nil
35.	Border Collie	8 yr.	Male	20	nil
36.	Cairn Terrier	9 yr.	Female	43.5×10^6	Staph. pyogenes var albus
39.	Toy Poodle	3 yr.	Female	0.4×10^6	Staph. epidermis.
40.	Keeshond	9 yr.	Male	0	nil
41.	Springer Spaniel	8 yr.	Male	0	nil
43.	Border Collie	9 yr.	Male	42,000	nil
45.	Mongrel	4 yr.	Male	10	nil
46.	Miniature Poodle	4 yr.	Male	20	nil
47.	Miniature Poodle	5 yr.	Male	0	nil
49.	Basset Hound	3 yr.	Male	10	nil
52.	Miniature Poodle	5 yr.	Male	30	nil
53.	Cairn Terrier	4 yr.	Male	10	nil
55.	Dalmatian	2 yr.	Male	0	nil

organisms per ml. and 16 more than one million organisms per ml; again the bimodal character of the distribution is apparent. In only one sample was a count between 1,000 and 100,000 organisms per ml. seem.

Assessment of the accuracy of the Examination of a Gram. Smear of fresh uncentrifuged urine in the determination of Significant Bacteriuria.

The accuracy of this test was assessed by comparison with the serial dilution, colony-counting technique in 139 observations on samples taken from animals that had no history of recent antibiotic treatment.

In 90 observations in which less than 100,000 organisms per ml. were present on colony counting, 87 were negative for bacteria and 3 were positive. In a further 49 in which more than 100,000 organisms per ml. were found on colony counting 5 were negative and 44 positive. (Table 30).

TABLE 30

ACCURACY OF THE EXAMINATION OF A GRAM- SMEAR OF FRESH UNCENTRIFUGED URINE AS A METHOD OF QUANTITATIVE BACTERIOLOGY.

Results of Colony Counting Technique	Number of Observations in which Bacteria were not seen in more than one field	Number of Observations in which Bacteria were present in more than one field	Total
Less than 100,000 orgs. per ml. urine	87	3	90
More than 100,000 orgs. per ml. urine	5	44	49
TOTAL	92	47	139

Thus/

Thus if 100,000 organisms per ml. is taken as the arbitary division between infection and contamination as is suggested by Kass (1956) for human urine the results of gram-smear examinations were correct in 93.2% of the observations.

Relationship between infection and the chemical type of Stone.

(a) Triple Phosphate Lithiasis.

Fifteen of the 16 cases of triple phosphate stones unassociated with recent antibacterial therapy had organisms present. (Table 24). In 13 of these 15 cases staphylococci were found. In the remaining 2 cases P. mirabilis and a mixed infection of Strept. faecalis and E. coli were found. The animal from which the Proteus organism was isolated had been presented at the clinic 3 months previously. On this occasion radiographic examination of the urinary tract failed to reveal the presence of calculi, but a mixed infection of Staph. pyogenes var albus and Strept. faecalis was found on quantitative bacteriological examination; this patient was also found to have a bladder neck abnormality and unilateral vesico-ureteral reflux.

(b) Other Chemical Stone Types.

In contrast with the high infection rate found in triple phosphate lithiasis, only 7 of 34 cases of the other chemical stone types that had not received recent antibacterial therapy were found to be infected. In 4 of these 7, staphylococci were isolated. In the remaining 3, E.coli or coliforms were found.

In/

In order to assess whether the apparent differences in the frequency of infection in the different chemically distinct types of stone was statistically significant an X^2 test was performed on the figures recorded. As a result of these calculations a total X^2 value of 27.73 was found. Thus a statistically significance difference ($P < 0.01$) in the association of infection to the different chemical types of stone existed in this series. Similar statistical analyses were carried out on the frequency of association of staphylococcal infection with the different chemical stone types, and with the frequency of occurrence of staphylococci in triple phosphate stones compared with all the other chemical stone types. A significant correlation ($P < 0.01$) was found in both these calculations.

Relationship of infection to sex in cases of urolithiasis.

Of the 36 male and 14 females with urolithiasis that had not received antibacterial therapy prior to presentation at the clinic, 22 were found to be infected; 14 of these animals were females and 8 were males. The X^2 test of frequency analysis was applied to these figures. A total X^2 value of 21.43 resulted. Thus a statistically significant difference ($P < 0.01$) between the distribution of infection in the sexes in urolithiasis was present. This difference was due to a higher incidence of infection in females than males.

Relationship/

Relationship Between the Incidence of Bacterial Genera in Urinary Tract Infections (unassociated with calculi) and in Infected Urolithiasis Cases.

Mosier and Coles (1958) and Mosier (1965) found that in urinary tract conditions in the dog, the most common organisms isolated are gram negative bacilli. In 53 cases of/urinary tract infection Mosier and Coles (1958) found that 17 (32.1%) were associated with Proteus organisms, 12 (22.6%) with E. coli, 11 (20.7%) with Streptococci and only 10 (18.9%) with Staphylococci. In order to determine whether a similar distribution of organisms with urinary tract infections occurred in this area, 21 animals with signs of urinary dysfunction (unassociated with urolithiasis) in which significant numbers of bacteria were present on quantitative bacteriological examination were selected and the organisms present isolated in each case with the following results:

E. coli and coliforms	8
Proteus	8
Staphylococci	4
Paracolon	1
Streptococci	1
Pseudomonas	1
Total	23 *

*In two of these animals mixed infections were present and both bacterial genera were recorded.

These results do not differ greatly from Mosier's (1958) figures. A comparison of the distribution of the different bacterial genera in urinary disorders unassociated with urolithiasis and with cases of this latter condition reveal considerable discrepancies, the most apparent of which is the greater frequency of staphylococcal organisms in patients with infected urolithiasis.

In/

In order to determine whether the difference in frequency of occurrence of staphylococci in urolithiasis and non-urolithic urinary tract disorders was significant, an X^2 analysis was applied. A statistically significant difference ($P < 0.01$) was found. Since 13 of the 17 Urolithiasis patients with staphylococci present contained triple phosphate stones, it is obvious that the increase of incidence of staphylococci in infections concurrent with urolithiasis is associated with this chemical stone type. However, staphylococcal organisms were also found in 4 of the 7 infections occurring with the other chemical types of stone, and it seems possible that an increased frequency of staphylococcal infections may also be related to calculi other than the triple phosphate type. To test this hypothesis an X^2 analysis was applied to the comparison of the distribution of staphylococci in infected urolithiasis cases containing stones other than triple phosphate and the distribution of staphylococci in urinary tract infections unassociated with urolithiasis. The apparent difference was not statistically significant.

Discussion.

Quantitative Bacteriological Techniques.

The technique of colony-counting carried out as described in this study is probably the most accurate method of determining the number of viable organisms present in the urine (Brumfitt and Percival, 1964). It depends on the fundamental assumption that each organism in the urine/

urine will form a colony on suitable culture media. The two media used (Nutrient agar and MacConkey's agar), seemed to be suitable for this purpose as the majority of urinary organisms appeared to thrive on one or the other. The diluent used (physiological saline) also appeared to be satisfactory.

Cases of urolithiasis and other urinary disorders, were divided by this technique into two groups (Figs. 5 and 6), representing infected and non-infected animals. Most infected animals had more than one million organisms per ml. of urine present; the uninfected group had, in the majority of cases, less than 100 organisms per ml. of urine. The number of cases between these limits represented approximately 10% of the total number of animals investigated. It is obvious therefore, that in approximately 90% of observations made, the division between infected and uninfected urines is distinct. In 16 normal animals the urinary colony counts did not exceed 20,000 organisms per ml. and in the majority of the samples examined less than 100 organisms per ml. of urine were found.

Although the accepted number of organisms indicating a significant bacteriuria in humans is 100,000 or more bacteria per ml. of urine (Brumfitt and Percival, 1964) a number of auhhors prefer to accept a much lower figure (King, 1961).

Effessøe and Jensen (1963) have suggested that in human urine, contamination during the collection procedure at the most can give a urinary colony-count of 10,000 colonies per ml. In the dog, however, due to the unco-operative nature of many patients, the presence of long hairs/

hairs in the preputial and vulval areas in many breeds and the large numbers of resident bacteria in the prepuce of many male dogs, the chances and the extent of gross contamination at the time of collection are likely to be much greater than in man. Until further work can be carried out, it seems reasonable to accept a level of 100,000 organisms per ml. in fresh urine as an indication of infection and to repeat the examination on specimens of a doubtful nature, i.e., specimens containing between 1,000 and 100,000 organisms per ml. The disadvantages of the colony counting technique are that it is time consuming and expensive, and is thus unsuited for routine use in Veterinary Practice. Simpler screening tests are required for this purpose and 2 such tests were used in this investigation. The triphenyl tetrazolium chloride test was only applied on 3 occasions because of the difficulty in interpreting the results when large amounts of blood are present in the urine as in many cases of urolithiasis. The second screening test used was the examination of a fresh smear of uncentrifuged urine after gram staining. The presence of organisms in more than one oil immersion field being taken as an indication of the presence of more than 100,000 bacteria per ml. The accuracy of this simple test was assessed by comparing the results with those of the dilution colony-counting technique. In 139 comparisons the gram smear technique was found to be accurate in 93.2%. It seems, therefore, that this procedure may well have an application in general veterinary practice since it is inexpensive, simple to perform and sufficiently accurate for clinical purposes.

Relationship/

Relationship of Infection to Urolithiasis.

Probably the most significant fact emerging from this study on the relationship of infection to urolithiasis is the obvious association between staphylococcal organisms and triple phosphate calculi. In the majority of cases of triple phosphate lithiasis staphylococcal organisms were isolated, whereas in urolithiasis due to other chemical types of calculi these organisms were only occasionally found. The difference in frequency of occurrence of staphylococci in the urine of patients with triple phosphate lithiasis and in patients with other chemical types of stones was statistically significant ($P < 0.01$). These results support the observations of Brodey (1955) who found a similar relationship in 52 cases.

Hellström in 1924 suggested that Staphylococcal infection played a predominant role in the formation of certain phosphate stones in human patients and that calculi of this type contained a framework of bacteria. This author was of the opinion that the presence of staphylococcal organisms was essential for the development of this type of stone. Brodey (1955) also observed the presence of staphylococci in some phosphate calculi of canine origin and expressed a similar opinion that they were an aetiological factor.

In the present study the fact that one animal with a urinary tract infection with staphylococcal organisms later developed phosphate calculi gave further evidence to support this theory. Similarly the decreased incidence of staphylococcal infection in cases with calculi other than triple phosphate suggests that the presence of stones alone does not lead to a urinary infection with this type of organism. Although the clinical evidence now indicates/

indicates that staphylococcal infection is a likely cause of triple phosphate stones in dogs, the proof by the experimental production of these calculi is still lacking.

In one case of triple phosphate lithiasis recorded in the present study, no bacterial infection was found even by repeated urine examinations. In two further cases infection was present but the organisms involved were not staphylococci nor were they urease-positive. Brodey (1955) recorded similar cases in his series and suggested that in these animals either the body defence mechanism had succeeded in eradicating the bacteria or that they had received prior antibacterial therapy. Whilst this may be the case, the fact remains that occasional cases of triple phosphate lithiasis occur which do not seem to be related to the presence of either staphylococcal or other urea-splitting organisms.

The relationship of infection to the other types of calculi has been shown to be statistically significantly different to that of triple phosphate lithiasis. In the 13 cases of cystine stone no infection was found. Approximately a third of the oxalate stones were in infected urine, but in half of these cases organisms other than staphylococci were isolated. These findings indicate that bacterial infection probably plays little part in the aetiology of the majority of either cystine or oxalate stones.

The position with regard to calcium phosphate stones is not clear as only four cases were encountered, one of which had staphylococci present in the urine.

No/

No bacteria were isolated from the Dalmatian dog suffering from the urate calculi. This result is of interest as the presence of urea-splitting organisms in the urine of these cases has been recently suggested as a possible aetiologic factor in the genesis of this stone type (Porter, 1963).

Although it is generally believed that the urea-splitting ability of the organisms is the major factor involved in calculus formation the actual mechanism by which this change is brought about is still unknown. The sequence of events is thought to be that the bacterial urease decomposes the urea present in the urine into ammonia, carbon dioxide and water; the presence of an increased concentration of ammonia in the urine then adversely influences the solubility of some stone-containing compounds (nobably magnesium ammonium phosphate) by two mechanisms; firstly by the increase in urinary pH and secondly by the law of mass action.

The data reported in this investigation casts some doubt on the validity of this simple concept. From the literature and from the results of the present study it appears that in urinary tract infections unassociated with calculi, proteus organisms are more commonly isolated than staphylococci, yet the reverse is true when calculi are present. Furthermore, if the urease activity was the important factor one would expect a greater incidence of Proteus than Staphylococci in calculous disorders as Proteus organisms generally contain the greater quantity of urease and thus split urea more rapidly. It seems likely, therefore, that factors other than the/

the ability to split urea are necessary for bacteria to influence calculi formation.

Many other theories regarding the action of micro-organisms on calculous formation both in man and dogs have been expounded. The more usual theories are listed below:

(a) By damaging the epithelial lining of the urinary tract, causing large quantities of cellular debris to be present in the urine which may act as a nucleus for stone formation (Krabbe, 1949).

(b) By destroying urea which may act as a protective colloid and which has been shown to influence the solubility of urates and oxalates (Medes, 1932).

(c) By possibly increasing the renal calcium excretion (Suby, 1954).

(d) By the production of high molecular-weight hyaluronic acid, which according to Butt, Seifter and Hauser (1954) and Butt (1954) has a marked precipitating and gluing action.

(e) By causing inflammation of the urinary conduit system, thus interfering with its contracting ability thereby resulting in urinary stasis and a tendency to calculus formation (von Lichtenberg,1923).

It seems possible that the method of action of Staphylococci on triple phosphate stone formation could be profitably investigated in dogs by a properly controlled experiment involving the establishment of bladder or renal infection with these organisms.

The relationship of infection to sex in cases of urolithiasis is of considerable interest in that a significantly greater number of females than males with this disease were found to have concurrent urinary infections ($P < 0.01$). Indeed all 14 females that had not received antibacterial therapy prior to sampling were infected, whereas less than 25% of the 36 males were similarly affected. These results suggest that urinary infection is the rule in bitches with urolithiasis, but is a relatively infrequent occurrence in male dogs with this disorder.

The/

The sex distribution of urinary infection is not well known in the dog. In man, however, females are much more prone to urinary infection than males (Carroll, 1963; Innes Williams, 1965). This predisposition of females to urinary infection has been related to the difference in urethral length between the two sexes (Brumfitt and Percival, 1964). This reason seems unlikely to account for the increased incidence of infection in bitches with urolithiasis as in these animals the increase is associated with only one specific bacterial genus and not with the more wider variety of organisms associated with urinary infections in the dog.

Post Treatment Bacteriology in Urolithiasis.

In the cases treated in this study the therapy was standardised to the removal of calculous material from the urinary tract and to five consecutive days of appropriate antibiotic therapy as determined by sensitivity tests. The fact that 29% of the animals tested in the immediate post-operative period still contained organisms in the urine indicated that the antibacterial therapy was not entirely effective in clearing the infection. It is of interest to note that in most of these animals no bacteria were isolated when they were again re-examined at a later date, even though no further therapy was instituted. This finding suggests that at least in some cases, once the calculi are removed from the urinary tract, the body defenses can overcome the urinary infection. However, this is not always so, as when 11 cases of initially infected urolithiasis were examined at periods of more than one month following treatment there were 27% still infected. In all these/

these cases the initial bacterial genus was not found on the later examinations. The assumption is that re-infection with a different organism occurred.

In 5 infected cases with triple phosphate calculi which were examined bacteriologically at intervals of six months or more, following treatment, 2 showed radiographic evidence of reforming calculi and in both of these animals a urinary infection was present. This finding concurs with that of Brodey (1955) who recorded that most cases of recurrent phosphate stones were infected.

With regard to oxalate and cystine lithiasis the absence of infection in recurrent cases supports the initial bacteriological findings and the opinion that infection does not seem to play an important part in the formation of these calculi.

The fact that in 15 animals with initially uninfected urolithiasis only 1 case of post-treatment infection was found, suggests that at least under the conditions in this study the introduction of infection by surgical interference in these cases is of infrequent occurrence.

2. CYSTINURIA.

Review of the Literature.

History

Cystine was first isolated from 2 human vesical calculi by Wollaston (1810) and just 13 years later its occurrence in a canine urolith was reported by Lassaigne (1823). The second report of cystine calculi in the dog was by Gross (1861) who found it in an 8 year old male Scottish Terrier. Gross recorded the case because he thought the condition was common in the dog. Until White in 1944 demonstrated the relatively frequent occurrence of the disease, most authors had considered it to be rare.

The first occasion on which cystine stone disease was diagnosed pre-operatively in the dog was in 1935 when a Biochemist, Brand, who was at that time engaged in research on cystinuria in man, diagnosed it in an Irish Terrier (Morris, Green, Dinkel and Brand, 1935). He found the urine of this dog to contain the typical transparent flat hexagonal crystals that had been described in human cystine stone disease more than a century previously by Prout and Stomeyer (1824). Since the report of Morris et al. (Loc. cit.) clinical cases of cystine stone disease in the dog have been recorded by Stephenson (1939), Sjoberg and Nilsson (1940), Pollock (1955) and Harding and Sumner (1955).

White (1944) in Liverpool recorded 19 cases of cystine stone disease in 103 cases of canine urolithiasis. The majority of these cystine stones were found in the urethral site and the author suggested that they were the commonest calculi necessitating urethrotomy in male dogs.

Pathogenesis/

Pathogenesis.

Although this condition has been known to occur in dogs for more than 100 years its aetiology has only been reasonably understood in the last decade.

Dent (1949) when examining the urine of 5 cystinuric human patients found that all excreted large amounts of 4 related amino-acids 1 of which was cystine and he postulated that a defect in amino-acid transport in the renal tubules was present in these cases. In order to substantiate this hypothesis, it was necessary to demonstrate that the blood levels of these amino-acids were either normal or low. This Dent and Rose accomplished in a further study in 1951. Dent's hypothesis assumed that the 4 amino-acids implicated had at least 1 stage in their tubular reabsorption which was common to all. If this was correct then the artificial raising of serum levels of one of these amino-acids would overload this reabsorption mechanism and cause increased excretion of the others. That this did occur was demonstrated experimentally in both normal human subjects (Beyer, Wright, Skeggs, Russo and Shaver, 1947) and dogs (Webber, Brown and Pitts, 1961). Further evidence that the defect was mainly renal-tubular in nature was documented by Dent, Senior and Walshe (1954) who found that the rate of excretion of these amino-acids by the cystinuric patient was similar to the glomerular filtration rate.

The urine and serum amino-acid pattern of a cystinuric Labrador dog was studied by Crane and Turner (1956) who found it to be virtually identical to that occurring in man. A few years later Treacher (1962) repeated this study using quantitative chromatographic techniques on 11 dogs with cystine stone disease. He found that in addition to the basic/

basic amino-aciduria an abnormally high excretion of citrulline and theonine was also present.

It is known that active amino-acid transport mechanisms occur at three sites in the body, namely:- In the renal tubules; at the cellular membrane level, and in the intestinal mucosa. Since it has been shown that identical groups of amino-acids, such as the dibasic amino-acids, Cystine, Lysine, Arginine and Ornithine share the same transport mechanisms in both the intestinal mucosa and the proximal renal tubules in experimental animals (Wiseman, 1953; Hagihira, Lin, Samiy and Wilson, 1961), it was postulated that in some conditions in which defects in renal amino-acid transport were present, similar defects may also be present in the transport systems at the other two sites.

With regard to the transport systems at the cellular membrane level, Becker and Green (1958) were unable to show competition for uptake of these amino-acids in human leucocytes from normal and cystinuric patients. This indicated that in cystinuric humans no ~~such~~ defect such as that in the renal tubules was present at the cellular membrane level. However evidence that an amino-acid transport defect is present in the intestinal mucosa in this condition was recorded in man by Milne, Asatoor, Edwards and Loughridge (1961) and in dogs by Treacher (1962). Thus, in the dog, the defect in active amino-acid transport has been shown to occur in both the intestinal mucosa and the renal tubules.

This disorder in amino-acid transport might well be harmless to the dogs were it not for the fact that of the various amino-acids excreted in excessive amounts by the kidney, one, namely cystine, is extremely insoluble and precipitates in the urine.

The/

The Nature of the Biochemical Defect.

Some evidence disputing the existence of a common pathway of amino-acid transport for cystine and the basic amino-acids was presented by Rosenberg, Downing and Segal (1962) who found, from studies on rat-kidney cortex slices that lysine, arginine and ornithine did in fact share a common transport mechanism and were competitive inhibitors of each other, but that cystine did not share this pathway. This work was repeated and confirmed on Kidney slices from control and cystinuric humans (Fox, Thier, Rosenberg, Kiser and Segal, 1964). The results of these observations led the authors to believe that cystinuric tissue demonstrated a defect in ability to transport lysine and arginine but that cystine transport was unimpaired. They further suggested that the reason for the excessive cystine excretion in the disease was unknown, but cite evidence suggesting an abnormality of cystine-cysteine interrelations in patients with cystinuria. Further studies in the same laboratory, by Thier, Fox, Segal and Rosenberg (1964) on jejunal mucosa obtained by intestinal biopsy on cystinuric human subjects indicated a defect in the intestinal transport of cystine as well as lysine in this disease. Thus the defect in the transport of these amino acids in the intestinal mucosa appears to be different from the defect in the renal tubules. The actual nature of these defects is not known but in all cystinuric human patients studied by Collini, Mattern, Shapiro, Harris and Zinsser (1965) the presence of a pyridoxine deficiency was considered by these authors to be confirmatory evidence that the defect was in the process of transamination.

Since cystinuric patients excrete large amounts of cystine and basic

amino-/

amino-acids in the urine, it was thought that the concentration of these amino-acids in the renal vein was likely to be much lower than in the renal artery. However, Frimpter (1963) found relatively little difference between the cystine levels in the renal artery and vein, although the difference in cysteine concentration was much greater. He therefore suggested that the large quantities of cystine excreted in the urine might result from abberant cysteine metabolism in the kidney. Rosenberg, Durant and Holland (1965) also found no significant difference in the renal arteriovenous concentration of cystine in both cystinuric and normal human patients. However these authors also found no significant difference in cysteine arteriovenous concentrations and on the basis of these results postulated that the faulty renal tubular reabsorption in this condition may be followed by a greater production of cystine in the parenchymal cells to maintain equal arterial and venous concentrations and that this enhanced turnover of cystine could be accomplished by acceleration of *de novo* synthesis or tissue protein catabolism.

It is obvious that the above evidence is insufficient to clearly establish that abnormal cystine - cysteine interrelationships are present in this disease and further observations on renal arteriovenous concentrations are required to elucidate this and to determine whether renal cystine production occurs.

Associated Abnormalities.

With regard to the clinical condition in dogs no signs of abnormality apart from the tendency to stone formation have been reported. This is surprising since these animals are not only losing large amounts of amino-

acids/

acids in the urine but they also have a defect in intestinal absorption of the same amino-acids. In human cystinurics a number of associated abnormalities have been recorded. These include pancreatitis (Gross, Ulrich and Maher, 1962) osteogenesis imperfecta together with mental retardation (Berry, 1959) and protein intolerance (Perheentupa and Visakorpi, 1965).

Incidence.

The percentage of the canine population that suffers from cystinuria and the number of cystinuric dogs in which calculi formation occurs is not known. In man it is thought that more than 50% of cystinurics eventually form stones (Dent and Senior, 1955). Treacher (1962) has suggested that one in every 300 dogs suffers from cystine stone disease. The condition certainly appears to be widespread in dogs, White (1966) having recorded its presence in 20 different breeds.

Cystine stone disease may affect dogs at a younger age than phosphate calculi (Treacher, 1962) but older animals are occasionally affected. In man, cystine stone formation usually occurs in early adult life and it has the youngest age incidence of the calculous disorders. In human patients with this condition survival beyond the age of forty years is not common (Zinsser, 1956). The complete lack of reports of the occurrence of the disease in animals less than one year old has not as yet been adequately explained.

Inherited Nature.

In 1935 when Morris, Green, Dinkel and Brand first diagnosed cystine stone disease pre-operatively in the dog it was realised that the condition in/

in man had a familial incidence and thus the genetics of the condition in dogs was studied by some of these authors. By adopting a breeding programme using cystinuric animals, 300 dogs were obtained and 12 were found to be cystinurics (Brand and Cahill, 1936; Brand, Cahill and Kassell, 1940). All the cystinuric dogs were found to be males and it was thought that a sex-linked recessive factor was responsible for its transmission. The defect in active amino-acid transport in this condition may therefore be present from birth.

Diagnosis.

From the literature it would appear that most clinical cases of this disease in dogs detected prior to the removal of stones are diagnosed on the presence of cystine crystals in the urine (Morris et al., 1935; Harding and Sumner, 1955, and Pollock, 1955). The majority of cases, however are diagnosed by simple clinical tests on the uroliths removed at operation. These tests include; (a) heating of the powdered stone which burns with a recognisable sulphurous odour (White, Treacher and Porter, 1961) and (b) solution of the powdered stone in ammonia which is then allowed to evaporate leaving typical cystine crystals which can be identified microscopically.

In the human condition, the cyanide-nitroprusside test of Brand, Harris and Biloon, (1930) or similar tests (Fischl, Sason and Segal, 1961) are used in the detection of a raised urinary cystine concentration. A variety of methods for the determination of the amino-aciduria associated with this condition have also been described (Vassel, 1941; Reed, 1942; Yeh,/

Yeh, Frankl, Dunn, Parker, Hughes and György, 1947; Dent and Rose, 1951; Fischl and Segal, 1963).

In dogs, the cyanide-nitroprusside test (Brand, Cahill and Kassell, 1940; Pollock, 1955) and chromatographic amino-aciduria detection techniques (Treacher, 1962) have been applied.

Natural History.

Some authors are of the opinion that cystine stone disease is characterised by recurrent episodes of urolithiasis (Bloom, 1954; Treacher, 1962). Although this seems likely, due to the persistent nature of the underlying abnormalities, there is very little recorded evidence regarding the natural history of the clinical condition. The expected interval between recurrences of cystine stone formation is not known.

Material, Methods and Results.

No previous clinical series of cystine stone disease dogs have been described in the literature. In the 55 cases of urolithiasis under discussion in this study, 13 contained cystine stones. The age, sex and breed incidence together with the incidence of the disease in the total clinic population and the position of lodgement of calculi in the urinary tract have been discussed in the previous chapter.

Clinical Features of Canine Cystine Stone Disease.

All thirteen of the cases diagnosed had urethral calculi present. Ten of/

of these animals also had calculi present in the bladder. The characteristic syndrome of urethral obstruction was thus obvious in most cases. Two cases, however, (case 47 and 54) did not show typical signs of obstruction, but were presented for investigation following periods of haematuria. All cases occurred in male dogs and all except 2 experienced the initial episode of the disease before reaching the age of 6 years.

Diagnostic Features of Canine Cystine Stone Disease.

(1) Radiographic Features.

Some authors are of the opinion that cystine calculi are radio-translucent (Archibald, 1965; Uvarov, 1956), others suggest that they are either slightly radio-opaque (Uller, 1959; Jubb and Kennedy, 1963; Schlaaff, 1962 a & b) or are characterised by a variable radio-density (Clark, 1966).

All dogs in this series were radiographed in laterial recumbency; both lateral and ventro-dorsal x-rays of the entire urinary tract being taken. In 6 of 10 cases where both vesical and urethral calculi were present the stones were clearly seen in both anatomical positions. In 2 of the 3 cases where the calculi were present only in the urethra, no calculi were seen on the radiographs; in one of these animals the calculi were lodged at the ischial arch and in the other they were within the groove of the os penis. In each case the stones appeared to be lodged in an area, the radiographic shadow of which was superimposed on bone. In one animal a bladder calculus was not observed on the plain radiographs, but/

but in a pneumocystogram of this same animal the stone was clearly seen. (Figs. 8a and b). In all except one animal multiple calculi were observed in the urinary tract. They were in general small, rounded in appearance and not strongly radio-opaque. In most animals several of the large stones were of à similar size.

Radiographic exposure of 5 isolated stones, (selected because of their similar dimensions - 4mm. diameter) from different animals did not show obvious variability in radio-density. Similarly radio-graphic exposure of an isolated stone together with a similar thickness of pure cystine, showed that whilst the pure cystine was only very faintly radio-opaque, the stone cast a much more dense shadow. (Fig. 9). A radiographic comparison of a cystine and a triple phosphate stone of a similar size showed the cystine stone to be less opaque than the phosphate.

(2) The Occurrence of Cystine Crystals in the Urine.

The examination of the urinary sediment was carried out on initial presentation in all cases. For the most part the urine was taken at operation for the removal of stones. In 11 of the 13 animals more than 1 sample was examined during the period of hospitalisation. In 5 of these 11 animals no cystine crystals were seen in any of the repeated samples. In the remaining 6, crystals were observed in some samples but not in others. In the two cases in which only one sample was taken the typical crystals were seen in both cases. (Fig. 10).

(3) The Detection of Increased Concentration of Urinary Cystine.

(a) Using the Test devised by Brand, Harris and Biloon (1930). This test was/

Fig. 8a. Plain Lateral Radiograph of a 3 year old Male Cairn Terrier with a Vesical Cystine Calculus. The Calculus is not Discernible.

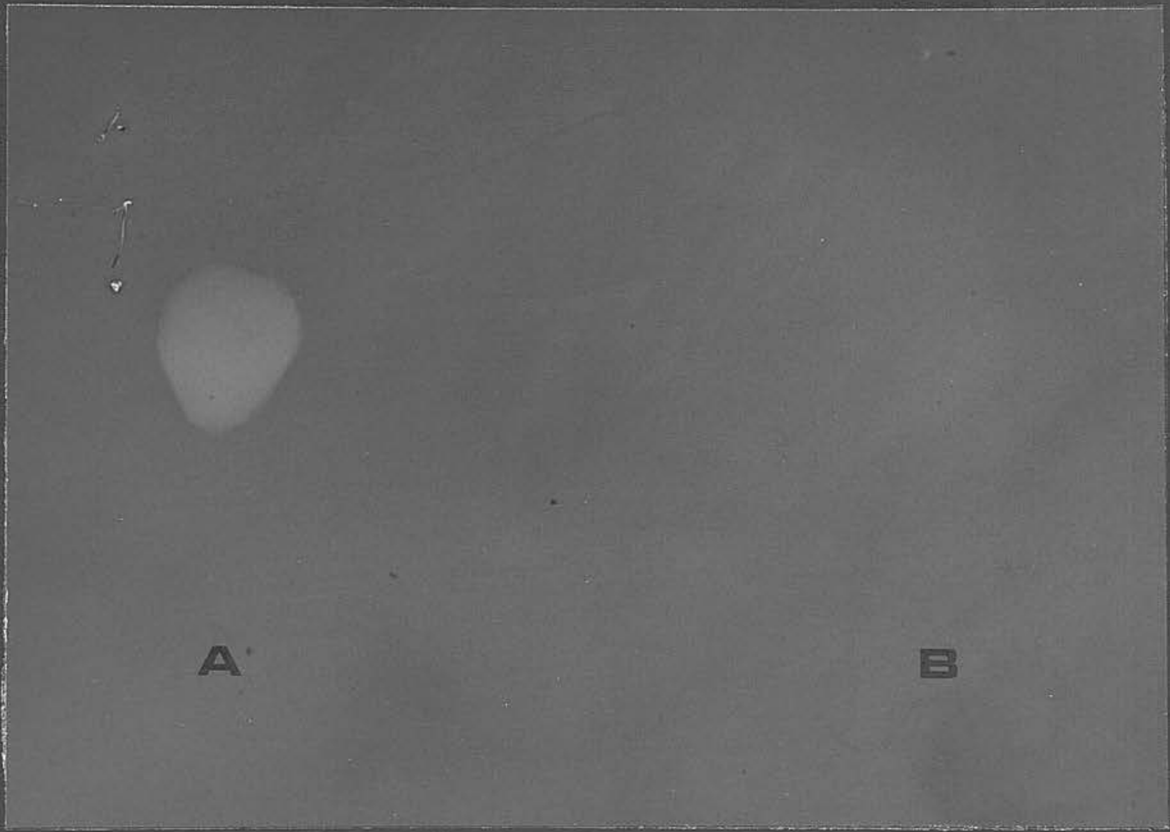
Fig. 8b. Pneumocystogram of the same Animal. A single round faintly-opaque Calculus can be seen in the Posterior Portion of the Bladder.

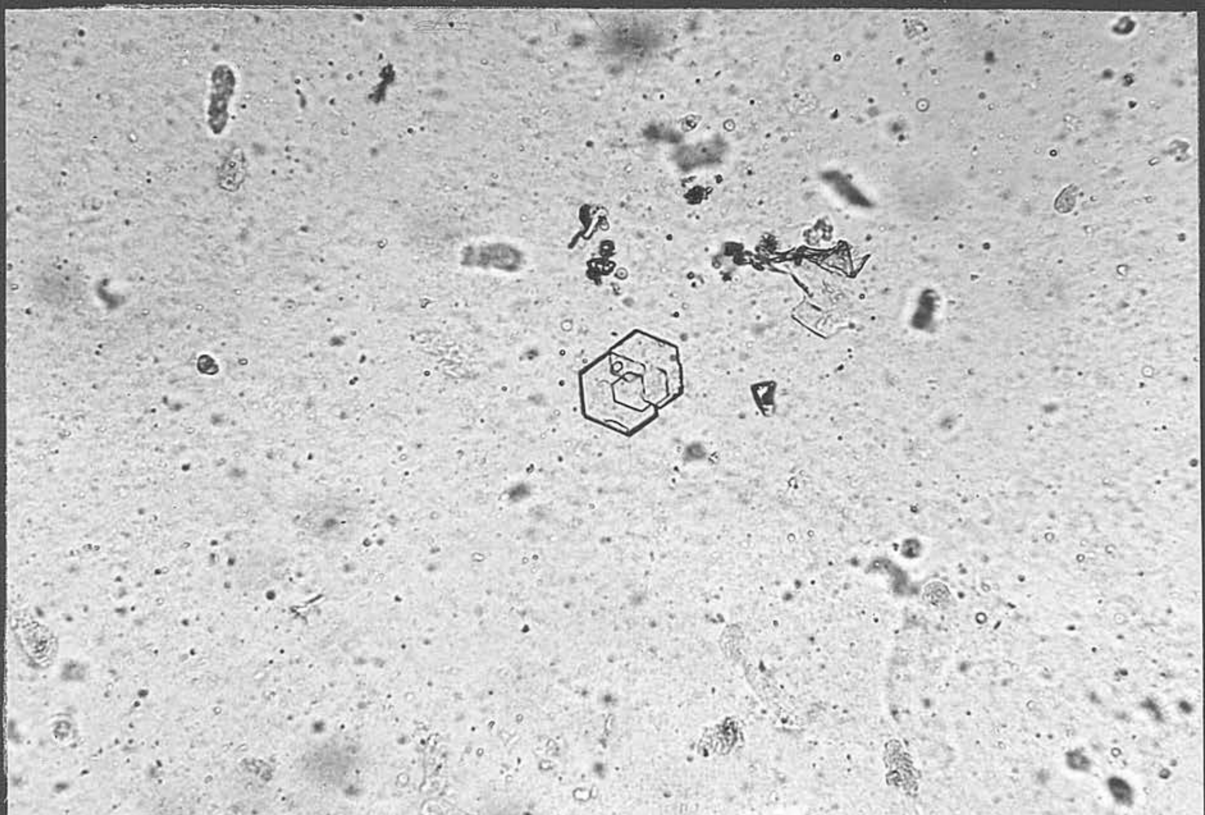
Fig. 9. Radiographic Comparison of a Cystine Stone with Pure Cystine.

A - Cystine Calculus of 5 mm. Diameter.

B - Equivalent Thickness of Pure Cystine.

Fig. 10. Typical Cystine Crystals seen in Canine Urine.


A
B



was performed in the following manner; to 3 - 5 ccs. of fresh urine, 2 ccs. of 5% ($^W/v$) aqueous sodium cyanide were added; the mixture shaken and allowed to stand for 10 minutes; Six drops of a 5% ($^W/v$) aqueous sodium nitroprusside were then added. A positive result was recorded when a magenta (pink-purple) colour developed within half-a-minute and persisted for three minutes or longer. A brown or light pink colour was regarded as negative. A control solution of urine with two ccs. of water added in place of the 5% ($^W/v$) sodium cyanide was recorded and compared with the test solution on each occasion. The nitroprusside solution was freshly prepared on the day of use. The cyanide solution was kept in a well-stoppered bottle and was replaced every few months.

Results.

The test was carried out on one urine sample in 6 animals and on more than 1 sample in the remaining 7. In the former group the test was negative in 1 case and positive in 5. In the latter group the test was positive on all samples in 5 animals and was negative on some occasions and positive in the others in the remaining 2.

(b) Using Low Voltage Paper Electrophoresis. This test, as described by Goulden and Leaver (1966) was carried out on 5 cystinuric, 12 normal and 15 dogs with various urinary disorders. In three cystinuric animals the test was performed on more than 1 sample.

Results.

The increased concentration of the basic amino-acids and cystine in this disease was obvious on the electrophoresis papers and the difference between/

between the pattern in these dogs and normal animals was unambiguous. In the cystinuric animals in which repeated examinations were performed the patterns in serial electrophoreograms were found to be almost identical. Thirteen of the 15 dogs with various other urinary disorders showed a pattern similar to that in normal dogs. The remaining 2 had patterns similar to those with cystine stone disease. In 1 of these animals a nitroprusside test of Brand et al. (1930) had been positive on two previous occasions and a history of repeated urinary disorders was noted. The Blood urea level was within normal limits (26.3 mgm.%) and the urinary sediment examination did not reveal any abnormalities. Calculi were not observed on radiographic examinations which included a pneumocystogram and an intravenous pyelogram. These findings together with the cystinuric pattern observed on electrophoresis indicated that this dog suffered from the specific defect in active transport of the basic amino-acids and cystine in the renal tubules usually associated with cystine stone formation. In the second animal in which an abnormal amino-acid pattern was discovered on electrophoretic examination of the urine, a terminal nephritis, as indicated by an excessively elevated blood urea level (more than 400 mgm.%) together with a proteinuria and glucosuria (unassociated with a raised blood sugar level) was diagnosed. In this animal an increased density of neutral amino-acids was present at the origin of the electrophoresis paper and this was considered indicative of a generalised inability in tubular reabsorption of amino-acids rather than the specific defect found in cystinuria.

(4)/

(4) Other Diagnostic Tests. The Bacteriology, Urinary pH, Blood Biochemistry, diet, physical characteristics and chemical analysis of stones in these cystinuric animals is described elsewhere in this report.

Treatment.

In these animals the treatment was standardised to operating for the removal of stones from the urinary tract and 5 days of penicillin and streptomycin therapy. No measures to prevent reformation of calculi were instituted.

Results.

Of the 12 animals on which operations were performed 6 died or were destroyed on the owners request in the immediate post-operative period (2 months); a very high percentage of failure when compared with the 2 deaths which occurred in 36 patients operated on for the removal of other chemical stone types. The application of the x^2 frequency analysis to these figures demonstrates that a statistically significant difference ($P<0.01$) is present. Therefore, in this series a significantly greater number of cystine stone disease patients died or were destroyed than occurred with other forms of urolithiasis.

Factors associated with the death of these 6 cystine stone disease cases were:-

(a) Rupture of the bladder wall immediately following urethrotomy (case 43). In this animal the rupture was probably present at the time of the operation, although a few hours prior to the urethrotomy, an x-ray had revealed an intact bladder wall.

(b)/

(b) In 2 animals disruption of the cystotomy suture line occurred consequent to obstruction of urinary outflow by small calculi inadvertently left in the bladder at cystotomy. (Cases 47 and 53).

(c) After removal of stones from the urethra by urethrotomy in 2 animals (Cases 51 and 52), post-operative obstruction by further calculi (presumably from the bladder) occurred and terminal uraemia resulted. The remaining animal (Case 50) was destroyed *in extremis* with uraemia after repeated episodes and operations for stone removal.

Cases 50, 51 and 52 had been suffering from repeated episodes of the disease; cases 43, 47 and 53 were initial episodes.

Natural History.

50% of the 12 cases in which the post-operative history was known were dead within 18 months of the initial clinical episode of this condition. The average number of clinical episodes was 2, but ranged from 1 to 5. Repeated episodes, may have been due to calculi left at the initial operation. Only 2 (17%) of the 12 cases in which the post-treatment history was known remained free of radiographically detectable calculi for two years following clinical episodes of this condition. One of these animals developed a persistent intractable proteus infection in its urinary tract, the urinary pH of this animal being above 7.4 on at least 5 occasions during this infection. In 7 cases with a history of previous episodes, or in which repeated episodes occurred during the period of observation, the interval between episodes did not in any case exceed 18 months. It seems likely, therefore, that the majority/

majority of animals that survive the initial episode of this disease may be expected to have a recurrence within 18 months.

Discussion.

From the material presented in the 13 cases described here, it seems that the typical clinical picture of canine cystine stone disease is a young 2 - 6 year old male dog with urethral obstruction, which on examination is found to be caused by multiple, smooth, round, yellow calculi. Occasionally single stones or Mulberry-like stones may occur and older dogs may be affected. Cystine Kidney stones have also been reported in this disease (Treacher, 1962). In two animals in the present series on which post mortem examinations were carried out, small macroscopic calculi were seen in the kidney parenchyma (Fig. 11). Calculi occurred usually in the urethra and bladder and were in general sufficiently radio-opaque to be observed in these positions on plain radiographs. If however they are superimposed on a bony silhouette in the radiographs they may remain undetected. Similarly in occasional cases vesicular cystine stones may not be observed unless pneumocystography is performed.

Pure cystine was found to be virtually radio-translucent and certainly seems unlikely to be detected within the animal body on plain radiography, however naturally occurring cystine calculi are considerably more radio-opaque than pure cystine. This difference may be due to the presence within the calculi of radio-opaque impurities or to the dense packing of the cystine crystals. The difficulty experienced in identifying cystine stones/

Fig. 11. Sectioned Kidney Specimen of a Dog with Cystine Stone Disease. Six Small Macroscopic Calculi can be seen in the Medullary Parenchyma near the Cortico-Medullary Junction.

stones on clinical radiographs does not seem to be due to variations in the density of the stones but rather to variations in the size of the calculi, differences in the radio-opacity of the tissue in which the calculi rest and, of course, variations in the radiographic quality of the films taken.

Little reliance can be placed on the presence of cystine crystals in the urine as a method of diagnosis. In more than 40% of the cases studied no cystine crystals were observed on examination of the urinary sediment in repeated samples. Conversely, the presence of cystine crystals in the urine sediment seems reasonable presumptive evidence that cystinuria with its associated basic amino-aciduria is present. Cystine crystals have been reported in the urine of humans in conditions other than the specific amino-acid transport defect discussed here, but these conditions which include cystinosis and the De Toni-Fanconi Syndrome have not been reported in dogs.

The test of Brand, Harris and Biloon (1930) will detect most cases of cystinuria on repeated examinations but it is not specific for cystine and it has a number of disadvantages in routine clinical use (Goulden and Leaver, 1966). A more exact diagnosis may be made by the method described by Goulden and Leaver (1966). Using this method a specific basic amino-aciduria associated with cystine stone disease can be readily detected. One adult animal with a cystinuric pattern on the electrophoretogram had no clinical evidence of cystine stone disease. This finding together with previous reports of similar cases by Blamey and Bland (1939), Pollock (1955)/

(1955) and Treacher (1964) may indicate that, as in man, not all cystinuric dogs form cystine stones. It must be remembered, however, that an apparently small proportion of cystine stone diseased animals may not show clinical signs of stone formation until late in life.

It has been shown in the present study that the mortality rate in cystine stone diseased patients on which operations were performed is higher than in patients with other chemical types of urinary calculi. This may be attributable to a number of factors, the most obvious of which is the rapid recurrence which results in an increased number of obstructive episodes and consequent kidney damage.

From the information recorded here it appears likely that most cases experience repeated episodes within 18 months. However it is difficult to affirm that these episodes are not due to failure to remove all the calculi at the initial intervention. Two cases have however, survived for over two years subsequent to the removal of the calculi and as yet no radiographic signs of stone reformation are detectable. Further studies on the natural history of cystine stone disease are certainly necessary to assess the value of different methods of preventative therapy.

3. PRIMARY HYPERPARATHYROIDISM.

(and Observations on Blood Chemistry in Cases of Urolithiasis.)

Review of the Literature.

Primary hyperparathyroidism was first described as a disease entity in man by Barr, Bulger and Dixon (1929) who grouped under this heading a series of conditions that had been hitherto described as osteomalacia, multiple cystic tumours of the bone or parathyroid tumours.

In the dog, Krook (1957) reported 3 cases of parathyroid adenoma on post-mortem and, according to him, 3 cases of parathyroid neoplasm were recorded in the literature prior to his report but the histopathology of these cases did not accord with 600 cases of parathyroid tumours which had been described in man. In the 3 cases of primary hyperparathyroidism reported in Krook's thesis, 2 had nephrocalcinosis and 1 had, in the urinary tract, a small number of calculi largely composed of calcium and phosphate with lesser amounts of magnesium, ammonium, oxalate and cystine.

The effects of parathyroid hyperfunction were produced experimentally in 31 dogs by Leberman (1940) who used intermittent subcutaneous injections of increasing amounts of parathormone over a period of 18 months. On autopsy, calcium phosphate calculi were found in the bladder of 3 dogs and 2 had developed a calcium plaque on the side of the renal papilla similar to that described by Randall(1937).

Since Barr, Bulger and Dixon (1929) first described the condition in man, several authors have considered primary hyperparathyroidism as a possible cause of urolithiasis in the dog (Milks, 1935; Bloom, 1954; Medway/

Medway, Archibald and Bishop, 1955; Hickman, 1958) but it was 1962 before Fasnacht and Maksic described the first clinical case in a one year old English Setter. The characteristic features in this case were posterior weakness and loss of reflexes and skin sensation in the hind limbs, followed by rib-fracture and a shifting front leg lameness. Radiographic examination revealed a fracture of the ulna with rarefaction of all the long bones, and the dog later broke his mandible when chewing a bone. According to the authors, the blood calcium was "slightly higher than normal". A diagnosis of osteitis fibrosa cystica disseminata (primary hyperparathyroidism) was made on the radiographic signs and blood chemistry but no post-mortem examination was recorded. It seems possible that the condition described in the above animal may have been due to some other derangement of calcium metabolism such as excessive Vitamin D intake or hypersensitivity to Vitamin D.

In 1965 Pearson, Dellman, Berrier, Case and Collier reported the first successful surgical treatment of this condition in the dog. They reported a case of primary hyperparathyroidism in a 3 year old spayed female beagle. The clinical syndrome in this animal was characterised by severe pain in the cervical region and sporadic lameness of the left forelimbs. Radiographic findings indicated a generalised decalcification of bone. Areas of rarefaction surrounding the teeth and radio-opaque material in soft tissue areas were found. Laboratory tests revealed a blood calcium level of 27 mgm.% and a phosphorus level of 5 mgm.% Alkaline phosphatase level was 12 Bodansky units; no abnormalities were found in an examination of the urine. On surgical exploration

of the/

the neck, the left 4th parathyroid gland was found to be enlarged, and was removed. Histological finding did not reveal the presence of neoplastic tissue, but a 72.4% increase in active (clear chief) cells in comparison to normal dogs was recorded.

In neither the case recorded by Fasnacht and Maksic (1962) nor that recorded by Pearson et al. (1965) were urinary calculi reported.

In primary hyperparathyroidism in humans, the following pathological entities have been found:- single adenoma; double adenoma; carcinoma; primary water-clear cell hyperplasia and hypertrophy and primary chief-cell hyperplasia (Clark and Nordin, 1965).

Method of Detection.

In order to detect the presence of gross metabolic disorders, and more particularly, parathyroid dysfunction in cases of urolithiasis, blood calcium estimations were carried out in 38 of the 55 cases. In most of these animals, serum inorganic phosphorus (36 cases) and serum magnesium (33 cases) were also noted. Since primary hyperparathyroidism is commonly associated with kidney dysfunction in humans, the blood urea level was also determined (47 cases). Since generalised bony abnormalities in adult humans were also frequently associated with this condition, adult dogs with generalised bone disease were examined radiographically for the presence of urinary calculi and if present, the foregoing blood chemical determinations were made.

Samples for blood urea estimations were taken immediately on initial presentation whilst samples for calcium, magnesium and inorganic phosphorus were/

were taken at operation. The blood urea samples were collected from the cephalic vein into disposable polythene syringes. Immediately on removal, the blood was transferred to disposable plastic bottles containing potassium oxalate*. Estimation on these samples were completed during the following day. Samples for calcium, inorganic phosphorus and magnesium estimations were usually collected from the jugular vein. The area over the vein being suitably prepared and the blood collected into "Vactuainer" syringes**.

Blood urea levels were determined using the urease Nesslerization method (Varley, 1962); Calcium estimations were carried out by the method of Clark and Collip (1925); Inorganic Phosphorus by the method of Fiske and Subbarow (1925) using Gomori's (1942) modification and Magnesium by the method described by Moodie and Walker(1963).

Results.

Two cases of primary hyperparathyroidism were detected, and the results of the biochemical tests employed are shown in Table 31.

1. Serum Calcium Determinations.

Only one animal (Case 41) had a level above 12.7 mgm.% and this dog was found to be suffering from primary hyperparathyroidism. In the second case of this disorder, (Case 40) serum calcium estimation was not completed, but the addition of ammonium oxalate to a serum sample/

* Stayne Laboratories Ltd.
** Bectin Dickinson Ltd.

TABLE 31

TABULATED RESULTS OF BLOOD BIOCHEMISTRY IN CASES OF UROLITHIASIS

Case No.	Blood urea mgm.%	Calcium mgm.%	Inorganic Phosphorus mgm.%	Magnesium mgm.%	Chief Chemical Constituent
1	39.3				Triple Phosphate
2	45.9	10.7	6.3	2.2	
3	80.2				
4	24.3	10.3		3.9	
5	39.7	11.8	4.2	2.7	
6	36.2	9.9	4.9	4.3	
7	35.1	9.1	12.2	2.3	
8	36.2	11.8	3.5		
9	21.6	8.9	4.0	2.1	
10	46.6	11.0	6.9	4.5	
11	29.3	9.8	5.7	3.4	
12	31.9	10.0		3.6	
13	27.7	10.0	5.6	2.6	
14	30.6	10.2	3.7	1.8	
15	23.5	12.2	3.9	3.2	
16	-	-	-	-	
17	11.7	11.2	5.6	3.4	
18	-	-	-	-	
19	29.2				
20	33.3				Calcium Oxalate
21	29.2	11.5	6.9	4.1	
22	32.6				
23					
24	15.3	12.1	3.0	4.4	
25		11.4	2.2	3.7	
26	55.7	10.4	5.2	3.9	
27		10.4	3.0	4.2	
28		11.2	4.6	3.6	

TABULATED RESULTS OF BLOOD BIOCHEMISTRY IN CASES OF UROLITHIASIS (contd)

Case No.	Blood urea mgm.%	Calcium mgm.%	Inorganic Phosphorus mgm.%	Magnesium mgm.%	Chief Chemical Constituent
29	33.3	12.7	5.6	4.1	Calcium Oxalate
30	25.0	10.8	6.8	3.1	
31	28.6	10.7	3.4		
32	29.2	10.7	4.0		
33	47.2	11.5	4.7		
34	42.3				
35	25.7	9.0	2.9		
36					
37	42.1	11.0	3.4	2.4	
38	35.1	11.9	2.4	2.7	Calcium Phosphate
39	44.6	9.5	3.2	1.9	
40	172.0				
41	150.2	14.2	3.9	3.6	
42	18.9				Cystine
43	62.9				
44					
45	38.9				
46	27.1	11.4	6.2	3.2	
47	22.2				
48	82.9	10.4	2.9	2.7	
49	18.1	10.6	3.5	4.1	
50	25.6				
51	184.2	9.0	11.3	3.8	
52	35.1	12.7	18.6	5.1	
53	13.9	9.2	4.1	2.5	
54	16.5	10.7	5.1	2.1	
55	433.3	9.9	11.0	3.3	Urate

sample of this animal caused a heavy deposit indicative of a raised calcium concentration. Unfortunately a laboratory accident prevented the completion of this test.

2. Serum Inorganic Phosphorus Determinations.

A wide variation in the serum inorganic phosphorus levels (2.4 mgm.% to 18.6 mgm.%) was found; three adult animals and one 18 week old puppy having levels above 8 mgm.%.

3. Serum Magnesium Determinations.

A variation in Magnesium levels ranging from 1.8 - 5.1 mgm.% was found.

4. Blood Urea Determinations.

Of the 47 cases examined on initial presentation, 14 (30%) had levels of more than 40 mgm.%, the accepted upper limit of normality in dogs (Hoe and O'Shea, 1965).

From the results recorded in the above estimations, the relationship between the serum concentration of each of these substances and the chemically distinct types of stones were assessed.

(a) Relationship Between Serum Calcium Levels and the Chemical Stone Types.

Only a small variation between the mean serum calcium level of each chemical stone types (with the exception of urates) was observed. (Table 32).

TABLE 32

RELATIONSHIP BETWEEN SERUM CALCIUM LEVELS AND THE CHEMICAL STONE TYPES

Chief Constituent of Stone	No.of Cases	Variation in Serum Calcium levels, Mgm.%	Mean Serum Calcium Levels, Mgm.%
Triple Phosphate	14	8.9 -12.2	10.49
Calcium Oxalate	13	9.0 - 12.7	11.03
Calcium Phosphate	3	9.5 - 14.2	11.87
Cystine	7	9.0 - 12.7	10.57
Urate	1	9.9	

When an analysis of variance was applied to these findings (with the exception of the urate case) no statistically significant difference between the serum calcium levels in each stone type was found.

(b) Relationship Between Serum Inorganic Phosphorus Levels and the Chemical Stone Types.

The relationship between serum inorganic phosphorus levels and the chemical types of stones is shown in Table 33.

TABLE 33

RELATIONSHIP BETWEEN SERUM INORGANIC PHOSPHORUS LEVELS AND THE CHEMICAL STONE TYPES

Chief Constituent of Stone	No.of Cases	Variation in serum inorganic Phosphorus levels Mgm.%	Mean serum Inorganic Phosphorus level. mgm.%
Triple Phosphate	12	3.5 - 12.2	5.54
Calcium Oxalate	13	3.0 - 6.9	4.28
Calcium Phosphate	3	2.4.- 3.9	3.17
Cystine	7	2.9 - 18.6	7.39
Urate	1	11.0	

The/

The difference in serum inorganic phosphorus levels between the types of stones (with the exception of urates) were found to be not statistically significant when analysed by the analysis of variance.

(c) Relationship Between Serum Magnesium Levels and the Chemical Stone Types.

The extent of the variations of the mean serum magnesium concentration in the different chemical stone types was less than 1 mgm.%

TABLE 34

RELATIONSHIP BETWEEN SERUM MAGNESIUM LEVELS AND THE CHEMICAL STONE TYPES

Chief Constituent of stone	No.of Cases	Variation in serum Magnesium level. Mgm.%	Mean Serum Magnesium level Mgm%
Triple Phosphate	13	1.8 - 4.5	3.08
Calcium Oxalate	9	2.4 - 4.4	3.72
Calcium Phosphate	3	1.9 - 3.6	2.73
Cystine	7	2.1 - 5.1	3.35
Urate	1	3.3	

Statistical analysis (analysis of variance) revealed that the difference in serum magnesium levels between the different chemical stone types (with the exception of urates) was not statistically significant.

(d) Relationship between Blood Urea Levels and the Chemical Stone Types

The relationship between the blood urea levels and the chemical types/

types of stone is shown in Table 35.

TABLE 35

RELATIONSHIP BETWEEN BLOOD UREA LEVELS AND THE CHEMICAL STONE TYPES

Chief Constituent of stone	No.of Cases	Variations in B.U. levels mgm.%	Mean B.U. Levels mgm.%	No.of Cases with B.U. Levels above 40 mgm.%	% Cases With B.U. Levels above 40 m.g.m.%
Triple Phosphate	17	11.7 - 80.2	34.6	3	18
Calcium Oxalate	13	15.3 - 55.7	33.8	4	31
Calcium Phosphate	4	35.1 - 172.0	100.5	3	75
Cystine	12	13.9 - 184.2	45.5	3	25
Urate	1	433.3	-	1	-

Statistical analysis (analysis of variance) applied to the Blood Urea levels in the above table (with the exception of urates) revealed that no statistically significant difference in the blood urea levels of each type of stone occurred. The high mean blood urea level occurring in cases of calcium phosphate stones being offset by the small number of animals present in this group. Similar analysis of the difference between blood urea levels in calcium phosphate patients compared with that occurring in all other types of stones, however, did reveal a statistically significant difference ($P < 0.01$).

Observations on the 2 cases of Primary Hyperparathyroidism.

Case 1.

Subject: Nine year old male Keeshond. (case 40)

History: This animal showed evidence of lumbar pain for a few days when 5 years old (January 1961). Since that time occasional short intermittent bouts of illness, characterised by pain over the lumbar region, were noticed; the/

the penultimate episode occurring in July 1963. In September 1965 he was presented at the Clinic with diffuse symmetrical bilateral swellings of the upper jaw and nose, which, according to the owner, had suddenly appeared 6 weeks previously; polydipsia had been noticed over a period of several years, and during the few days prior to admission he had refused to eat solid food.

Clinical Examination: The animal was depressed and reluctant to move; the mucous membranes exhibited a muddy appearance and the breath had a slightly unpleasant ammoniacal odour. Bilaterally symmetrical diffuse firm swellings extending the entire length of the maxillae were present. The teeth were moveable in their alvoeli when digital pressure was applied to their crowns. The lower jaw was flexible and had a rubbery texture. Pain could be elicited by deep palpation over the kidneys.

Radiographic Examination: Ventro-dorsal and lateral radiographs of the skull; cervical region; abdomen; and the phalangeal region of the right forelimb together with a micturating urogram were taken on the live animal. Further radiographs of the isolated entire urinary tract and separate radiographs of the kidney were taken at autopsy. The significant findings were as follows:-

(a) Skull - Radiographic evidence of generalised decalcification of the entire skull together with mottling of the cranial vault; obvious areas of increased decalcification around the apicies of some of the teeth were especially noticeable in the lower jaw. (Fig. 12a and b).

(b) Cervical Region - Increased density of the tracheal rings was observed.

No/

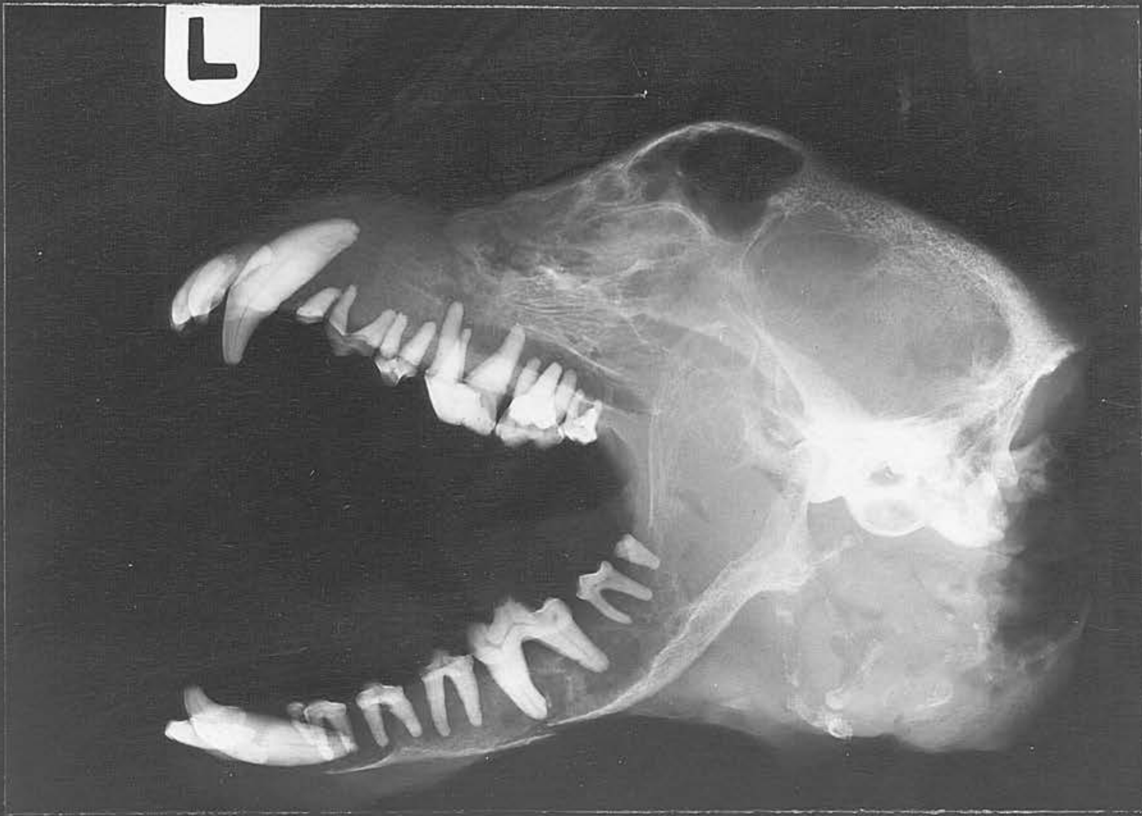
Fig. 12a. Lateral Radiograph of the Skull in Primary Hyperparathyroidism. (Keeshond). A Sectioned Skull showing:-

(a) Generalised Decalcification of Bones of the Skull.

(b) Increased Mottling of the Parietal Bones.

(c) Areas of increased Decalcification around the Apicies of the Teeth. Especially obvious around the Carnassial Tooth in the Lower Jaw.

Fig. 12b. Dorso-Ventral Radiograph of the Skull in the same Animal. Showing obvious Decalcification of the Anterior Portion of the Mandibles and an Area of Increased Decalcification around the Teeth.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No abnormalities were seen in the area occupied by the parathyroid glands.

(c) Abdomen - Radio-dense shadows could be seen in the area of both kidney pelvises. (Fig. 13a).

(d) Phalangeal area of right forelimb - No radiographic abnormalities were obvious.

(e) Micturating Urogram - Prostatic enlargement was observed.

(f) Isolated specimen of the entire urinary tract - Radio-dense areas were seen only in the region of the kidney pelvises.

(g) Isolated specimen of kidneys - Bilateral renal calculi were seen but no evidence of nephrocalcinosis was obvious (Fig. 14a).

Clinical Laboratory Examinations

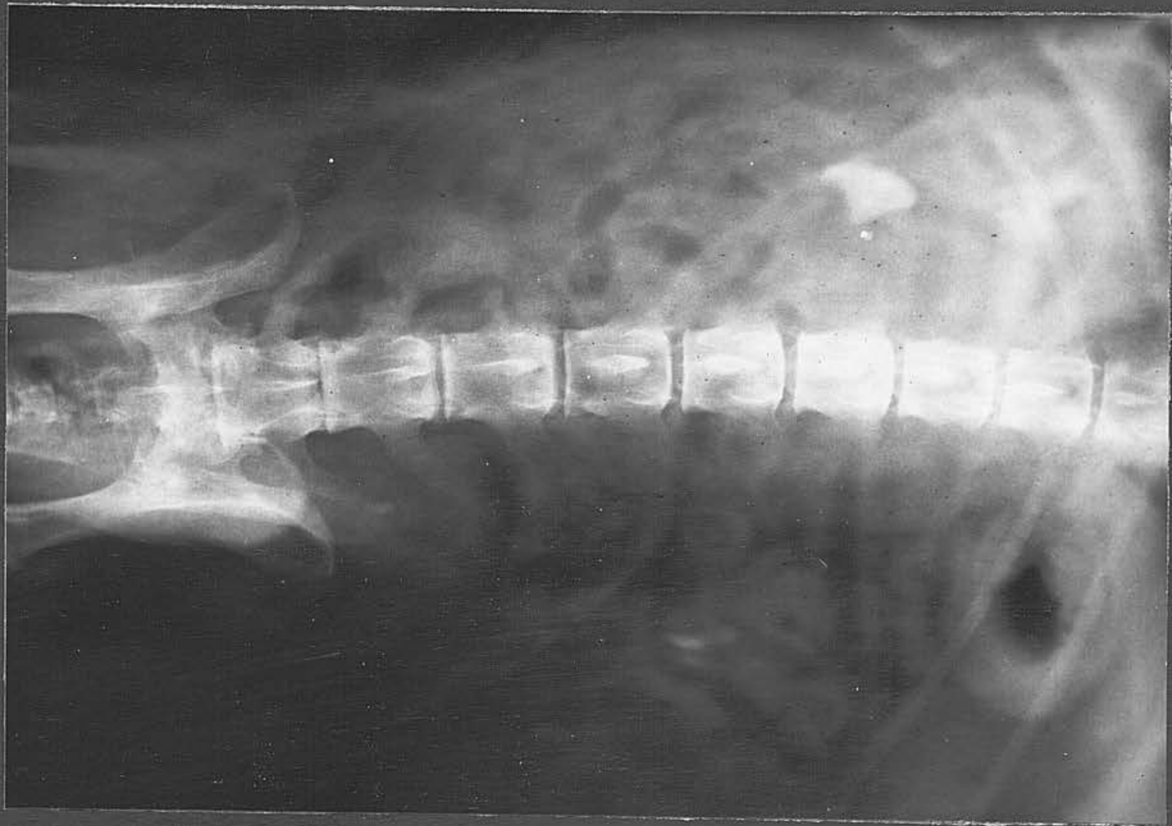
From the live animal blood samples were taken for haemocytology and blood chemical examinations and urine samples were taken for urinalysis, urinary sediment examination and for bacteriological examination.

Haemocytology - The total red blood cell count (4.68×10^6 per c.m.m.) was slightly lower than normal; the erythrocyte sedimentation rate (1 mm. per hour) packed cell volume (36%), haemoglobin (13.8 grams. per 100 ml.), total white cell count (7,500 per c.m.m.) and differential white cell count (lobulated neutrophils 6,000/c.m.m.; eosinophils 75/c.m.m.; Lymphocytes 1,350/c.m.m. and monocytes 75/c.m.m.) all being within the normal limits described by Baer, Peck and McKinney (1957).

Blood Chemistry - The blood urea level (172.0 mgm.%) was found to be considerably higher than normal. As previously stated a laboratory accident prevented the completion of the serum calcium estimation, but the presence of an extremely heavy deposit after the addition of ammonium oxalate to a serum sample from this animal was indicative of/

Fig. 13a. Ventro-Dorsal abdominal Radiograph of a Keeshond with Primary Hyperparathyroidism. Showing Bilateral Renal Lithiasis.

Fig. 13b. Lateral Radiograph of the Abdomen of a Springer-Spaniel with Primary Hyperparathyroidism. Showing Small Calculi in the Area of both Kidneys.



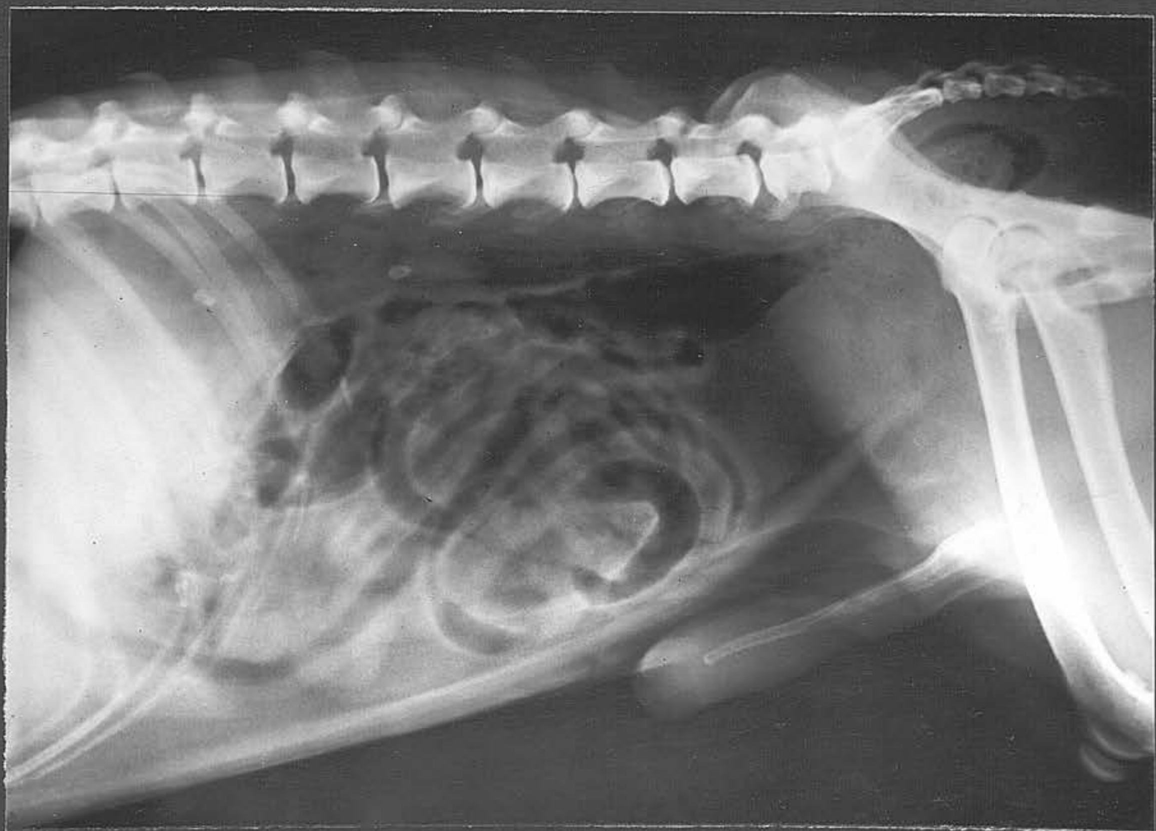
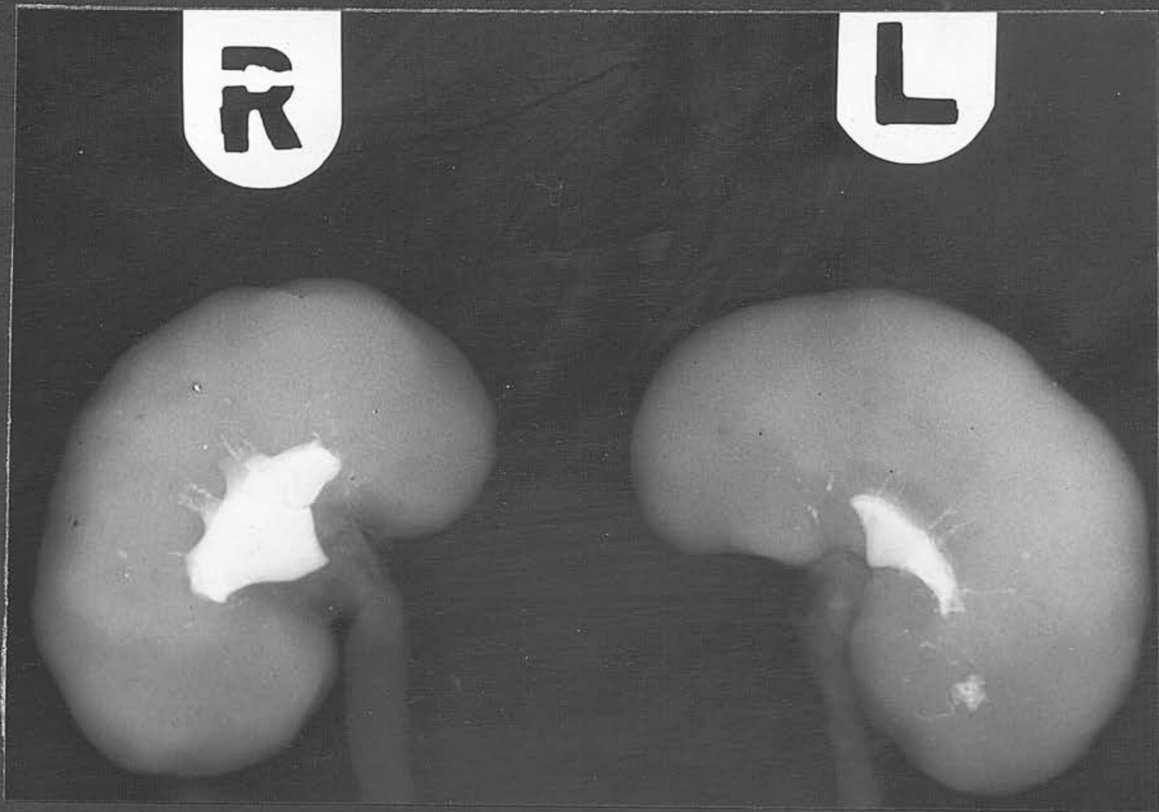


Fig. 14a. Radiograph of Kidney Specimen in a Keeshond with Primary Hyperparathyroidism. Showing large Calculi present in the Area of the Kidney Pelvises.

Fig. 14b. Radiograph of Kidney Specimen in a Springer-Spaniel with Primary Hyperparathyroidism. Showing Small Discrete Calculi present in the Renal Pelvises.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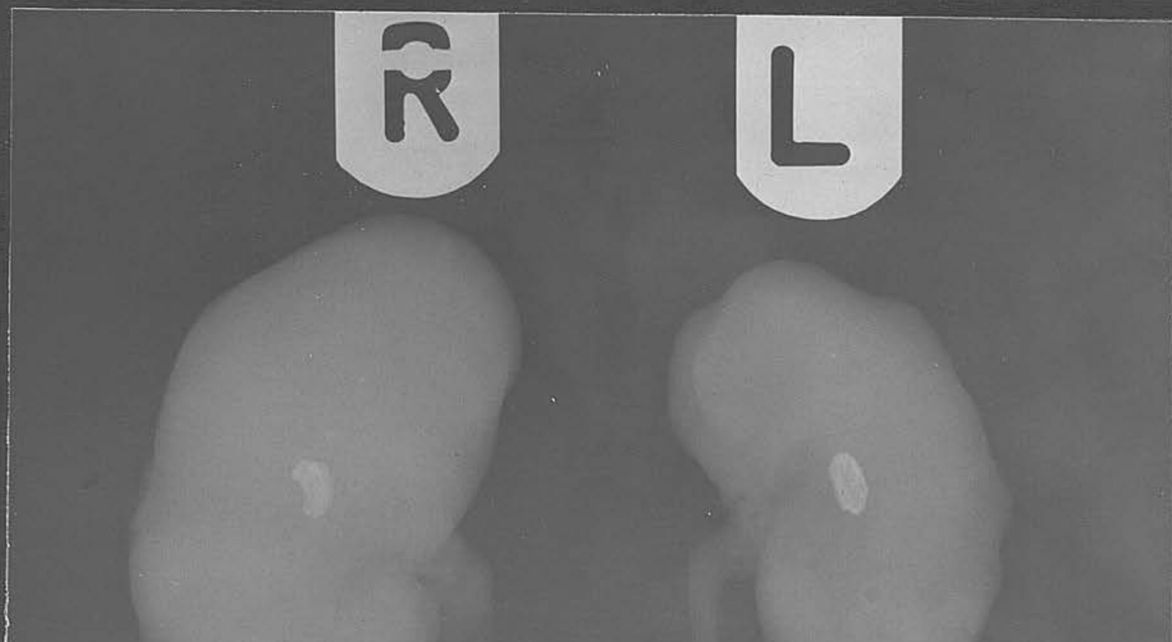
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of a raised calcium concentration. The serum inorganic phosphorus (3.44 mgm.%), sodium (144.5 mEq/litre) and potassium (4.3 mEq/litre) were all within the normal limits described by Baer et al (1957).

Urine Examinations.

(a) Urinalysis - a low specific gravity (1.010) and a high pH (7.9) were noted. Blood and protein were detected using Haemacombistix*. An extremely cloudy deposit was observed in the Sulkowich's test indicating a raised urine calcium concentration. No sugar, Ketones or cystine were detected.

(b) Bacteriology - No organisms were isolated in the colony counting quantitative bacteriological examination of the urine.

(c) Urinary sediment - After centrifuging 10 c.c. of urine for 5 minutes at 1500 revs./min. in an M.S.E. minor centrifuge (maximum diameter $17\frac{1}{2}$ inches) o.5 ml. of white cellular deposit was found together with a clear supernatent. This deposit proved to be composed almost entirely of leucocytes (>100/H.P.F.) with some red blood cells, epithelial cells and some incompletely formed triple phosphate cyrstals also present. No casts or bacteria were seen.

Diagnosis: The concomitant occurrence of bilateral nephrolithiasis and generalised decalcification of the bones of the skull, together with a suspected rise in serum calcium concentration suggested the presence of the primary rather than the secondary form of hyper-parathyroidism.

Euthanasia was requested by the owner of this animal and a post mortem examination was performed.

Post/

* Ames Company

Post Mortem Findings.

(a) Morbid Anatomy

The animal was in apparently good condition. The swellings of the maxillae and looseness of the teeth previously described were confirmed.

Parathyroid Glands - The parathyroid glands on the left side were small, the largest being approximately 3 mm. x 2 mm. x 3 mm. One parathyroid gland on the lateral surface of the right thyroid was obviously enlarged 15 mm. x 11 mm. x 10 mm. Fig. 15a.

Kidneys - The surface of both kidneys was nodular and pitted. The left kidney was very slightly larger than the right but both organs were of approximately normal size. The capsules were removed with difficulty from the kidney surface and the cut surface of the kidneys was mottled with paler areas and whitish pinpoint spots overall. The pelvis of each kidney was slightly dilated and contained approximately 5 cc. of pus. In the pelvis of the right kidney was one large (21 mm. x 18 mm. x 10 mm.) and 3 small calculi which together almost filled the pelvis (Fig. 16a). The calculi were yellowish in colour with a smooth surface and irregular shape. One large calculus (20 mm. x 10 mm. x 5 mm.) was present in the pelvis of the left kidney; some small calculous material was also present. Occasional macroscopic crystals shaped like large oxalate crystals and yellowish in colour were also present. One whitish hard fibrotic area (10 mm. x 2 mm.) was present in the left kidney, and it extended from the pelvis to the cortico-medullary junction. (Fig. 16a).

Ureters - Both ureters appeared to be slightly dilated.

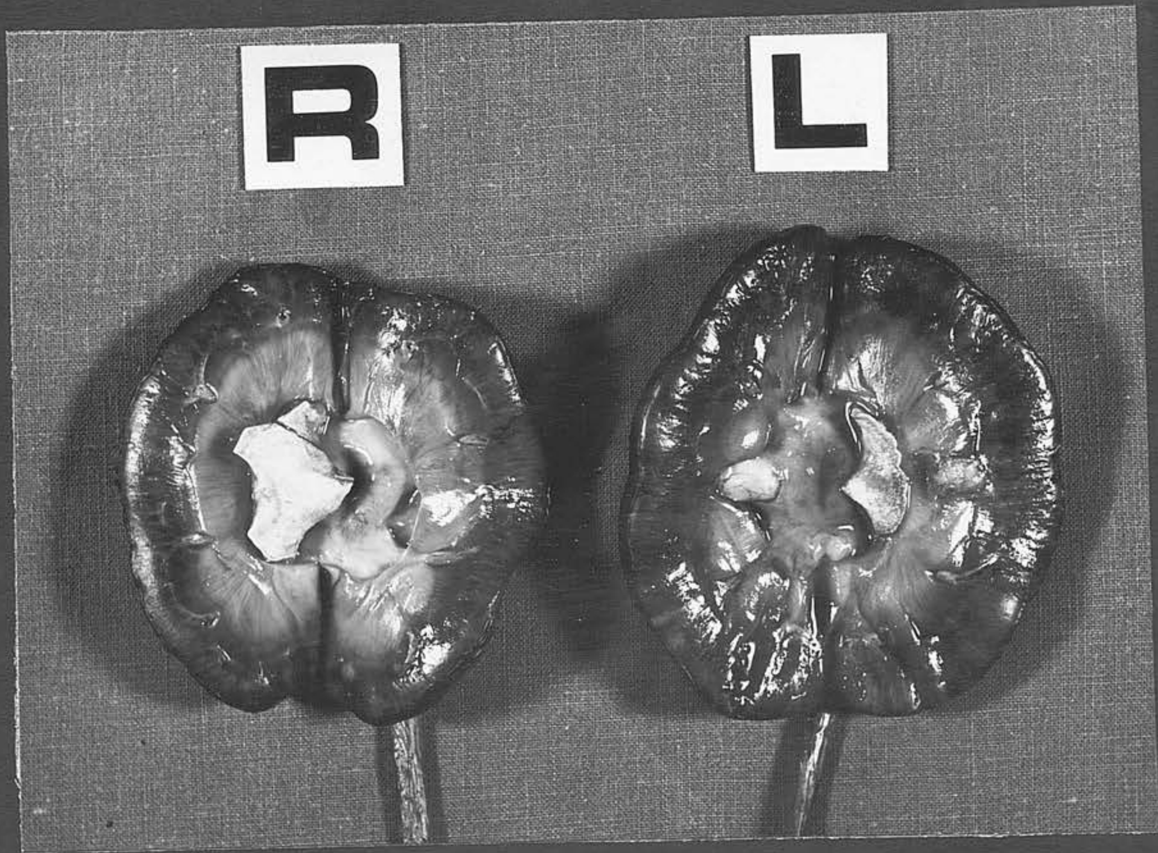
Bladder/

Fig. 15a. Enlarged Parathyroid Gland in a Keeshond with Primary Hyperparathyroidism. An extremely Enlarged Single Parathyroid Gland can be seen on the Lateral Surface of the Right Thyroid.

Fig. 15b. Enlarged Parathyroid Glands in a Springer-Spaniel with Primary Hyperparathyroidism. One enlarged parathyroid gland can be seen on the Surface of the Right Thyroid (lying on the under surface of the Trachea in this Photograph). Another can be seen on the Lateral Border of the Oesophagus as it passes Anteriorly under the Trachea.

Fig. 16a. Sectioned Kidney Specimens of a Keeshond with Primary Hyperparathyroidism. Showing Calculi Present in each Pelvis. Medullary fibrosis is obvious in the left Kidney.

Fig. 16b. Sectioned Kidney Specimens of a Springer Spaniel with Primary Hyperparathyroidism. Showing Bilateral Nephrolithiasis. A calcified plaque similar to that described by Randall (1937) can be seen below the urolith present in the Right Kidney pelvis.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Bladder - The bladder contained approximately 100 cc. of dark brown urine. The serosal surface was apparently normal. The mucosal surface contained many raised, very dark red, smooth nodular projections of varying sizes up to 15 mm. in length, which were apparently organising blood clots. In one area (5 cm. x 4 cm.) on the mucosa in the fundus of the bladder, sand-like particles of yellowish crystaline material were observed.

Prostate - The prostate was soft and approximately twice the normal size (6 cm. x 5½ cm. x 4 cm.)

The adrenal glands, heart, lungs, liver, intestinal tract, pancreas, spleen, genitalia, bone marrow and pituitary gland were macroscopically normal.

(b) Histopathology.

The majority of the tissues taken for histopatholgical investigation from this animal were destroyed by fire in the Pathology Department in November 1965. Kidney and Prostate samples, however, were salvaged.

Kidney - Focal wedges of interstitial fibrosis were present in the cortex. Some of the glomeruli were shrunken and showed hyaline fibrosis whilst others exhibited earlier changes with peri-glomerular fibrosis and eosinophilic protein casts in Bowman's Capsules. In the areas of fibrosis there were a few foci of plasma cells, lymphocytes, and some atrophic tubules. Other tubules were cystic and contained granular casts and desquamated epithelial cells. Similar but less obvious changes were present in other areas of the kidney where the tubules contained more casts and showed some pigmentation of their cytoplasm.

Prostate - A moderate degree of cystic hyperplasia with occasional foci/

foci of lymphocytes and a few plasma cells in the interlobular septi were observed.

Analysis of Calculi:

The renal stones were analysed by quantitative micro-chemical, X-ray diffraction and optical crystallographic techniques.

The result of the quantitative micro-chemical analysis was as follows:-

Calcium 25.64 gms.%; Phosphorus 14.94 gms.%; Magnesium 1.89 gms.%; Ammonia 2.44 gms.%; Oxalate 17.90 gms.%. These results indicate that the chief chemical constituent is a calcium phosphate compound and that significant proportions of calcium oxalate and magnesium ammonium phosphate were also present.

Xray diffraction analysis showed that this phosphate compound was hydroxylapatite ($Ca_5(PO_4)_3(OH)$). Minor amounts of Whewellite ($Ca\ C_2O_4H_2O$) and Struvite ($Mg\ NH_4PO_4, 6H_2O$) were also detected. Optical crystallographic findings confirmed that the crystals present were those detected by X-ray diffraction.

Case 2.

Subject - Eight year old male Springer-Spaniel. (case 41)

History: When 6 years old the animal was presented to the Clinic with pedal dermatitis and at this time was observed to have a Kyphotic appearance. One year later he was again brought to the Clinic, this time with a history of vomiting and diarrhoea for 10 days and urinary incontinence which had been noticed over a few days.

Clinical/

Clinical examination revealed bilateral retinal atrophy and a blood urea concentration of 125 mgm%. The animal was admitted to the hospital and blood and urine samples were examined. The significant changes in the blood were an increased sedimentation rate (38 mm. per hour) and a decreased total red blood cell count (3.54×10^6/c.m.m.). The P.C.V. was found to be 32% and the haemoglobin concentration 12.5 mgm%. The specific gravity of the urine was low (1.012) and the reaction alkaline. Some protein and blood were present. An examination of the urinary sediment revealed numerous leucocytes and epithelial cells together with a few red blood cells and finely and coarsely granular casts. Bacteriological examination of the urine at this time failed to reveal the presence of organisms.

At this stage a tentative diagnosis of nephritis was made and the animal treated with penicillin and streptomycin. Over the following 10 days blood urea estimations were recorded on 6 occasions; the level gradually increasing from 130.0 mgm.% to 225.0 mgm.%. During this time no significant changes from the original samples occurred in the blood and urine examined. The dog's general condition however improved clinically over this period and he was eating well. No vomiting or diarrhoea occurred but polydipsia was still evident. The animal was then examined at monthly intervals for four months during which time its general condition was maintained, although polydipsia was still obvious and the blood urea concentration settled at a level between 120-127 mgm.%.

Five months later the animal was again presented. Repeated vomiting and loss of condition had been noticed together with polydipsia and/

and difficulty in chewing biscuits.

Clinical Examination.

The animal appeared bright and lively. No changes in the temperature, heart rate, pulse or respiratory rate were obvious. Pressure applied to the crown of the teeth caused some movement in the tooth socket. Hyperparathyroidism was suspected but no palpable abnormality could be found in the region of the parathyroid glands. No pain could be elicited by palpation over the kidneys.

Radiographic Examination.

A s in the previous case ventro-dorsal and lateral radiographs of the skull; cervical region; abdomen; and the phalangeal region of the right forelimb were performed on the live animal. Later at a post-mortem examination further radiographs of the isolated entire urinary tract and separate radiographs of the kidneys were taken (Figs. 13b, 14b).

The findings were closely comparable to those recorded in Case 1.

Clinical Laboratory Examinations.

(a) Haemocytology - A marked anaemia was found in this animal. The packed cell volume was 17%; Haemoglobin concentration 5.8 gms/ 100 ml.; and total red blood cell count 1.67×10^6 c.m.m. The erythrocyte sedimentation rate was 69 mm/hour. No obvious changes were present in the total white cell count (5,550/c.m.m.) or the differential/

differential white cell count (neutrophils 4,440/c.m.m.; eosinophils 222/c.m.m.; lymphocytes 721/c.m.m.; monocytes 117/c.m.m.)

(b) Blood Chemistry - The blood urea level (221.0 mgm.%) was still very high. The serum calcium level was also raised (14.2 mgm%) as was the inorganic phosphorus level (9.2 mgm.%). The presence of a metabolic acidosis was indicated by a raised bicarbonate ion (19.0 m.mols./litre), but the lowered PCO_2 (32 mm.Hg.) present in the same animal was suggestive of respiratory compensation which was apparently maintaining the blood pH (7.412) within the normal range. A slightly lowered sodium ion concentration (130 mEq/litre) and total protein (5.45 gms.%) were recorded. The potassium (4.3 mEq/litre) and alkaline phosphatase activity (6.25 K.A. unit/100 ml) were within normal limits. The serum creatinine was estimated at 4.3 mgm.%. A repeat serum calcium examination was carried out and the calcium concentration was again found to be 14.2 mgm.%.

Urine examinations.

(a) Urinalysis - As in case 1, a low specific gravity (1.010) and high pH (7.5) was recorded. Some protein was present but no blood, cystine, sugar or ketones were detected. Only a slight cloudiness resulted on the addition of Sulkowich's reagent to the urine.

(b) Bacteriology - No organisms were isolated.

(c) Urinary Sediment - After centrifuging 10 cc. of urine as described in Case 1, 0.05 ml. of white cellular deposit was again found in a clear supernatant. The main constituent of the deposit was leucocytes (>100/H.P.F.), numerous epithelial cells, a few finely granular casts and occasional triple phosphate crystals were also observed. No bacteria or red blood cells were seen.

(d)/

(d) Urinary Chemistry - Low voltage electrophoresis examination for the detection of cystine and the basic amino acids (Goulden and Leaver, 1966) was negative. Urine calcium level was 16 mgm.%, Creatinine 57.1 mgm.% and Inorganic phosphorus 68.7 mgm.%.

The blood and urine samples for the estimations of calcium, inorganic phosphorus and creatinine in this dog were collected at the same time, so that the screening procedure recommended by Clark and Nordin (1965) for the detection of primary hyperparathyroidism in humans could be performed. This procedure involves the calculation of the phosphate excretion index (P.E.I.) using the following formula -

$$C_p/C_{cr.} = \frac{U_p \times P_{cr.}}{P_p \times U_{cr.}}$$

where C = clearance rate; U = Urine; P = Plasma; p = inorganic phosphorus concentration and cr. = creatinine concentration. The normal phosphate excretion index for dogs is not known, but in humans is $\pm$ 0.09. High values are found in primary and secondary hyperparathyroidism and low values in parathyroid insufficiency. The P.E.I. in this animal was found to be 0.56.

An electrocardiographic examination of this animal did not reveal obvious arrhythmias or abnormal electrographic intervals.

Euthanasia was requested by the owner and a post mortem examination was performed.

Post Mortem Findings.

(a) Morbid Anatomy

The animal was thin and dehydrated. The mucous membranes of the mouth were of a muddy appearance. The teeth were covered with tartar, and some movement of the teeth in the mandibles could be elicited by digital/

digital pressure on their crowns.

Parathyroid Glands - The Parathyroid Gland in the centre of the lateral surface of the right thyroid was enlarged and pale-lemon in colour (Fig. 15b). Another smaller parathyroid, present on the dorsal border of the same thyroid gland, was also enlarged but slightly darker in colour. A third parathyroid at the posterior pole of the right thyroid was small and not noticeably enlarged. On the dorsal border of the left thyroid a parathyroid gland was of similar size and colour to that present in the corresponding position on the right. The only other obvious parathyroid gland was seen approximately 3 cm. posterior to the thyroid on the ventro-lateral surface of the oesophagus. This gland measured 11 mm. x 6 mm. x 3 mm. and was pale fawn in colour.

Kidneys - The right kidney (6½ cm. x 3½ cm.) was larger than the left (5½ cm. x 3 cm.) very pale in colour and had a very uneven, pitted surface. The capsule stripped with difficulty. On cutting, the kidney was found to be harder than normal with a slightly enlarged pelvis, which contained one small, irregular shaped, smooth surfaced, yellow calculus (approximately 8 mm. x 4 mm. x 4 mm.) and about 2 cc. of pus. The cortex was narrow (cortex: medulla ratio was 1:3) and whitish in colour. Some white ulcerated areas were present on the surface of the renal papillae (Fig. 16b).

The left kidney was nodular and dark coloured with diffuse whitish areas present throughout. The capsule stripped with difficulty, being firmly adherant to the pits on the kidney surface. On cutting, the consistency was found to be firmer than normal. A small, irregular-shaped/

shaped calculus (6 mm. x 4 mm. x 3 mm.) with a smooth yellow surface together with a considerable amount of pus (approximately 5 cc.) was present in the pelvis. The cortex of the gland was narrow (cortex :medulla ratio was 1:3).

Ureters - The right ureter was approximately twice the diameter of the left which appeared to be normal. The greatest dilatation of the right ureter appeared to be at the anterior end (Fig. 17).

Bladder - The bladder was contracted and contained very little straw-coloured urine. The bladder wall was thickened and the mucosal surface thrown up into folds. Small haemorrhages were present on the ridges of these folds.

Prostate - The prostate was large and whitish in appearance; the entire surface being nodular and undulating (Fig. 17). The consistency of the gland was putty-like. Purulent fluid exuded from the cut surface.

Pancreas - The pancreas was covered with patchy whitish and reddish, poorly defined areas which were of similar texture to the rest of the pancreatic tissue and which spread into the substance of the gland. Occasional small pin-head to lentil sized yellow spots were also observed.

Stomach - The stomach contained a small amount of sanguinous fluid. Small ulcerated areas on the mucosa, on the ridge of the folds were present in the fundus.

Trachea - The cartilaginous rings of the trachea showed small, (about 2 mm. diameter) yellowish-white spots.

The adrenal gland, heart, lungs, liver, intestines, spleen, genitalia, and/

Fig. 17. Urinary Tract Specimen of a Springer Spaniel with Primary Hyperparathyroidism. Showing a Contracted Left Kidney, a dilated Right Ureter and an Enlarged Prostate.


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and pituitary gland were macroscopically normal.

(b) Histopathology.

Parathyroid Glands - A 4 mm. x 3 mm. section of an enlarged Parathyroid Gland was studied. It showed a thin collagenous connective tissue capsule and a few thin trabeculi which contained major veins and arteries and which subdivided the gland. There were a few fat globules inside the capsule at one point only. The gland was divided into irregular sized packets by capillary sinusoids containing little blood in their lumina. A few small acinar structures were found near one edge with a little eosinophilic secretion in their lumina. The cells had large round or oval nuclei with none or one nucleolus. A thin but distinct nuclear membrane was present and discrete but fine nuclear chromatin dots and threads, arranged as if to suggest vacuolation, occasionally covered the whole nucleus. The cystoplasmic limits to the cells were ill-defined. There appeared to be a syncytium of fine eosinophilic threads forming a foamy or vacuolated mass in which the nuclei lay.
Kidney - Contained focal wedges of interstitial fibrosis. Some glomeruli showed fibrosis and hyalin changes, some tubules were cystic with eosinophilic hyalin casts, whilst others were hyper-plastic and without such casts. There were some foci of plasma cells and lymphocytes. The more normal tubules, lying between the areas of interstitial fibrosis, contained granular casts and showed some encircling fibrosis. Medullary fibrosis with cystic hyperplasia of the collecting tubules was observed. Varying degrees of necrosis of the pelvic epithelium, with neutrophil and macrophage infiltration and some pinkish fibrin, were also present.

Bladder/

Bladder - Desquamation of the eipithelium was observed together with a plasma cell and lymphocyte infiltration of the somewhat fibrosed subepithelial connective tissue.

Prostate - Showed slight cystic hyperplasia. Some oedema of the interlobular septi, with a few plasma cells and lymphocytes, were seen. The capsular arterioles were very thick walled.

Trachea - The central areas of the cartilage stained pale pink with haematoxaline and eosin whilst peripheral regions were a deeper pink. Some vacuoles surrounded by deep purple coloured cartilage were observed indicating that they may have been areas of calcification which had not survived the sectioning process.

Analysis of Calculi.

The techniques of analysis used in Case 1 were again applied. The results of quantitative micro-chemical analysis were - Calcium 27.60 gms.%; Phosphorus 15.89 gms.%; Magnesium 1.69 gms.%; Ammonia 3.54 gms.%; Oxalate 14.50 gms.%. These results exhibit a very close similarity to those recorded in Case 1 and suggest that the chief constituent was a calcium phosphate compound; some calcium oxalate and magnesium ammonium phosphate also being present.

X-ray diffraction analysis revealed that, as in Case 1, hydroxylapatite was the chief constituent, with Whewellite and Struvite as minor constituents. In addition to these, however, Weddellite ($Ca\ C_2O_4,\ 2H_2O$) was also detected. Optical crystallographic observations supported these findings.

Discussion/

Discussion.

(a) Blood Chemistry in cases of urolithiasis.

Calcium.

In the animal with a serum calcium level in excess of 12.7 mgm.% primary hyperparathyroidism was found. The calcium level in this case was 14.2 mgm.% on two consecutive samples. This level is not considered indicative of a hypercalcaemic state by some authors (Hoe and O'Shea, 1965; Persson, Persson and Asheim 1961) but since it is higher than the upper limit of normality usually quoted (lebermann, 1940; Dukes, 1947; Brodey, 1954; Hoskins, Lacroix and Meyer, 1959; Simesen, 1963; Campbell, 1964; Goldsmith, Wulsin and Wiester, 1965) and since it is higher than the level found in dogs with histologically normal kidneys (Hoe and O'Shea 1965) it seems likely to be an abnormally high level.

The mean serum calcium levels recorded in the present study were slightly higher in the calcium-containing stones than in the other chemical types but the difference was not statistically significant. Also the variation of calcium levels within each stone type was similar except for the case of hyperparathyroidism. These results suggest that the majority of urolithiasis cases have serum calcium levels within normal limits; occasionally, however, a raised serum calcium level is encountered and is indicative of parathyroid dysfunction.

Inorganic Phosphorus.

A wide variation in the serum inorganic phosphorus levels was found in these animals. Such a variation could be anticipated in a condition that results in renal disturbances. The normal serum inorganic phosphorus level in dogs has been recorded by several authors/

authors; Albritten (1957) suggests a normal range of 3.2 - 6.0 mgm./ 100 ml.; Campbell (1964) however, suggested a slightly higher level of 4 - 9 mgm/100 ml.; Simesen (1963) suggested that the level of inorganic phosphorus in the serum varied with age and that in adult dogs the normal level is 4.3 mgm/100 ml., but in puppies levels of up to 9 mgm/100 ml. may be found. Hoe and O'Shea (1965) suggest that the majority of normal dogs have levels of less than 4 mgm./100 ml. and in renal conditions the inorganic phosphorus value does not exceed 8 mgm/100 ml. in animals with a blood urea value of less than 40 mgm/100 ml.

Of the four animals in this study with serum inorganic phosphorus levels above 8 mgm/100 ml. only one was a puppy. Thus only 3 of the 36 cases could be stated to have definitely raised inorganic phosphorus levels. These results indicate that the serum inorganic phosphorus levels in most cases of urolithiasis remain within normal limits. The occasional raised level was probably associated with kidney dysfunction. Unfortunately, in the results recorded here, no comparison between serum inorganic phosphorus levels and kidney dysfunction, as measured by the blood urea concentration, can be made as the blood samples for these two estimations were not always collected at the same time.

Magnesium.

Greta Hammerstein (1956) suggested that magnesium may influence oxalate excretion and that magnesium deficiency may cause a high oxalate excretion in rats. The magnesium levels encountered in this series however did not indicate a deficiency in any of the animals, nor did they vary significantly between the different chemical types of stone.

Urea/

Urea.

It is of interest to note that approximately 30% of the animals presented with urolithiasis had uraemia. Although statistically there was no significant difference in the blood urea levels of the various chemical stone types when analysed together, a significant difference did occur when blood urea levels in the calcium phosphate stones were analysed against the other stone types. This result is of limited meaning, however, as the number of cases involved in the calcium phosphate group were few. However, two of these cases had bilateral nephrolithiasis, associated with very high blood urea levels; no other similar cases of bilateral renal stones occurred in any of the other groups.

It was thought that a difference may occur between the type of stones most frequently associated with urinary outlet obstruction (cystine and oxalate) and the type most commonly associated with vesical lithiasis (triple phosphate). Whilst both oxalate and cystine stone cases did have a slightly higher percentage of raised blood urea levels than did triple phosphate cases the difference was not statistically significant.

(b) Primary Hyperparathyroidism.

The occurrence of secondary hyperparathyroidism following renal dysfunction has been commonly recorded in dogs (Eichholtz and Ojemann, 1941; Gratzl, 1941; Méry, 1944; Hogg, 1948; Platt, 1951; Neilsen and McSherry, 1954; Brodey, 1954; Trevino, 1955; Dammrich, 1958; Brodey, Medway and Marshak, 1961; Persson et al., 1961). In these animals pathological bone changes and the clinical "rubber-jaw" syndrome is common.

This/

This report deals with a similar clinical syndrome but suggests that the underlying pathology may be due to a primary rather than a secondary form of hyperparathyroidism. The major difference in clinical features of these two forms is the presence in the primary form, of a raised rather than a lowered serum calcium level and (in the 2 cases described) bilateral nephrolithiasis. Several previous authors have mentioned the possibility of primary hyperparathyroidism as a aetiologic factor in canine urolithiasis but no previous reports describing clinical cases in which urinary calculi have formed in the presence of this condition could be found in the literature. Experimentally, however, urinary calculi have been produced by the administration of parathormone in the dog (Leberman, 1940) and on an autopsy study, the association between the 2 conditions has been recorded (Krook, 1957).

In humans, primary hyperparathyroidism is an important cause of renal lithiasis, approximately 1 - 5% of renal stones being associated with this disorder (Clark and Nordin, 1965). The finding of 2 cases of this condition in the present series of 55 cases of urolithiasis in the dog indicates that its incidence as an aetiologic factor in urinary stone formation may be similar to that occurring in humans.

Some human cases of primary hyperparathyroidism do not appear to be associated with renal lithiasis or even clinically obvious renal complications and similarly skeletal changes do not occur in all cases (Black, 1960).

A number of conditions are considered in the differential diagnosis of the human disease; these include Vitamin D intoxication; malignant diseas especially of the breast and kidney; the milk-alkali syndrome; myelomatosis/

matosis; hyperthyroidism and sarcoidosis (Clark and Nordin, 1965). The history with careful reference to diet and a post mortem examination of these two dogs failed to reveal the presence of any of these disorders.

Apart from the clinical signs (which are variable and often vague in the human disorder) the main diagnostic aid used in the detection of this condition is the estimation of serum calcium concentration. Of the two cases reported here, it seems likely that the total serum calcium level was raised in both cases. The rise recorded in the Spaniel (14.2 mgm%) was not nearly so marked as in the Beagle with primary hyperparathyroidism reported by Pearson et al. (1965), in which a serum calcium level of 27.5 mgm./100 ml. was found.

The total serum calcium concentration is known to be contained in 2 major and 1 minor fractions. The majority of the calcium being either in ionized form or bound to plasma proteins. The smaller portion exists in the undissociated state mainly as calcium citrate. In parathyroid overactivity the action of parathyroid secretion affects mainly the ionized fraction. However, in cases complicated by renal dysfunction, abnormal plasma protein levels may exist. In such cases the raised ionic calcium concentration may well be masked by the effect of a lowered serum protein level accompanied by a lowered protein-bound calcium fraction. Consequently in these animals the total plasma protein levels should also be determined. The total plasma protein concentration in Case 2 was found to be 5.45 gms./100 ml.. Normal plasma protein levels for dogs being reported as 6.2 ± 0.6 (Baer et al., 1957). A more exact diagnosis, however, might have been achieved by the determination of serum ionized calcium or ultrafilterable calcium (ionized calcium together with calcium

existing/

existing in the undissociated state).

The radiographic changes seen in the two patients seem similar to that described by Brodey et al. (1961), in dogs with secondary hyperparathyroidism. The only obvious difference being the presence of bilateral renal stones.

Whilst the haemocytology in Case 1 was notable only for a slightly lowered red blood cell count, a severe anaemia was observed in Case 2. The reduced production of a renally produced substance (erythropoietin) whose function is to stimulate bone-marrow, has been suggested by Forsell (1958) and Naetz (1958), as the cause of anaemia in chronic renal disorders and in Case 2 a raised blood urea level was known to be present for several months and it is possible that the anaemia in this case may well have bemn due to a similar phenomenon.

The metabolic acidosis present in Case 2 may have also been the result of renal dysfunction.

A surprising finding in this dog was the relatively low alkaline phosphatase level. In cases with generalised decalcification of bone the alkaline phosphatase activity is expected to be raised above the normal level.

No obvious abnormalities were observed in an electro-cardiographic tracing taken in Case 2 although in man a shortening of the Q - T interval is commonly observed in the condition (Pyrah et al., 1966).

In both cases described here the urine specific gravity was low. The parathyroid secretion is thought to be a potent diuretic and the polyuria and polydipsia associated with hyperactivity of the parathyroids in man is thought to be the result of a primary action of his hormone

(Black/

Black, 1960). Carone, Epstein, Beck and Leviten (1960) showed that hypercalcaemia of brief duration in dogs treated with parathyroid extract, caused specific renal tubular lesions and an impairment of renal concentrating ability. In the two cases reported here, however, the renal pathology may well have played some part in the lowered urine specific gravity. As well as an increased water excretion, fixed base and electrolytes (including calcium and phosphorus) are excreted in excess in hyperparathyroidism (Black, 1960). Thus a constantly high urinary pH can be expected and is frequently associated with the condition in humans. In these 2 cases urinary pHs of 7.9 and 7.5 were recorded. In the latter case a consistently alkaline urine was excreted over several months, a pH of 7.4 being recorded on several occasions.

The formation of urinary calculi during increased parathyroid secretion may be due to several factors, the most obvious of which is the hypercalciuria associated with this condition.

In their excellent review of primary hyperparathyroidism in man, Pyrah, Hodgkinson and Anderson (1966) suggest that calcific deposits in the renal pyramids and ducts of Bellini may also play some part in initiating the stone forming process.

Analysis of the calculi in both dogs showed that Apatite was the chief constituent and that some oxalate and magnesium ammonium phosphate was also present. The calculi experimentally produced in dogs by subcutaneous injections of parathormone (Leberman, 1940) and the calculi from previous cases of this condition (Krook, 1957) also contained a calcium phosphate compound as their chief constituent.

As/

As previously stated a number of pathological conditions of the parathyroid are known to be related to primary hyperparathyroidism in man. In the first case studied here the macroscopic appearance of the enormously enlarged gland was indicative of a single adenoma. Unfortunately, however, histopathological studies could not be performed. In the second animal, the macroscopic appearance indicated either multiple adenomata or a hyperplastic condition, both of which are implicated in the disease complex in man (Pyrah et al., 1966).

The chief cellular component of the enlarged parathyroids in the spaniel apparently resembled the water clear cells of Pyrah et al. (1966) and the active cells described by Pearson et al. (1965) which occurred in increased numbers in the Beagle suffering from this condition. These cells,however, are not distinctly different from the chief cells described by Platt (1951) and the water clear cells described by Dammrich (1958) in secondary hyperparathyroidism.

Since the parathyroid glands in dogs appear to be structurally different from the corresponding glands in man and since so little has been recorded on the pathology of the parathyroid in dogs suffering from primary hyperparathyroidism it is not surprising that confusion exists regarding the histopathologic criteria for diagnosis of this condition. Pearson et al., (1965) have attempted to overcome this problem by quantitatively measuring cell size and numbers.

In man primary hyperplasia with a uniform histologic appearance characterised by large water clear cells (Pyrah et al., 1966) has been reported and the second dog in this study appeared to present a comparable histologic picture.

The/

The pathogenesis of secondary hyperparathyroidism in the dog has been discussed by Persson, et al. (1961) and Brodey et al (1961) and a resume of the present knowledge of the mode of action of the parathyroid hormone has been included in a review on primary hyperparathyroidism in man by Pyrah et al. (1966).

4. DIET

Introduction

The method available for the study of the influence of diet on canine urolithiasis in this clinical survey was by questioning dog owners as to the feeding arrangements and gross constituents of the food given to affected animals. Such a method does not allow the collection of precise data concerning the amount of various components in the diet such as Vitamin A etc., that are thought to play some part in the process of urolithogenesis. For these to be investigated, facilities for balance studies or intricate biochemical estimations are necessary, and, at the time of this study were not available to the author. Consequently, only a few simple observations regarding the diet of these animals could be made. However, for future investigations into the aetiology of certain calculous disorders, balance studies will need to be performed, and knowledge of the influence of possible dietary factors on stone formation is a necessary pre-requisite for such studies. For this reason, a resume of the present knowledge of the effect of these dietary factors on urinary calculi formation is included in the review of the literature.

Review of the Literature.

Diet has been suspected as an aetiological factor in human urinary stone disease for many hundreds of years, the Persians having considered the cause of urinary calculi to be the habitual consumption of sour milk, fruits and acid drinks and foods (Garrison, 1939).

The observation that, during the early part of this century, a decrease/

decrease in the incidence of lower urinary tract stones coincided with improvement in nutrition and dietary habits in the industrial areas of the United Kingdom and Europe was considered by Joly (1931) as evidence that dietetic factors may be implicated in their aetiology.

It is thought that diet may influence the formation of calculi by the following mechanisms:-

(a) By the presentation to the kidneys of excessive calculous constituents with consequent oversaturation of the urine and resultant precipitation.

(b) By the imbalance or deficiency of certain substances such as vitamins which may cause changes in the urinary tract leading to conditions favourable to calculi formations.

(c) By causing changes in the urinary pH which in turn may influence the solubility of calculi-forming substances.

(d) By interference with the intestinal absorption of certain calculous constituents.

(e) By causing changes in the type and amount of protective substances in the urine.

The first mechanism was investigated in one of the earliest experimental studies on urinary stone disease in dogs by Studensky in 1877. Studensky inserted foreign bodies in the bladder of dogs and fed one group with water heavily impregnated with lime salts. He found that a heavy layer of lime salts soon formed over the foreign bodies and that the results differed greatly from the animals fed with pure water and meat.

Muller and Glass (1911) interpreted these results as proving that foods and fluids may have a certain influence on the formation of stones in the bladder.

However Smith and Jones (1961) stated that experimentally, waters of high mineral content and irritant and unnatural foods, have all failed/

failed to cause urolithiasis provided there was no inflammatory process present in the urinary tract and providing the supply of Vitamin A was adequate.

According to Uller (1959), the relationship between diet and stone formation in dogs is very noticeable. He states that particularly in small dogs which are fed liver there is an increased incidence of urinary concrements, and that excess feeding of particular substances in the food is a factor in the aetiology of urinary calculus formation.

Other veterinary writers also implicate diet as a factor in stone formation in the canine urinary tract. Joest (1924) quoted Hébrant as saying that uroliths occur particulary in overfed, underexercised dogs and Bloom (1954) wrote "In monotonous, ill-balanced diets there may be an indirect influence on the chemical composition of the calculi, as well as the presence of increased concerntrations of easily precipitable urinary constituents". He continued, "It is well established that the incidence of calculi is diminished when animals are fed a suitable balanced diet. Clinical observations indicate that urinary stones are more frequent in dogs given a vegetarian diet instead of meat". This last opinion was also expressed by Klarenbeek et al.(1935). However the literature offers no satisfactory evidence to support such statements.

1. Effect of Diet on the Formation of Stones that have Either Calcium Salts or Phosphates as Their Chief Constituent.

Stones that have a calcium-containing compound as their chief component in the dog are usually of the calcium oxalate or calcium phosphate type and the majority of phosphate stones have magnesium ammonium phosphate as their main constituent.

Dietary/

Dietary excesses of the ionic constituents (Calcium, Oxalate and Phosphate) of calcium-containing uroliths have been studied in humans and experimental animals and a resume of the present knowledge of their influences on urolithogenesis is as follows:-

Excess Dietary Calcium.

In normal humans, the influence of dietary calcium levels on the urinary excretion of calcium has been shown to be small (Bogdonoff, Shock and Nichols, 1953; Nicolaysen, Eeg-Larsen and Malm, 1953; Hodkinson and Pyrah, 1958; Nordin, 1959; Carruthers, Copp and McIntosh, 1964). Similarly, Keyser (1923) found that the forced feeding of a high calcium diet to rabbits did not result in an increased crystalline content of the urine of these animals, and he concluded that this was good evidence against a "hard-water" or "lime-soil" factor in the aetiology of stones. Gill, Finlayson and Vermeulan (1959) however, showed that the addition of calcium lactate to the diet of rats caused an increase in their urinary calcium excretion, but paradoxically this inhibited the formation of calculous material on foreign bodies placed in their bladders.

Higgins and Straffen (1963) stressed the importance of the dietary relationship of calcium and phosphorus and the fact that excess dietary calcium may combine with phosphate in the gastro-intestinal tract and produce a non-absorbable compound with consequent reduction of the amount of phosphorus available to the body. Jolly and Worden (1966) fed 4 young male Welsh Corgis on a diet with a calcium-phosphorus ratio of 19:1 and found that calculi formed but were mostly composed of a proteinaceous/

proteinaceous material and were unlike naturally occurring uroliths.

Attention was drawn to the secondary effects of normal dietary calcium levels in certain human urolithiasis patients by Dent and Watson (1965). These authors describe a patient with "idiopathic hypercalcinuria" due to an intestinal over-absorption of calcium in which the urinary calcium concentration was greatly influenced by changes in dietary calcium.

No reports of dietary excess of calcium associated with an increase in the incidence of the usual chemical types of uroliths in dogs could be found in the literature, but it is possible that similar cases to that described in humans by Dent and Watson (loc. cit.) may also occur in dogs.

Excess Dietary Oxalate.

According to Hammersten (1956) dietary oxalate is only partly absorbed, the rest passing in the faeces as insoluble calcium oxalate. Consequently large amounts of oxalate in the food may cause a loss of calcium to the body.

The soluble oxalate salts, (e.g. Sodium Oxalate) however are partially absorbed, but Archer, Dormer, Scowen and Watts (1957) found that only 2.3 - 4.5% of the total amount of Sodium Oxalate administered to humans is excreted in the urine. Thus the oxalate present in the urine would appear to originate mainly in endogenous sources. Its precursors and its metabolism are poorly understood. Higgins (1951) however claimed that oxalate stones were produced in Vitamin A deficient dogs/

dogs fed on oxamide. Calculi had previously been experimentally produced in the urinary tract of dogs by the feeding of oxamide alone (Nicolaier and Ebstein, 1891; Thomassen, 1893; and Keyser, 1923). In Keyser's experimental animals the calculi were found to contain oxamide and not calcium oxalate as in the clinical condition. The excessive feeding of oxalates to dogs with smooth aseptic foreign bodies in the bladder failed to produce urinary concretions (Tuffier, 1893).

Excess Dietary Phosphate.

In human urolithiasis it has been shown that the urinary excretion of inorganic phosphates does not differ appreciably from that of normal individuals (Hodgkinson and Pyrah, 1958).

Reduction of dietary phosphate has been advocated by some authors in the treatment of phosphate calculi in dogs (Brodey, 1955; and Schlaaff, 1961). Conversely the oral administration of orthophosphate has been found by several authors (Howard, 1962; Thomas, Bird and Tomita, 1963; and Edwards, Russell and Hodgkinson, 1965) to have a beneficial effect on reducing the recurrence rates of calcium-containing stones in humans. Similarly, Care and Wilson (1956) reported that sodium hexametaphosphate prevented the formation of experimental bladder stones in rats. The mechanism of this beneficial action is poorly understood. Vermeulen, Lyon, Gill and Chapman (1959) suggested that the phosphates reduce intestinal absorption and urinary excretion of calcium in much the same manner as sodium phytate (Sodium inositolphosphate) but evidence recorded by Edwards, Russell and Hodgkinson (1965) suggests that increased dietary/

dietary phosphates result in an increased urinary excretion of orthophosphate, pyrophosphate and citrate, all of which have been found to influence the deposition of calcium salts in the urine and to decrease urinary calcium and magnesium levels. The effect on these latter two elements could not be fully explained by the interference with intestinal absorption of these compounds. Paradoxically therefore it seems that increases in certain phosphates in the diet may have a beneficial rather than an adverse effect on some phosphate stones. Whether such an effect exists in dogs is not established, but it may be stated that at present the relationship of dietary phosphate levels and canine urolithiasis is not known.

Several dietary constituents, that are known to influence the urinary levels or solubility of either calcium oxalate or phosphate, have been investigated in humans and experimental animals in order to assess their influence on urinary calculous formation. These substances include citrate, pyridoxine, sodium phytate, magnesium and Vitamin D. Another dietary constituent that has been discussed as an aetiological factor in urolithogenesis, usually in relation to phosphate stone formation, is Vitamin A.

Influence of Citrate.

Citrate is known to conjugate with calcium in the urine to form a soluble complex. Consequently some attention has been focused on the urinary citrate levels in calculous disorders.

In/

In human urolithiasis, Boothby and Adams (1934), Kissing and Locks (1941) and Shorr, Almy, Cloan, Tanssky and Toscani (1942) showed that the citrate concentration of the urine was below normal in many patients. Conway, Maitland and Rennie (1949) suggested that this was not due to a deficiency of citrate excretion by the kidney but was associated with urinary infection.

Having found that physiological levels of citrate had only minor effects on calcium solubility in the urine, Yarbero (1958) postulated that the lowered urinary citrate levels in human urolithiasis were related to a metabolic defect and he considered it unimportant as an aetiological factor in urolith formation. McIntosh, Seraglia, Uhlemann and Kore (1963) however, in a study on the effect of the alteration of dietary calcium content in normal humans and on idiopathic calcium-stone-formers, found evidence to support the concept that urinary citrate may be of importance in the prevention of calcium precipitation and hence in the pathogenesis of stones.

From these studies, it can be seen that the significance of urinary citrate in urolithogenesis is not clearly established.

Influence of Pyridoxine (Vitamin B_6).

Vitamin B6 deficiency has been found to cause hyperoxaluria in both cats (Gershoff, Faragalla, Nelson and Andrus, 1959) and rats (Andrus, Gershoff, Faragalla and Prien, 1960). Renal deposits of calcium oxalate were found in both these studies but gross calculi were not found in the cat whilst in the rat crystal deposition was entirely/

entirely restricted to the apex of the renal papilla and gross concretions with their obstructive sequelae were noted. Prien (1961) suggested that 10 mgm. of Vitamin B_6 daily decreased oxalate excretion in normal and oxalate stone-forming humans. The position of Vitamin B_6 in the formation of oxalate stones however remains unknown.

It is of interest to note that Vitamin B_6 deficiency was found in all cystinuric patients studied by Collini et al. (1965). These authors suggested that this was the result of a transamination defect in this disorder.

Influence of Sodium Phytate.

According to Vermeulen, Lyon, Gill and Chapman (1959), the oral administration of sodium phytate results in its combination with dietary calcium to produce a non-absorbable compound in the gastro-intestinal tract. The reduced urinary calcium excretion consequent to sodium phytate administration was thought by Boyce, Garvey, Gowen and Winston-Salem (1958) to be the cause of reduced incidence of calculi in a series of human patients. Similarly, Henneman, Benedict, Forbes and Dudley (1958) found that the urine calcium excretion in human idiopathic hypercalcinuric patients was lowered by about 100 mgm. per 24 hours by the oral administration of sodium phytate. Vermeulen et al. (1959) found that the oral administration of other phosphates (orthophosphate and hexametaphosphate) as well as sodium phytate (sodium inositol phosphate) resulted in a decreased urinary calcium. These authors however/

however, found that a simultaneous increase in phosphate excretion was occurring. Fleisch, Bisay and Care (1964) found that the oral administration of some phosphates (orthophosphate) to healthy subjects induced an increase in urinary pyrophosphate which they found to be a potent inhibitor of the precipitation of calcium phosphate and calcium oxalate. Thus the method of exerting a beneficial influence on calculi formation by these phosphatic compounds may be twofold; firstly the urinary calcium level may be decreased and secondly the urinary levels of certain phosphates which inhibit the precipitation of calcium-stone-containing salts, may be increased.

Influence of Magnesium.

A magnesium deficiency in rats was shown by Greta Hammersten (1937) to lead to an increase of oxalate in the urine in some cases, even on a diet free of oxalate. She also demonstrated that the solubility of calcium oxalate in the urine was very low at low levels of urinary magnesium. Since this publication conflicting reports on the influence of magnesium in stone formation have appeared in the literature. The reduction of urine oxalic acid by administration of magnesium to some human oxalate-stone formers was reported by Hammersten (1956) and Albuguerque and Tuma (1962) but similar results on both human subjects and animals could not be obtained by Gershoff (1964).

The ability to calcify rachitic rat cartilage by urine from renal stone formers was found by Mukai and Howard (1963) to be abolished by increasing the urinary magnesium concentration. Vermeulen, Goetz, Ragins and Grove (1951) however, found that low dietary magnesium levels/

levels actually reduced the rate of calculous deposition on foreign bodies placed in the bladder of rats.

The extent and the nature of the influence of magnesium on stone formation is therefore confused and further work is necessary for its clarification.

Influence of Vitamin D.

Hypervitaminosis D or an oversensitivity to Vitamin D is known to cause a hypercalciuria in humans, (Melick and Henneman, 1958; and Jackson and Dancaster, 1959) and is thought to be associated with some cases of urolithiasis. This condition has not been reported in the dog although the possibility of its occurrence as an aetiological factor in the formation of vesical calculi in bitches has been mentioned by Hickman (1958).

Influence of Vitamin A.

Probably the best known dietary constituent associated with urolith formation is Vitamin A.

In 1917 Osborne, Mendel and Ferry observed that rats fed on a Vitamin A deficient diet developed calcium phosphate and calcium carbonate calculi in the kidney or bladder and discussions as to the role of Vitamin A as a causative factor in clinical urolithiasis have been continuous since this time.

Fujimaki (1926) confirmed Osborne and his co-workers' findings and noted that in all rats in which calculi were present the urine was alkaline and that on administration of Vitamin A the calculi dissolved. Fujimaki (1926) also subjected a litter of 5 pups to

a/

a Vitamin A free diet and found that one animal that lived 101 days on this diet had both bladder and kidney stones. No controls were kept.

In 1933 Higgins repeated the experiments on mice and suggested that as a rule concurrent urinary tract infection was present. Higgins later performed similar experiments on dogs (Higgins, 1935) and after 11 months, found multiple stones in the bladder of 3 out of 4 of these animals. The resultant stones were composed of ammonium and calcium phosphate with small amounts of carbonates. No bacteriological findings were reported. In a subsequent paper he claimed that with a Vitamin A deficient diet he had successfully developed uric acid stones in a Dalmatian on a high purine diet, cystine stones in a cystinuric dog and oxalate stones in dogs when oxamide was fed (Higgins, 1951). It would therefore appear possible to produce calculi in the urinary tract of the dog under certain experimental conditions by reducing the amount of Vitamin A in the diet.

An interesting investigation indicating that the method of action of Vitamin A deficiency in urolithogenesis in rats was through its association with infection, was reported by Hedenberg (1954). This author produced experimental bladder calculi in albino rats by feeding a diet deficient in Vitamin A. In all cases in which calculi were produced, a urinary infection was present, but in cases in which antibiotic administration had prevented infection, no calculi developed.

The/

The occurrence of Vitamin A deficient signs in the clinical canine patient with calculous disease, appears to be rare. Indeed, in the literature, only Uller (1959) described its possible occurrence in Pekingese and Maltese terriers in which corneal defects could be demonstrated.

Thus, whilst Vitamin A deficiency may be capable of producing urolithiasis in both dogs and laboratory animals, the evidence that it is a factor in clinical canine urolithiasis is inconclusive.

2. Effect of Diet on Cystine Stone Formation.

That the level of urinary cystine in human cystinuric patients was influenced by diet was shown by Brand and Cahill (1934). Brand, Cahill and Harris (1935) and Dent, Senior and Walshe (1954). These authors demonstrated that the ingestion of cystine itself had no effect on the urinary levels of this amino-acid, but methionine and cysteine were largely excreted as cystine.

Dent, Senior and Walshe (1954) recorded only a small rise in cystine excretion with the oral administration of methionine but a much larger rise with oral cysteine in both cystinuric and normal subjects.

In the cystinuric dog, Hess and Sullivan (1942) investigated the effects of oral administration of these three amino-acids at various levels of protein intake on cystine excretion. Their results collaborated the findings of the workers in the human field in that while the cystine had little effect, both methionine and cysteine caused an increase in urinary cystine excretion.

The /

The phenomenon was more obvious at low protein intake levels. They claimed that on a diet consisting of only 5% protein in the form of casein, cystine excretion of 2 cystinuric Irish terriers was reduced to 8 mgm./day. At a level of 25% protein in the diet, the excretion was 80 mgm. per day.

The apparent conversion of methionine to cystine was found by Tarver and Schmildt (1943) to occur more rapidly in cystinuric than in normal dogs. Dent, Senior and Walshe (1954) when dealing with human cystinuric patients found that the restriction of dietary protein only mildly influenced cystine excretion, and Dent and Senior (1955) reported that no more cystine was excreted on a high protein diet than on a normal diet. Dent and Senior (1955) also demonstrated that a very low protein diet did reduce the urinary cystine level, but found that such a diet was difficult to prepare and suggested that it may be positively harmful due to the likelihood of inducing an essential amino-acid deficiency in subjects that were already excreting very large quantities of some amino-acids.

Treacher (1962) suggested that since cysteine and methionine formed a considerable portion of the protein of red meat, liver, heart, kidney, fish and egg, the substitution of these foods with protein of vegetable origin may have some effect on cystine stone formation by reducing the sulphur intake and disposing towards a more alkaline urine.

No results of clinical trials to elucidate the effect of such diets on canine cystine stone disease patients have appeared in the literature.

3./

3. Effect of Diet on Urate Calculi Formation.

Included in the first report of the unusual uric-acid excretion pattern of the Dalmatian Dog by Benedict (1915-16) was a study of the effect of diet on the urinary uric-acid level. Benedict found that, following a four-fold increase in the nitrogen content of the food, the uric-acid output was not increased. He also reported that the feeding of a purine-free diet to a Dalmatian for nearly a year had little effect on the urinary uric-acid level and he concluded that they could synthesize purine from non-purine material.

Young, Conway and Crandall (1938) investigated the effects of synthetic diets in Dalmatians. They found that even the feeding of uric acid or nucleic acid had little or no effect on the excretion of uric-acid and they suggested that there existed no dietary precursor in the synthesis of uric-acid.

From these reports the extent of dietary factors in the formation of urate stones, by affecting urinary uric-acid levels, would appear to be minimal. It is conceivable however, that an acid-ash diet may in fact increase the ammonium ion concentration of the urine which according to Porter (1963b) is an important factor in the formation of urate stones.

Observations on the Diet of 32 Cases of Urolithiasis.

Information concerning the diet of 32 of the 55 cases of urolithiasis was recorded. Owners of affected animals were questioned on the feeding arrangements including the constituents of the main daily meal and auxiliary meals. Specific questions on the frequency of availability of/

of tinned food, fresh meat, table scraps, dog biscuits, vegetables, fish, eggs, milk, bone, vitamin or mineral supplements, bread, biscuits and sweets were directed to each of the owners. It was realised that such information would not contain accurate data on the quantity of specific food ingredients but the questions were designed to assess whether Gross dietary abnormalities were present in cases of urolithiasis.

No obvious clinical syndromes of dietary deficiency conditions were seen in these animals. Similarly no animal appeared to be suffering from malnutrition and only two were found to be grossly obese.

All but 6 of the 32 cases received a mixture of meat and cereals as the major portion of their daily diet. The meat was available in either the raw, cookedor tinned form and the cereals were mainly in the form of biscuits, bread or proprietary breakfast cereals. The remaining 6 dogs received an almost entirely meat diet. No animal received a diet composed mainly of vegetables and in fact only 7 of the 32 animals regularly received vegetables in the form of table scraps.

One animal received regular vitamin supplements which consisted of a teaspoonful of cod liver oil weekly and another received regular mineral supplements in the form of a yeast preparation fortified with minerals.

The diets in 9 cases of triple phosphate lithiasis, 11 cases of calcium oxalate lithiasis, 9 cases of cystine lithiasis and 3 cases of calcium phosphate lithiasis were known.

(Table 36).

TABLE 36 /

TABLE 36

RELATIONSHIP OF DIET TO THE DIFFERENT CHEMICAL TYPES OF CALCULI.

Chemical Type of Calculi.	No. of Cases.	Mainly Meat Diet.	Meat and Cereal Diet.	Dogs Regularly Receiving Vegetables.	Dogs Regularly Receiving Mineral or Vitamin Supplements.
Triple Phosphate	9	4	5	3	2
Calcium Oxalate	11	1	10	3	-
Calcium Phosphate	3	-	3	1	-
Cystine	9	1	8	-	-
Total	32	6	26	7	2

With regard to diets fed to these animals the following observations were made:-

(a) In no case of triple phosphate or calcium phosphate lithiasis was an alkalising diet fed.

(b) In no case of calcium oxalate lithiasis was either a high calcium or high oxalate diet fed.

(c) In only 1 cystinuric animal was a diet almost entirely of purine-rich meats consistently fed.

(d) Dogs forming different chemical types of calculi were receiving similar diets.

Discussion.

Observations on diet have not revealed a relationship between a particular type of diet and a specific chemical type of calculi. The diets/

diets received by most animals could have been low in certain vitamins and unbalanced with respect to certain minerals. The mineral content in many of the diets was apparently low.

The animals in which an almost entirely meat diet was fed would possibly have had a calcium-phosphorus imbalance with a low calcium high phosphorus ratio.

Jolly and Worden, (1966) have recently drawn attention to the fact that in the dog at least 2 types of calculi disease, namely cystine and urate lithiasis are inheritable and a third type viz: magnesium ammonium phosphate calculi occur in association with urea-splitting infections which cannot be attributed to nutrition and therefore diet cannot be considered as a primary factor in the aetiology of a substantial percentage of urolithiasis cases in the dog. However Hedenberg (1954) demonstrated in rats that the formation of calculi in vitamin A deficient animals was associated with urinary infections. It is thus possible that a dietary deficiency of this vitamin may lead to an increased susceptibility to urinary tract infections in dogs. Calculi forming in vitamin A deficient laboratory animals however usually occurs in animals with clinical signs of the deficiency, and similar signs were not found in the dog studies here, but it is possible that sub-clinical deficiencies of vitamin A may have been present.

According to Bulcott (1966), the diet of the majority of dogs in this area consists of a mixture of meat and cereals. Such a diet was received by most dogs with urolithiasis. From these observations it seems unlikely that diet is a primary factor in the formation of most uroliths in this area; the possibility of its playing a secondary role however, cannot be denied.

5. URINARY HYDROGEN ION CONCENTRATION.

Review of the Literature.

For more than a century Veterinary authors have discussed the role of urinary pH in calculus formation (Gamgee, 1862) and although, virtually all authors have considered pH either as a primary or secondary factor in the aetiology of urolithiasis, very few studies on this subject have appeared in the literature. The association of phosphate calculi and alkaline urine has been repeatedly discussed and the high pH has usually been attributed to the presence of urea-splitting organisms. Brodey (1955) suggested that urate or uric-acid stones form in acid urine but this opinion has been recently disputed by Porter (1963b). Treacher (1962) was of the opinion that cystine stone formation also occurred in acid urine and he showed that the solubility of cystine in canine urine increased rapidly as the pH rose above 7.6. At lower hydrogen ion concentrations the influence on cystine solubility was less marked. The urinary pH is thought to have little influence on the formation of oxalate calculi (White, Treacher and Porter, 1961).

The effect of urinary pH on the solubility of the calculi containing substances is thought to be the chief mechanism involved in its relationship to calculus formation. Elliott, Quaide, Sharp and Lewis (1958) have shown that Struvite crystallised from human urine only when the pH was above 7.2; Apatite crystallised only when the pH was above 6.6 and Brushite only when the pH was less than 6.6. Porter (1963) when discussing urate stone formation suggested that pH did not greatly influence/

influence the solubility of the chief constituent of these stones, but that it may affect their formation by influencing the concentration of the ammonium ions in the urine, which in turn influences the flocculation of colloidal urate solutions.

Materials and Methods.

The pH was recorded in each of the 55 cases of urolithiasis. The urine was collected into chemically clean universal containers using sterile disposable Nelaton catheters. Occasionally the urine was obtained at the time of operation either immediately following release of the urethral obstruction or at cystotomy.

pH measurements were recorded with a Beckman zeromatic pH meter as soon as practical after collection. In most cases recording was completed within 15 minutes, but in others a delay not exceeding 2 hours occurred.

Results.

The pH occurring in the different chemical types of calculi and in relation to bacteria was recorded and the results tabulated in tables, 37 and 38 respectively.

Relationship of Urinary pH to Chemical Stone Types.

The mean urinary pH (7.44) of Triple Phosphate cases at or prior to treatment was found to be sligtly higher than for the other types of/

of urolithiasis (Table 37).

TABLE 37

RELATIONSHIP OF URINARY pH TO CHEMICAL STONE TYPES

Chemical Type of Stone	No.of Cases	Variation in pH	Mean pH
Triple Phosphate	19	6.1 - 8.0	7.44
Calcium Oxalate	18	5.8 - 7.6	6.51
Calcium Phosphate	4	6.4 - 7.9	7.33
Cystine	13	6.4 - 7.4	6.94
Urate	1	5.9	

The mean urinary pH of Calcium Phosphate calculi was also alkaline. Two of these cases were associated with primary hyperparathyroidism.

Seven of the 13 cases of cystine stones had alkaline urine present when examined initially. The mean pH for these cases, however, wasslightly acid. Only one case in which Calcium Oxalate stones were found had a urinary pH greater than 7.0. The urine of the Dalmatian in which the urate stone was found was markedly acid. Statistical analysis (Analysis of Variance) applied to these figures (excluding the urate) showed that the differences between the pH's of the chemically distinct stone types were statistically significant ($P < 0.01$).

Other statistical comparisons using the same type of analysis resulted as follows:-

(a)/

(a) Triple Phosphate vs Calcium Oxalate - Statistically Significant ($P < 0.01$).

(b) Both Phosphate Types vs Cystine plus- Calcium Oxalate - Statistically Significant ($P < 0.01$)

(c) Cystine vs Calcium Oxalate - Probably Significant ($P < 0.05$)

(d) Calcium Phosphate vs Calcium Oxalate- Not Significant.

Relationship between Urinary pH and Urinary Organisms.

Cases in which urinary organisms were isolated were divided into two groups; (a) those in which urea-splitting organisms were found and (b) those in which non-urea-splitting organisms were isolated. The initial urinary pH in each of these groups was compared with that occuring in cases in which no organisms were isolated. (Table 38).

TABLE 38

RELATIONSHIP OF URINARY pH AND ORGANISMS

Type of Organisms	No. of Cases	Variation in pH	Mean pH
Urea-splitting Organisms	17	6.9 - 8.4	7.59
Non-Urea-Splitting Organisms	5	5.8 - 7.0	6.36
No Organisms Isolated	28	5.2 - 7.9	6.72

The 5 cases that had received antibiotic treatment prior to presentation were not included in the above table.

The/

The mean urinary pH in animals from which urea-splitting organisms were isolated (7.59) was found to be considerably higher than those in which non urea-splitting organisms (6.36) or in which no organisms (6.72) were isolated. In all cases in which urea-splitting organisms were present in the urine, a pH of 6.9 or above was recorded.

Statistical analysis (Analysis of Variance) indicated a significant difference between these groups ($P < 0.01$). Further statistical comparisons resulted as follows:-

(a)	Urea-splitting groups vs Non-urea splitting	- Statistically significant ($P < 0.01$)
(b)	Urea-splitting group vs Non infected	- Statistically significant ($P < 0.01$)
(c)	Non urea-splitting group vs Non infected.	- Not significant

Urinary pH in Cases of Urinary Retention Unassociated with Urolithiasis or Infection.

Brodey (1955) and Bentinck-Smith (1963) have suggested that urinary pH is influenced by urine retention. Brodey (loc. cit.) suggested that retention for more than 24 hours always resulted in alkaline urine. To test this hypothesis six animals in which retention was known to occur for a period of 24 hours or more, in which no urinary organisms were isolated and in which no calculi were observed, were catheterised and the urinary pH determined. (Table 39). In no cases was an alkaline pH recorded.

TABLE 39

RELATIONSHIP OF URINARY pH TO URINARY RETENTION

Breed	Age (Years)	Sex	Duration of Retention	pH	Cause of Retention
Labrador Retriever	3	Female	48 hours	6.4	Neurogenic Bladder
Fox Terrier	9	Female	48 hours	6.9	Tumour at External Urethral Meatus
Alsatian	4	Female	48 hours	6.8	Neurogenic Bladder
Mongrel	13	Male	24 hours	5.3	Prostatic Enlargement
Mongrel	7	Female	24 hours	6.1	Neurogenic Bladder
Mongrel	6	Male	24 hours	5.7	Retroflexion of the Bladder.

Discussion.

The difference in the urinary pH recorded in the chemically different types of stone has been shown to be statistically significant. The findings confirm the generally accepted opinion that phosphate stones form mostly in alkaline urine and that oxalates form in acid urine. The position of cystine lithiasis appears to be intermediate between these two types; the mean urinary pH recorded in cystine stone disease (6.94) being probably significantly ($P < 0.05$) higher than in oxalate patients. The reason for this is unknown, but it may be related to the large amounts of basic amino-acids excreted by the cystinuric animals.

Whilst the raised urinary pH is for the most part associated with urinary infections by urea-splitting organisms in these cases, it/

it may also be related to the presence of primary hyperparathyroidism.

Organisms of the non-urea-splitting variety, do not appear to cause significant pH changes. Indeed most pHs recorded in animals with these organisms fell within the normal range of 5-7 (Krabbe, 1949; and Bloom, 1960).

Little is known of the daily variation in canine urinary pH but a few initial observations on apparently normal dogs by Goulden, (1969) show that the effect of normal physiological phenomena such as the post-prandial alkaline tide may cause considerable changes in the urinary hydrogen ion concentration resulting in marked alkalinisation for several hours. Further studies are required to assess the significance of these changes and their influence, if any, on urolithogenesis.

Previous authors Brodey (1955) and Bentinck-Smith (1963), have suggested that retention of urine per se leads to alkalinisation of the urine within short periods. Results recorded in 6 animals with retention unassociated with urinary calculi or urinary infection do not support this concept and it would seem unlikely that the retention of urine would be sufficient to cause alkalinisation, unless perhaps it existed for very prolonged periods.

A possible inaccuracy in the procedure of testing for pH may have resulted from some loss of CO_2 from the time of catheterisation to the time of recording. In order to minimise this possible inaccuracy/

inaccuracy rapid post-catheterisation recording was carried out whenever possible.

The low urinary pH recorded in the single animal with urate calculi is of interest as it has been recently suggested that urate stones are associated with alkaline pH and that this change in urinary pH is possibly due to the presence of urea-splitting organisms (Porter, 1963a).

TABLE 40

RELATIONSHIP BETWEEN URINARY pH. URINARY ORGANISMS AND

CHEMICAL TYPES OF CALCULI.

Case No.	pH	Organisms Urease + ve.	Organisms Urease - ve.	Chemical Stone Type.	Case No.	pH	Organisms Urease + ve.	Organisms Urease - ve.	Chemical Stone Type.
1	6.1	-	-	T.P.	29	6.4	-	-	C.O.
2	7.8	+	-	T.P.	30	6.7	-	+	C.O.
3	7.9	-	-	T.P.	31	7.0	-	-	C.O.
4	7.3	+	+	T.P.	32	6.7	-	-	C.O.
5	6.3	+	-	T.P.	33	7.6	+	-	C.O.
6	7.8	+	+	T.P.	34	5.2	-	-	C.O.
7	7.3	+	-	T.P.	35	6.9	-	-	C.O.
8	7.0	-	-	T.P.	36	6.5	-	+	C.O.
9	7.9	+	-	T.P.	37	6.7	-	-	C.O.
10	7.9	+	-	T.P.	38	6.4	-	-	C.P.
11	7.6	+	+	T.P.	39	7.6	+	-	C.P.
12	7.3	+	+	T.P.	40	7.9	-	-	C.P.
13	7.9	+	+	T.P.	41	7.4	-	-	C.P.
14	6.9	+	+	T.P.	42	7.4	-	-	C.
15	8.0	+	+	T.P.	43	6.7	-	-	C.
16	7.5	+	-	T.P.	44	6.7	-	-	C.
17	8.4	+	-	T.P.	45	7.3	-	-	C.
18	7.0	-	+	T.P.	46	7.1	-	-	C.
19	6.5	-	-	T.P.	47	7.3	-	-	C.
20	7.0	+	-	C.O.	48	6.6	-	-	C.
21	6.4	-	-	C.O.	49	6.5	-	-	C.
22	5.8	-	+	C.O.	50	6.5	-	-	C.
23	7.0	-	-	C.O.	51	6.4	-	-	C.
24	6.8	-	-	C.O.	52	7.2	-	-	C.
25	5.8	-	-	C.O.	53	7.3	-	-	C.
26	5.8	-	+	C.O.	54	7.2	-	-	C.
27	7.0	-	-	C.O.	55	5.9	-	-	U.
28	5.8	-	-	C.O.					

T.P. = Triple Phosphate; C.O. = Calcium Oxalate; C.P. = Calcium Phosphate; C. = Cystine and U. = Urate.

6. URINARY STASIS.

Review of the Literature.

In the recumbent human patient, stasis is thought to play an important part in the formation of upper urinary tract stones (Higgins and Straffen, 1963). In the dog, it is considered that stasis, and other factors that may prevent removal of minute calculi, would also predispose to urolithiasis (Treacher, 1966). Conditions causing urinary outflow obstructions, and conditions associated with the retention of urine in any part of the urinary tract, must be included as such predisposing factors.

The possibility that confinement, by favouring retention, was a predisposing cause of primary calculi formation and, by making infection of the bladder more likely, was also a factor in secondary calculi formation was mentioned by White, Treacher and Porter (1961). Treacher (1966) re-expressed this opinion and presumed that there was a greater tendency for confinement to be associated with pure-bred animals and small dogs than with mongrels and large dogs. He suggested that confinement may therefore be a factor in the high incidence of urolithiasis in small pure-bred dogs.

Bloom (1954) considered that stagnation of urine, consequent to obstructive conditions in the urinary tract, was associated with infection as a predisposing cause of urolithiasis.

Clinical reports of the association of urolithiasis with conditions causing incomplete bladder emptying in the dog have been recorded by Eisenmenger/

Eisenmenger (1957) who found urolithiasis in a dog with a persistent urachus, and by Candioti, Ubach and Cler (1937a and b) who recorded the association of urolithiasis and prostatic enlargement. Other possible causes of outlet obstruction, such as bladder neck abnormalities or urethral conditions, have not been reported in canine urolithiasis patients. A number of other conditions, such as those leading to interference with motor nerve supply to the bladder, pregnancy and the occurrence of vesico-ureteral reflux (regurgitation of the vesical contents into the ureters) may also lead to a degree of stasis within the urinary tract and presumably may predispose to urolithiasis.

Materials and Methods.

In an endeavour to evaluate the part played by the aforementioned conditions, each animal in this investigation was placed in lateral recumbency and radiographed in both the ventro-dorsal and lateral positions. Cystograms were also performed in 14 animals. The methods used for these procedures being those described by Douglas and Williamson (1963).

In some animals, the entire urinary tract was radiographically studied during the act of micturition because it was considered that such an examination was necessary to detect conditions such as bladder neck abnormalities and vesico-ureteral reflux. The method used for this examination is described below.

Micturating Urography.

In human medicine radiographic visualisation of the lower urinary tract/

tract is performed by introducing radio-opaque material into the bladder and radiographing the area during voluntary urination. In the dog such co-operation between radiographer and patient is impractical. However research workers, using dogs as experimental animals, use a technique whereby the bladder of an anaesthetised dog is filled with radio-opaque material via a urethral catheter until the stimulus of a filled bladder causes urination around the catheter. Although this method allows outlining of the urethra, it has the disadvantage of catheter causing partial obstruction during micturition. A variation of this method, avoiding this disadvantage by using the well known post-anaesthetic micturition reflex,was evolved. A high percentage of dogs with full bladders urinate when returning to consciousness from anaesthesia and advantage was taken of this reflex to carry out radiographic examinations.

Method.

Patients were anaesthetised with thiopentone sodium to approximately the depth required for intra-tracheal intubation. They were then positioned in lateral recumbency on a cassette and grid which were protected by a thin sheet of plastic, so that they were not contaminated by the urinated radio-opaque fluid.

The film size used was sufficient to ensure visualisation of the entire urinary tract. Fast-screen film was used in order to obtain satisfactory definition with short time exposures. These exposures were in the order of 0.04 - 0.4 seconds using 65 Kilovolts and 100 milliamps. Some variation in Kilovoltage was necessary in the very large and very small dogs.

In/

In the male dog, the penis was extruded and the external urethral orifice washed with 1:5000 Hibitane digluconate aqueous solution. A sterile nylon disposable catheter was then inserted into the urethra using a no-touch technique. In the female, the vulva was washed with the hibitane solution and dried with sterile gauze. A lighted vaginoscope was inserted through the lips of the vulva and the polythene catheter passed through the external urethral meatus. A twisting motion was often employed to facilitate entry of the soft catheter into the urethra. On rare occasions, difficulty was encountered in passing this type of catheter, and in such cases, metal catheters were used.

Following catheterisation, the bladder contents were drained and 10% urographin* in normal saline was then instilled through the catheter using a flutter valve connection. The bottle containing the fluid was kept at a steady height, approximately three feet above the animal, in an effort to standardise the pressure at a relatively low level. In very small dogs it was more expedient to use a 20 ml. syringe to instil the contrast media.

The amountof fluid necessary to fill the bladder without excessive stretching of the musculature (estimated by abdominal palpation) varied with the size of the dog and with the state of the detrusor muscle. In the smaller breeds 50-150 ml. were instilled. In the larger breeds, up to 300 ml. was found necessary to stimulate micturition at the required time. When the bladder was estimated to be full, a clip was applied to the catheter and the flutter-valve disconnected.

The/

* 2g. sodium and 13.2g. methylglucamine salts of 3, 5 diacetyl - 2, 4, 6 - triiodobenzoic acid.

The anaesthesia was allowed to lighten until the palpebral reflexes were re-established and the tongue curled on opening of the jaws. The catheter was then removed and in some cases micturition followed immediately, most however did not micturate until a further lightening of anaesthesia had occurred. Occasionally animals commenced to urinate whilst the radio-opaque material was being instilled into the bladder. In these cases, the catheter was immediately withdrawn and the X-ray taken. If vigorous involuntary movements commeced before urination, a little more intravenous thiopentone was administered, and additional urographin was instilled into the bladder. In some animals, particularly those with abnormalities of the urinary tract which resulted in gross urinary retention, micturition did not occur.

In the male dog, removal of the catheter under very light anaesthesia sometimes resulted in an ejaculatory type of micturition; these cases still permitted satisfactory micturating urographs to be taken.

In some few animals, in which no micturitional efforts were made, gentle digital pressure, applied to the bladder through the abdominal wall, initiated the micturition reflex.

Results.

A number of conditions possibly associated with urinary stasis were/

were found in cases of urolithiasis. These were:-

(1) Prostatic Enlargement

(2) Urethral Abnormalities

(3) Bladder-neck Obstruction

(4) Pregnancy

(5) Vesico-Ureteral Reflux.

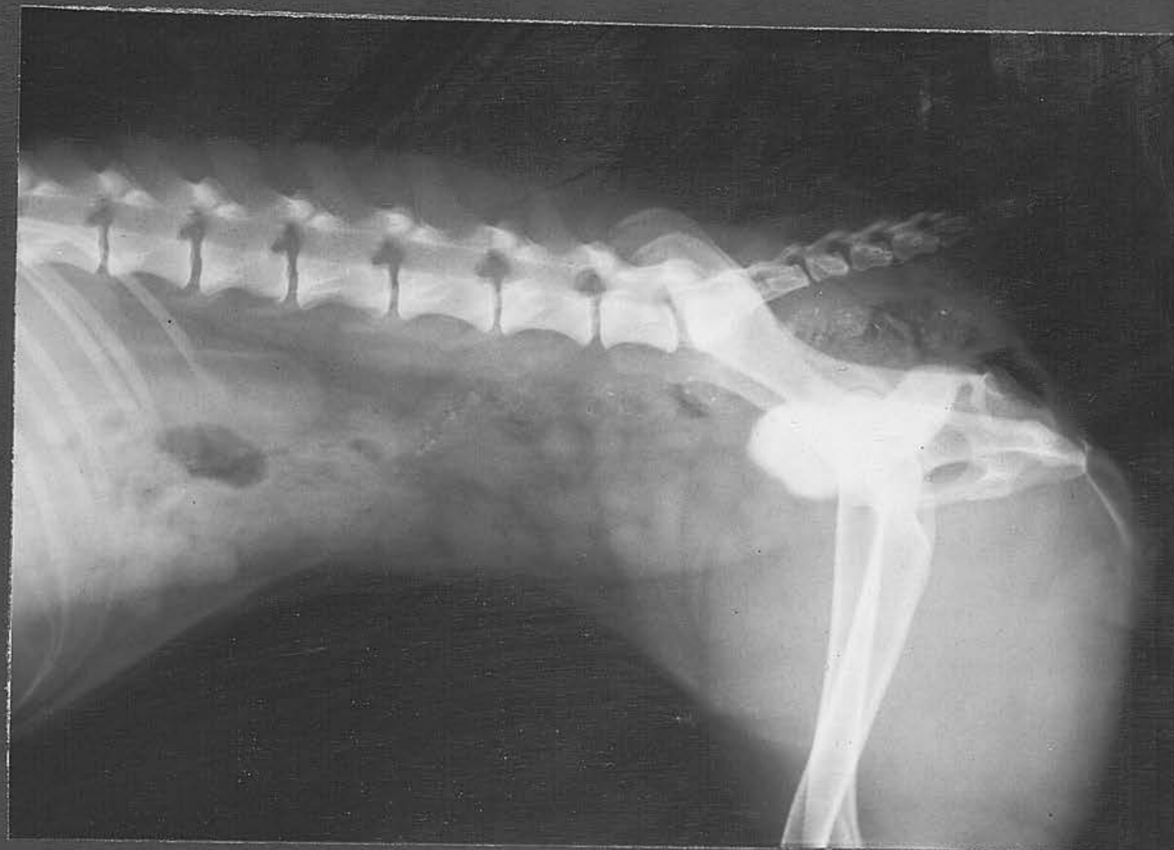
1. Prostatic Enlargement. Eight of the thirty-seven males with urolithiasis had apparently enlarged prostate glands. Enlargement of the prostate was diagnosed when the radiographic shadow of the anterior border of the prostate gland extended for more than 4 cms. beyond the shadow of the anterior border of the ilium on plain lateral radiographs or when other radiographic features of the prostate, rectal or abdominal palpation, or post-mortem indications of enlargement of this gland were observed. No attempt was made to differentiate various forms of prostatic enlargement. Five of the eight cases of prostatic enlargement occurred in patients with oxalate lithiasis; both dogs with primary hyperparathyroidism and a nine year old dog with cystine stone disease were also affected.

2. Urethral Abnormalities. These were diagnosed when abnormal changes in the urethral outline during micturating urography were observed. Two animals with urethral abnormalities were found in the 21 urolithiasis patients on which micturating urograms were performed. One animal (case 14) had an unusual step-like urethral course (Fig.18); the other (case 26) had a urethral diverticulum which was apparently the result of a previous ischial urethrotomy in this animal (Fig. 19).

(3)/

Fig. 18. Lateral Micturating Urogram in an Animal with a "Step-like" Urethra, Vesical Struvite Calculi and Concurrent Urinary Infection. Showing the Abnormal Urethral Outline and abnormal Bladder contraction. Compare with normal Urethral outline in Fig. 20c.

Fig. 19. Lateral Micturating Urogram in an Animal with Recurrent Oxalate lithiasis and a Urethral Diverticulum. The Diverticulum can be seen on the Posterior Aspect of the Urethra at the level of the Ischial Arch.




(3) Bladder-Neck Obstruction. Incomplete opening of the bladder-neck during micturition was detected in one animal (case 16).

Obstructions at the bladder-neck are common in man, but do not seem to have been reported in the dog. In this case the bladder-neck obstruction was diagnosed three-and-a-half months prior to the first clinical calculous episode. At this time no calculi were observed on radiographic examination but a mixed staphylococcal and streptococcal infection was present and the condition was treated with antibiotics; no treatment for the bladder-neck obstruction was performed. When presented with urolithiasis approximately three months later, a Proteus urinary infection was found. When this patient was first presented the features of the micturating urography were:-

(a) the presence of unilateral vesico-ureteral reflux unassociated with micturition. The reflux on this occasion did not extend throughout the entire length of the affected ureter (Fig.20a).

(b) During micturition the unilateral reflux was still present and a bladder-neck abnormality was also observed (Fig. 20b).

When this animal was later presented, on this occasion with urolithiasis, a repeat micturating urogram was performed and the following features noted:-

(a) Bilateral vesico-ureteral reflux was present; the radio-opaque material extending throughout the entire ureteral length and outlining the renal pelvises.

(b) The bladder-neck abnormality was again observed.

(c)/

Fig. 20a. Lateral Cystogram of a 6 year old Female Welsh Corgi with Urinary Infection. Showing Unilateral Vesico-Ureteral Reflux (unassociated with Micturition) extending into the Posterior Portion of the Ureter.

Fig. 20b. Lateral Micturating Urogram on the same Animal. Showing a Bladder Neck Abnormality and slight Unilateral Vesico-Ureteral Reflux. Compare with normal bladder neck in Fig. 20c.

Fig. 20c. Lateral Micturating Urogram of a 9 year old Female Dachshund with Vesical Struvite Lithiasis. Showing a normal Bladder Neck and Urethral Outline.

Fig. 20d. Lateral Micturating Urogram of the Welsh Corgi in Figs. 20a and 20b, taken three months later when the Animal was Presented with Vesical Struvite Lithiasis. Showing the original Bladder Neck Abnormality and Bilateral Vesico-Ureteral Reflux.

(c) The antero-ventral portion of the bladder outline was markedly irregular; a feature frequently seen in urolithiasis cases in this study. (Fig. 20d).

4. Pregnancy.

The association of pregnancy with the occurrence of significant bacteriuria in humans is well known (Pinkerton, Houston and Gibson, 1965). The increased incidence of infections during pregnancy is thought to be associated with partial obstruction in the upper urinary tract. It was thought that, assuming a similar situation occurred in dogs, the increased incidence of infection, may predispose to triple phosphate lithiasis in bitches. Details of the recent obstetrical history of females with urolithiasis were therefore recorded. Of the eighteen females with triple phosphate lithiasis found in this study, one (case 12) was pregnant at the time of a repeat clinical episode of this condition, 1 (case 3) had whelped 2 days prior to presentation for this condition, and another (case 1) had whelped 6 weeks prior to a clinical episode. The latter 2 cases had received antibiotics prior to presentation and no organisms were recovered from their urine; case 12 was found to contain significant numbers of both streptococci and staphylococci in the urine.

5. Vesico-Ureteral Reflux.

Nine normal dogs, 36 cases of various urinary disorders and 21 cases of urolithiasis were examined for the presence of this phenomenon.

Vesico-ureteral Reflux in Normal Dogs.

Results of the examination of 9 dogs with no signs of urinary dysfunction and a normal urinary sediment are shown in Table 41.

Table 41./

Table 41.

VESICO-URETERAL REFLUX IN NORMAL DOGS.

Breed	Age	Sex	Presence of Reflux
Mongrel	$5\frac{1}{2}$ mths	Male	Nil
Mongrel	Adult	Female	Nil
German Pointer	1 Year	Male	Nil
Border Collie	2 Years	Male	Nil
Mongrel	Adult	Female	Nil
Labrador Retriever	Adult	Male	Nil
Mongrel	Adult	Female	Nil
Mongrel	Adult	Female	Nil
Mongrel	8 mths	Male	Nil

Reflux was not observed in any of these animals.

Vesico-Ureteral Reflux in Miscellaneous Urinary Disorders.

The results of the micturating urographic examinations on 36 animals exhibiting signs of urinary dysfunction but in which no calculi were found are shown in Table 42.

Eleven cases of reflux were observed. The majority of which showed bilateral regurgitation of the vesical contents at micturition, to such an extent, that both kidney pelvises were outlined radiographically. Occasionally bilaterial or unilateral reflux unassociated with micturition was noted.

The/

TABLE 42

THE OCCURRENCE OF VESICO-URETERAL REFLUX IN MISCELLANEOUS URINARY DISORDERS

Breed	Age (year)	Sex	Urinary Condition	Bacteriology	Presence of Reflux	Other Observations
Cocker Spaniel	11	Male	Prostatic Enlargement	No organism	Nil	Passage of vesical contents into prostate
Welsh Corgi	5	Male	Urinary Infection	*P. mirabilis*	Nil	-
Miniature Poodle	Adult	Female	Intervertebral Disc Protrusion	No organism	Nil	-
Miniature Poodle	$1\frac{1}{2}$	Male	"Corkscrew Urethra"	No organism	Nil	-
Dachshund	8	Male	Prostatitis	No organism	Nil	Passage of vesical contents into prostate
Dachshund	$9\frac{1}{2}$	Female	Urinary Infection	*P. mirabilis*	Bilateral	Ventral Bladder Border Deformity
Boxer	8	Female	Urinary Infection	*E. coli*	Bilateral	-
Miniature Poodle	3	Male	Idiopathic Incontinence	No organism	Nil	-
King Charles Spaniel	Adult	Male	Idiopathic Urinary Disorder	No organism	Nil	Passage of vesical contents into prostate
Mongrel	Adult	Female	Urinary Infection	Klebsiella sp.	Bilateral	Irregular bladder outline
Mongrel	5	Female	Uraemia	No organism	Bilateral	-

Breed	Age (year)	Sex	Urinary Condition	Bacteriology	Presence of Reflux	Other Observations
Cairn Terrier	$7\frac{1}{2}$	Male	Idiopathic Urinary Disorder	No organism	Unilateral	Passage of vesical contents into prostate
Wire Haired Fox Terrier	2	Female	Urinary Infection	E. Coli	Nil	-
Border Collie	7	Male	Prostatic Enlargement	No organism	Nil	Passage of vesical contents into prostate
Bull Terrier	6	Female	Neurogenic Bladder	No organism	Nil	-
Springer Spaniel	11	Male	Cystic Prostate	No organism	Nil	-
Bull Terrier	7	Female	Tumour at Bladder neck	No organism	Nil	-
Cocker Spaniel	12	Male	Prostatitis	No organism isolated	Nil	Passage of vesical contents into prostate
Welsh Corgi	11	Female	Idiopathic Haematuria	No organism isolated	Bilateral reflux un-associated with mictur-ition	-
Doberman Pinsher	Adult	Female	Idiopathic urinary Disorder	No organism isolated	Nil	-
Miniature Poodle	1	Male	Urinary infection	Staphylococci	Nil	-
Yorkshire Terrier	11	Male	Cystic Prostate	No organism isolated	Nil	Passage of vesical contents into prostate

Breed	Age (year)	Sex	Urinary Condition	Bacteriology	Presence of Reflux	Other Observations
Miniature Poodle	$2\frac{1}{2}$	Male	Bladder outlet abnormality	No organism isolated	Nil	-
Mongrel	10 months	Female	Urinary infection	<u>E. coli</u>	Bilateral reflux	-
Welsh Corgi	6	Female	Urinary infection	<u>P. mirabilis</u>	Bilateral reflux	-
Alsatian	4	Female	Stenosis of external urethral meatus	<u>E. Coli</u>	Nil	-
Cocker spaniel	Adult	Male	Prostatic cysts	<u>E. coli</u>	Nil	Bladder neck obstruction and passage of vesical contents into prostate
Alsatian	3 months	Male	Hypospadias	<u>E. coli</u>	Nil	-
Pekingese	6	Male	Idiopathic urinary Disorder	E. coli	Bilateral reflux	-
Welsh Corgi	4	Male	Prostatic cysts	No organism isolated	Nil	-
Labrador Retriever	10	Female	Urinary infection	Proteus sp.	Nil	-
Mongrel	7	Male	Vesical tumour	Proteus sp.	Nil	-
Alsatian	10	Male	Prostatitis	Proteus sp.	Nil	-

Breed	Age (year)	Sex	Urinary Condition	Bacteriology	Presence of Reflux	Other Observations
Border Collie	11	Male	Prostatitis	Proteus sp.	Unilateral reflux without micturition. No reflux at micturition	Passage of vesical contents into prostate
Boxer	Adult	Male	Bladder tumour	P. mirabilis	Nil	-
Beagle	Adult	Male	Bladder Diverticula	P. mirabilis	Unilateral reflux	-

The Distribution of reflux in relation to infection and sex in these cases was subjected to the chi-square statistical analysis with the following results:-

(a) Infected vs Non-infected - Not significant ($X^2 = 0.38$)

(b) Males vs Females - Not significant ($X^2 = 2.66$)

During micturition 9 animals showed evidence of the passage of vesical contents into the prostate (Figs. 21, 22a, b, c and d).

Although the filling of a prostatic abcess by urine has been recorded in a dog (Archibald and Cawley, 1961), it is not generally realised that urine commonly passes into the prostate gland in these animals.

Micturating urography on animals with miscellaneous urinary disorders revealed the presence of two previously unrecorded urethral conditions. These are described in the appendix.

Vesico-Ureteral Reflux in Urolithiasis.

The results of the micturating urographic examinations for the presence of reflux in animals with urolithiasis are shown in Table 43. The table shows that 5 cases of vesico-ureteral reflux were found in the 21 urolithiasis cases examined. All were females, 4 of which were infected and 1 of which had recently been treated with antibacterial therapy. 4 of the cases were in animals with triple phosphate lithiasis. Bilateral reflux was observed in 4 of the 5 animals (Figs. 24a, b, c and d, 25); unilateral reflux was present in 1. In all cases the radio-opaque material was refluxed into the kidney pelvis. The animal with unilateral/

Fig. 21. Lateral Micturating Urogram in a 7 year old Cairn Terrier with an Idiopathic Urinary Condition. Showing slight changes in the outline of the Prostatic Urethra, some evidence of passage of the vesical contents into the prostate and Unilateral Vesico-Ureteral Reflux.

Fig. 22a. Lateral Micturating Urogram in an Adult Male Mongrel with passage of the vesical contents into the Prostate Gland. Slight changes in the outline of the Prostatic Urethra can also be seen.

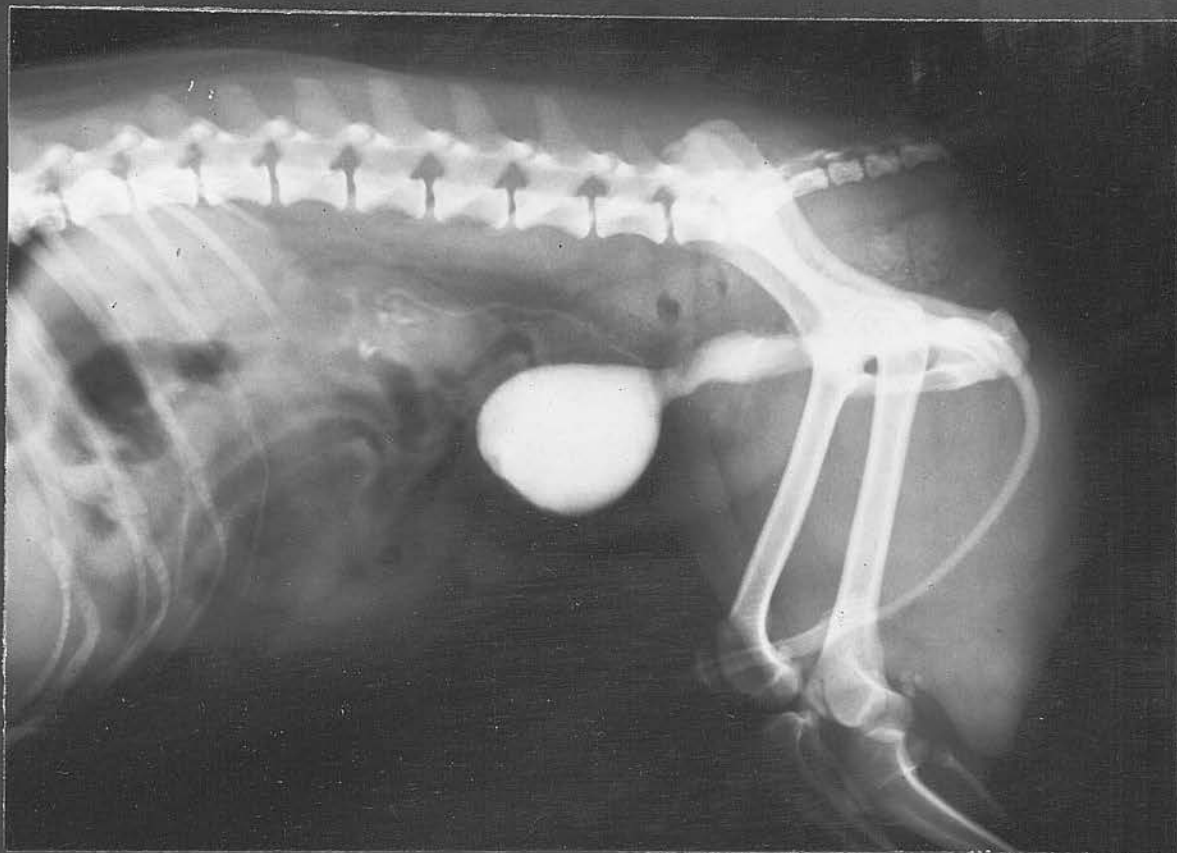


Fig. 22b. Lateral Micturating Urogram in an Adult Cocker-Spaniel with a Prostatic Cyst. Showing filling of the Cyst with Radio-Opaque Material during Micturition and a Bladder Neck Abnormality. This Animal was presented at the Clinic with Post-Micturitional Dribbling.

Fig. 22c. Lateral Micturating Urogram in an 11 year old Male Cocker Spaniel with Prostatic Enlargement. Showing passage of the Vesical Contents into the Prostate.


R·D·S·V·S

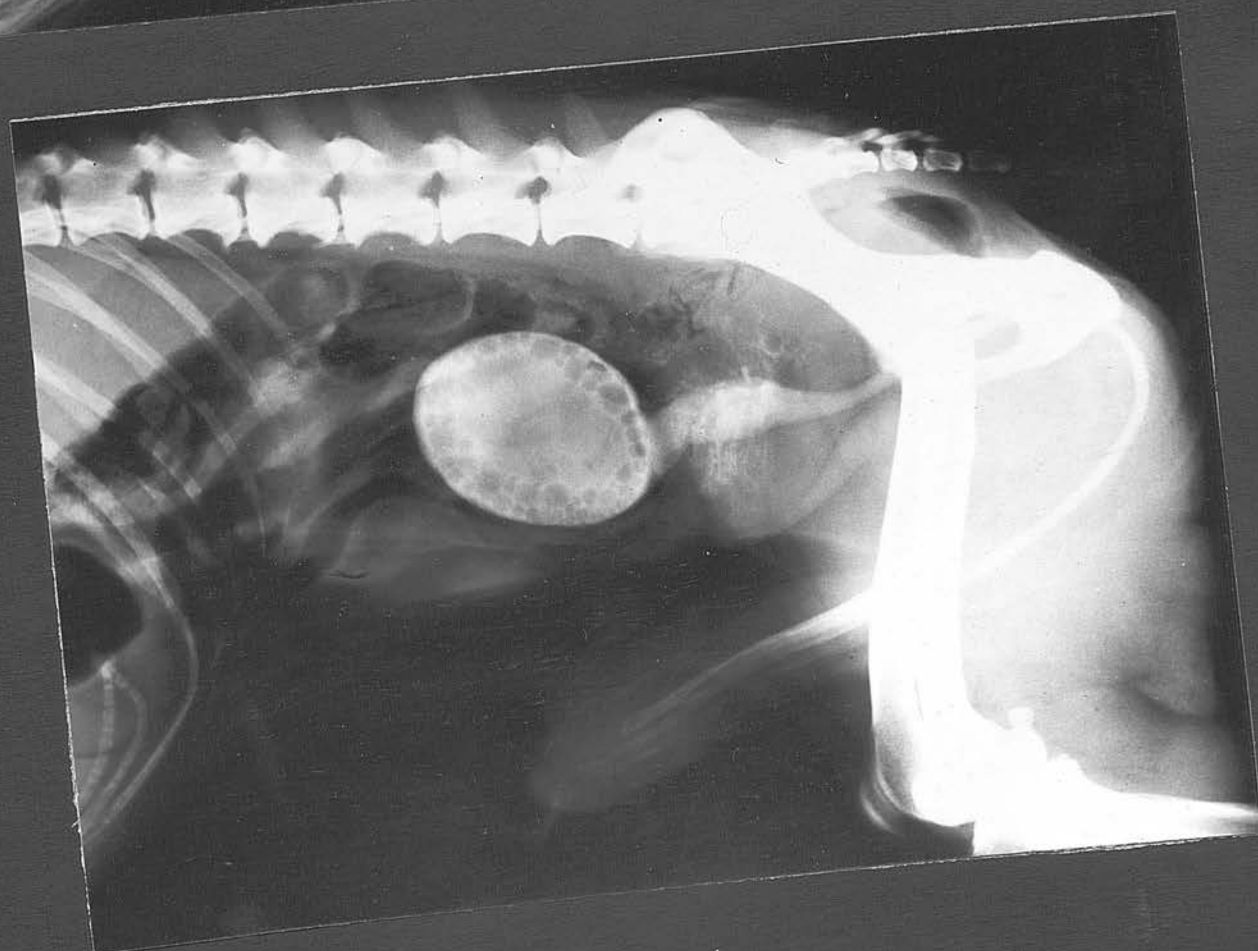


Fig. 22d. Lateral Micturating Urogram in a 12 year old Cocker Spaniel with Prostatitis. Showing passage of the Vesical Contents into the Prostate.

Fig. 23. Lateral Micturating Urogram in a 9 year old Dachshund Bitch with Vesico-Ureteral Reflux into Dilated Tortuous Ureters. The calyces of each Kidney are indistinct, probably indicating a Pyelonephritis. A Ventral Bladder Border Defect is also obvious.

Fig. 24a Micturating Urography on an 8 year old Welsh Corgi with Vesical Struvite Lithiasis and Urinary Infection. (a) Prior to Micturition. Showing a large single Laminated Calculus in the Bladder.

Fig. 24b. Micturating Urography on an 8 year old Welsh Corgi with Vesical Struvite Lithiasis and Urinary Infection. (b) During Micturition. Showing Bilateral Vesico-Ureteral Reflux and Abnormal Bladder Contraction.

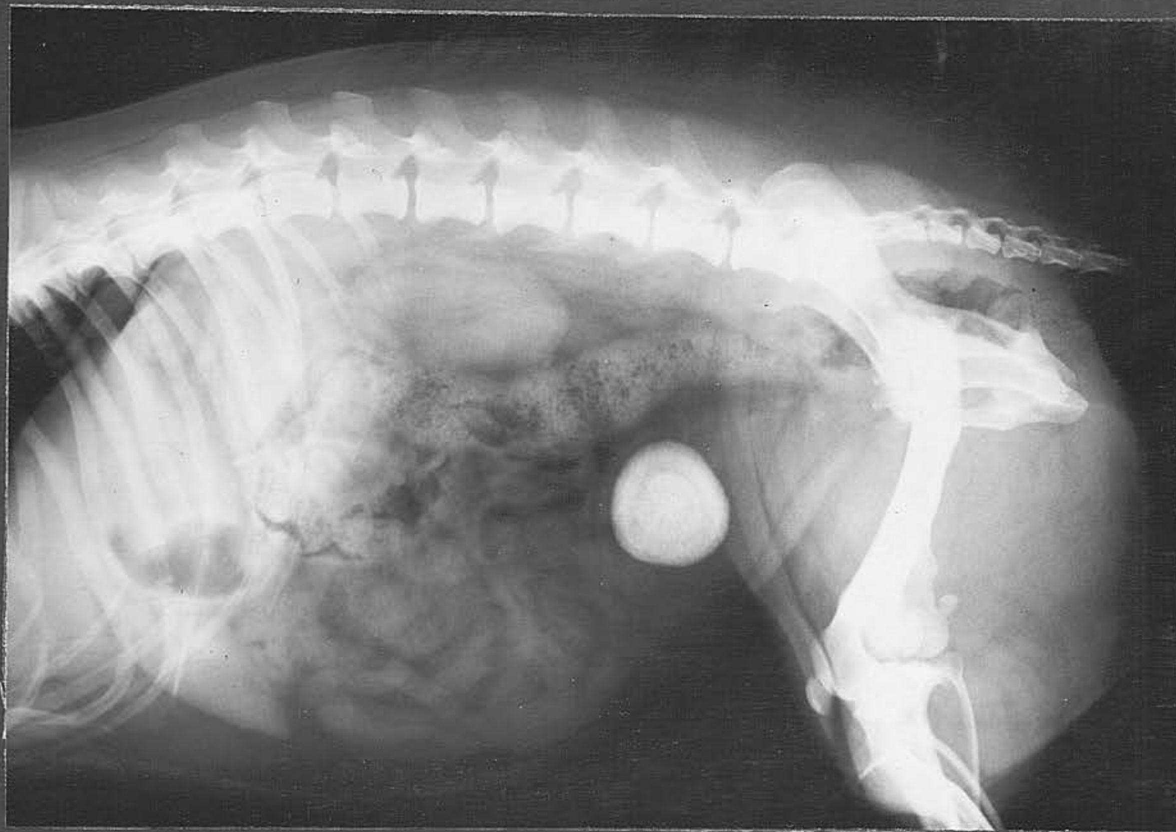


Fig. 24c. Micturating Urography on an 8 year old Welsh Corgi with Vesical Struvite Lithiasis and Urinary Infection. (c) Immediately following Micturition. Showing that most of the Radio-Opaque Material has been passed back from the Ureters into the Bladder where it remains as "residual Urine".

Fig. 25. Lateral Micturating Urogram in a 2 year old Female Miniature Poodle with Vesical Struvite Lithiasis. Showing Bilateral Vesico-Ureteral Reflux into normal Kidney Pelvises.

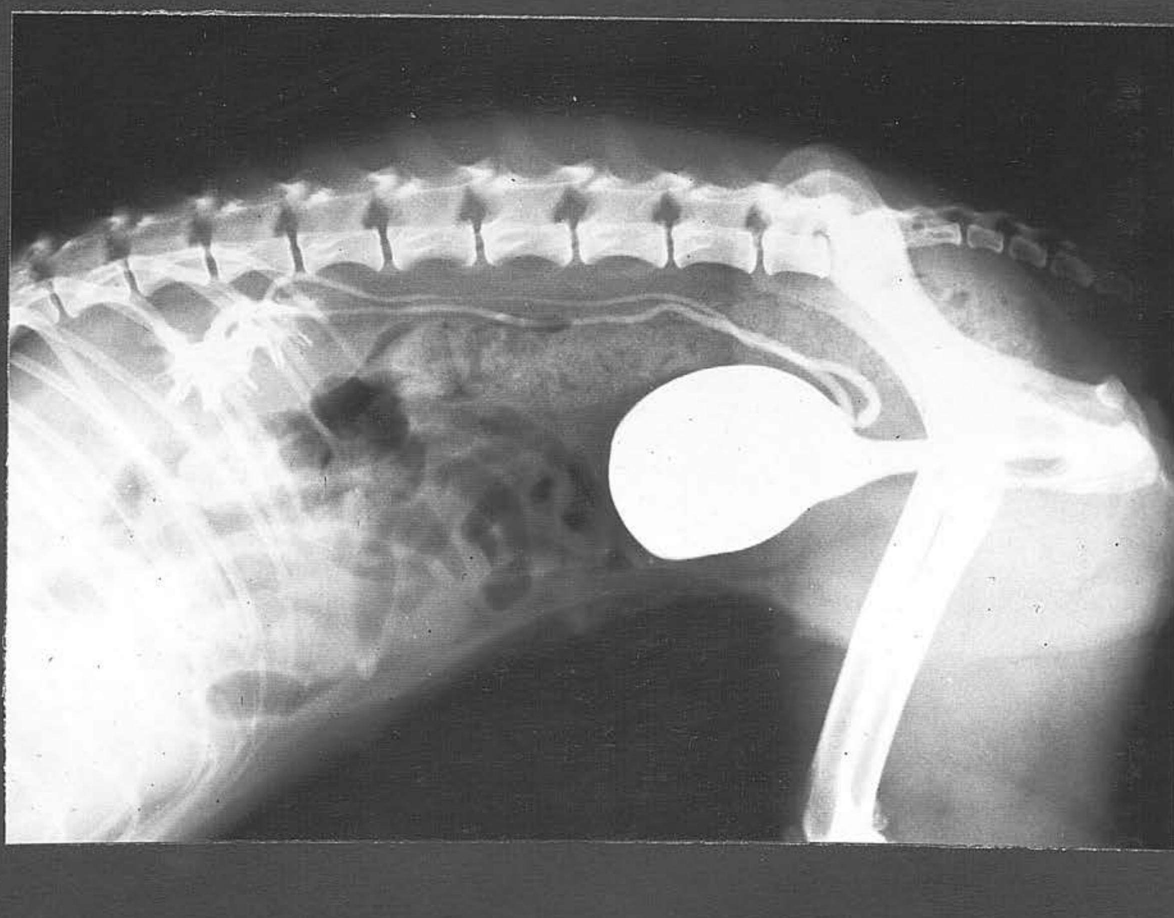


TABLE 43

RELATIONSHIP BETWEEN UROLITHIASIS AND VESICO-URETERAL REFLUX

Case No.	Sex	Chemical Stone Type.	Infection	Presence and Type of Reflux.
3	Female	Triple Phosphate	-*	Bilateral
4	Female	Triple Phosphate	+	Nil
5	Female	Triple Phosphate	+	Bilateral
6	Female	Triple Phosphate	+	Nil
12	Female	Triple Phosphate	+	Nil
13	Male	Triple Phosphate	+	Nil
14	Female	Triple Phosphate	+	Nil
15	Female	Triple Phosphate	+	Nil
16	Female	Triple Phosphate	+	Bilateral
17	Female	Triple Phosphate	+	Bilateral
22	Male	Calcium Oxalate	+	Nil
25	Male	Calcium Oxalate	-	Nil
26	Male	Calcium Oxalate	-	Nil
34	Male	Calcium Oxalate	-	Nil
36	Female	Calcium Oxalate	+	Unilateral
37	Male	Calcium Oxalate	-	Nil
38	Male	Calcium Phosphate	-	Nil
40	Male	Calcium Phosphate	-	Nil
47	Male	Cystine	-	Nil
53	Male	Cystine	-	Nil
54	Male	Cystine	-	Nil

* Antibacterial therapy had been administered to this animal prior to examination.

unilateral reflux (case 36) was found to have vesical stones, and renal stones in the kidney pelvis on the side affected (Figs. 26a and b).

The distribution of reflux, in infected cases, in the chemical stone types, and in the different sexes was analysed statistically with the following results:-

(a) Infected vs non-infected cases - not significant ($X^2 = 2.13$)

(b) Triple Phosphate lithiasis vs other chemical stone types - not significant ($X^2 = 1.27$)

(c) Males vs Females - probably significant ($X^2 = 4.65$)

Discussion

From the results recorded in this investigation, it appears that a significant proportion of animals with urolithiasis have concomitant urinary tract abnormalities. Approximately 30% of the 55 urolithiasis patients examined were affected in this manner. It would be reasonable to expect an even closer association between some urinary disorders and urolithiasis if more complete diagnostic investigations are performed in all affected cases. The nature of the relationship between these urinary conditions and urolithiasis is unknown. Some may play a part in the aetiology or natural history of urinary calculous diseases whilst others may be coincidental findings.

Prostatic Enlargement.

The fact that 5 of 8 cases of prostatic enlargement occurred in dogs with oxalate lithiasis may indicate a relationship between these two conditions,/

Fig. 26a Plain Lateral Radiograph of a 9 year old Female Cairn Terrier with Oxalate Stones in the Bladder and Left Kidney.

Fig. 26b. Lateral Micturating Urogram on the same Animal. Showing Unilateral Vesico-Ureteral Reflux into the Left Kidney Pelvis.


conditions, and it may, in part, explain the reason for the increased incidence of oxalate lithiasis in old male dogs. However, since oxalate formation and prostatic enlargement are more common in the aged male dog, the apparent association may merely reflect a similar age and sex distribution. The reason for the increased occurrence of oxalate lithiasis in older dogs however, is unexplained, and it seems possible that the conditions in the urinary tract in some animals with enlarged prostates may be more favourable for oxalate stone formation.

Urethral Abnormalities.

Only two cases of urolithiasis were found to have concurrent urethral abnormalities. These are of interest because neither the step-like urethral course seen in one animal nor the formation of a urethral diverticulum in the other has been previously recorded. Whether such conditions influence the formation of stone is unknown.

Bladder-Neck Obstruction.

The occurrence of incomplete opening of the bladder-neck at micturition, antedating the formation of triple phosphate calculi is also of interest. Experimentally, a correlation between obstruction of the urinary conduit system and the formation of infected uroliths has been recorded (Hryntschak, 1935; Suby and Suby, 1947), and the relationship between bladder-neck obstruction and urinary infection in children is well recognised (Stansfield, 1966). It is conceivable therefore that obstruction of the bladder-neck may influence struvite calculi formation by increasing the chances of urinary infection. No relationship between/

between the condition and urolithiasis however appears to have been recorded.

The radiographic features of this case (Fig. 20b) which show a collar-like impression at the bladder-neck protruding into the urethral lumen, is suggestive of the condition known in children as "Primary Bladder-Neck Hypertrophy" (Smellie, Hodson, Edwards and Normand, 1964).

Pregnancy.

The finding of urolithiasis in 3 bitches during pregnancy, or shortly following its termination, is of unknown significance as the number is not significantly different from that expected from a similar sized group of adult females.

Vesico-Ureteral Reflux.

It has been shown in this study that vesico-ureteral reflux is a relatively common occurrence in urinary disorders in dogs and that it occurs in cases of urolithiasis. Although the condition does not appear to have been recorded by veterinary clinicians, it has been observed in the dog by medical research workers (Barksdale and Baker 1930; Scott and de Luca, 1960), and its presence following ureteral transplantation has been suspected by Pearson, Gibbs and Hillson (1965).

Vesico-ureteral Reflux in Normal Dogs.

Modern authors, (Scott and de Luca, 1960; Levens and Metcalfe, 1964) using radiographic techniques for the detection of vesico-ureteral reflux, believe that it does not occur in the normal urinary tract of the adult dog. This opinion is shared by some earlier authors (Sampson, 1903/

1903: Gruber, 1929; and Barksdale, 1931) but is contrary to the opinion of others (Semblinoff, 1893; Coutarde and Guyon, 1894; Wislocki and O'Conor, 1920; and Graves and Davidoff, 1925). It may be significant that, the authors using more physiological techniques of vesico-ureteral reflux detection, tend to favour the hypothesis that reflux ofvesical contents into the ureters does not occur in the normal adult animal. Gruber (1929) thought that the occurrence of vesico-ureteral reflux in some previous experiments could be explained by the existence of overdistension of the bladder in the detection method used. He suggested that overdistension with consequent high vesical pressures tended to cause shortening of the ureteral valves which rendered them incompetent.

The fact that no reflux was observed in 9 normal dogs examined by micturating urography in this investigation suggested that this method per se did not lead to ureteral valvular incompetence.

Vesico-Ureteral Reflux in Miscellaneous Urinary Disorders.

Prior to this study, the occurrence of vesico-ureteral reflux in the dog has been noted only as a coincidental finding by research workers. Barksdale and Baker (1930) found it in 2 animals which, on autopsy, were found to have dense, post-operative adhesions in the bladder area, and Scott and de Luca (1960) recorded its association with an ectopic ureteral orifice in a mongrel bitch.

In man, reflux of the vesical contents is known to be a common concomitant of many urinary disorders, and the fact that, in the present investigation, 30% of 36 dogs with miscellaneous urinary disorders had concurrent/

concurrent vesico-ureteral reflux, indicates that it also commonly occurs in association with canine urinary disorders.

Again, in humans, reflux is known to be frequently associated with urinary infection (Johnston, 1962) and the findings in this investigation indicates that this may also be the case in the dog. The presence of urinary infection, in man, is thought to cause reflux by involving the intra-vesical portion of the ureters (the uretero-vesical valve) in an inflammatory process, thus causing it to become a more or less rigid tube, with consequent interference with its function as a valve.

Micturating urography on the animals in this series has also revealed that, during micturition, the vesical contents commonly flow into the prostate gland. This is another phenomenon that has not previously been considered common in the dog. Its significance is not known, but it is possible that it may play some part in the natural history of prostatic conditions.

Vesico-Ureteral Reflux in Urolithiasis.

The occurrence of vesico-ureteral reflux has not previously been reported in clinical canine urolithiasis. However, the possibility that calculi may cause reflux was suggested by Graves and Davidoff (1925). These authors found that, when sterile calculi were placed in the bladder of experimental animals, a slightly higher incidence of reflux, compared with control animals, was observed. In the present investigation, the fact that animals with uninfected urine and with non-triple phosphate calculi in the urinary tract, did not show reflux, indicates that the presence of calçuli *per se* does not cause this phenomenon.

As/

As in the animals with miscellaneous urinary disorders, a high percentage or urolithiasis cases with reflux were associated with urinary organisms, and it seems possible that its presence in these cases is related to concurrent urinary infection. Although the relationship between infection and reflux in urolithiasis patients was not statistically significant. The probable significance ($P < 0.05$) in the difference in distribution between the sexes in cases of urolithiasis is of interest, as a similar significant difference was not noted in the cases with urinary disorders other than lithiasis. Indeed half the 10 female urolithiasis patients examined were found to have reflux whilst no cases were observed in males with this condition.

The occurrence of reflux may influence the pathogenesis of urolithiasis: At micturition in this phenomenon some urine passes into the upper urinary tract, only to be returned to the bladder by ureteral peristalsis when micturition is completed. If certain organisms are present, the retained urine may assist in establishing a reservoir of infection which, in turn, may lead to urolith formation. Another possible influence on calculous formation may result in cases of infection. If, as is suggested by Suby (1954), renal infection is necessary for certain types of calculi formation, reflux may provide the mechanism whereby lower urinary tract infection may gain access to the kidneys.

PART 3.

ANALYSIS OF CALCULI

PART 3

ANALYSIS OF CALCULI

Review of the Literature

The methods used to determine the chemical composition of calculi are as follows:-

1. Appearance and Physical Characteristics.
2. Micro-Chemical Analysis.
   (a) Qualitative Micro-Chemical Analysis.
   (b) Quantitative Micro-Chemical Analysis.
3. Spectrographic Analysis.
4. Paper Chromatographic Analysis.
5. Crystallographic Analysis.
   (a) Petrographic Microscopy.
   (b) X-ray Absorption Analysis (Micro-Radiography).
   (c) X-ray Diffraction Analysis.
      (i) Debye-Scherrer Powder Camera Method
      (ii) Quantum-Counting Method.

1. Appearance and Physical Characteristics.

The simplest method of identification of the chemically distinct types of stone is by recognition of their appearance and physical characteristics. Several authors have described these properties in canine uroliths and a conspectus of their opinions is as follows:-

Phosphate/

Phosphate Stones.

Phosphate stones are usually described as being white or greyish-white in colour (Muller and Glass, 1911; Joest, 1924; Milks, 1935; White, Treacher and Porter, 1961; and Jubb and Kennedy, 1963) yielding a chalky powder when broken (White, Treacher and Porter, 1961; Jubb and Kennedy, 1963; and Verstraete, van der Stock and Mattheeuws, 1964). White, Treacher and Porter, (1961) suggest that single phosphate stones are usually rough whilst multiple stones are smooth, facetted and tetrahedral.

Oxalate Stones.

Most authors agree that oxalate stones are usually brown or blood-stained, with nodular, smooth or, more often, with sharp projecting plates. They are usually hard, heavy and brittle (Muller and Glass, 1911; Joest, 1924; Milks, 1935; White, 1944; White, Treacher and Porter, 1961; Jubb and Kennedy, 1963; and Verstraete, van der Stock and Mattheeuws, 1964).

Cystine Stones

Cystine stones are generally described as being yellowish in colour, small, smooth and spherical with a waxy appearance (Lentz, 1921; Joest, 1924; White, 1944; White, Treacher and Porter, 1961; and Jubb and Kennedy, 1963).

Treacher (1962) describes three distinct types of cystine stones occurring in the dog. The first conforming with the above description; the second being a larger, darker coloured stone with a nodular rough surface or a 'mulberry' appearance; the third type, found/

found in the renal pelvis, is also dark yellow to khaki in colour with a fairly smooth surface that may assume an irregular shape. The first and third types were found by Treacher, to be made up of concentric laminations, the second or 'mulberry' type having radial striations.

Urate Stones.

These have usually been described as being yellowish or brownish, small, smooth and spherical, and on section reveal concentric laminations (Muller and Glass, 1911; Joest, 1924; White, Treacher and Porter, 1961; and Jubb and Kennedy, 1963).

2. Micro-Chemical Analysis.

(a) Qualitative Micro-Chemical Analysis.

Analysis of human uroliths using qualitative micro-chemical techniques was carried out prior to the 19th century with similar analysis of canine urinary calculi being first attempted by Lassaigne in 1823. Qualitative chemical methods of analysis since that time have changed in detail, and recent methods of analysis of canine calculi have been published by White (1944), White, Treacher and Porter (1961), Schlaaff (1961) and Verstraete, van der Stock and Mattheeuws (1964).

The majority of authors using qualitative micro-chemical analysis on canine uroliths, report magnesium ammonium phosphate as the commonest chief constituent. Calcium oxalate, cystine and uric acid have also been commonly recorded. However, the reported occurrence of uric acid calculi has recently been questioned by Porter (1963), who suggests that stones of this type invariably consist/

consist of ammonium urate. Xanthine (Krook and Arwedsson, 1965) and Bilirubin (Wilkinson, 1962) have also been detected as constituents of canine uroliths when qualitative chemical methods of analysis were applied.

(b) Quantitative Micro-Chemical Analysis.

This type of analysis has been applied to urinary concrements in humans (Butt, 1956) Laboratory animals (Benjamin, Wilson and Leahy, 1945) and dogs (Verstraete, van der Stock and Mattheeuws, 1964). Variations in the techniques exist, but the principles of accurate sample weighing, dissolution in acid, and quantitative estimation of the ions present in the resultant solution, is the same throughout. From the results of analyses of this type the approximate percentage composition of calculi can be assessed. Using this method of analysis on canine uroliths, Verstraete and his co-workers (1964) found that there were a number of ions present other than those forming the main constituent, and for this reason they considered that canine uroliths were usually of mixed composition.

Porter (1963b) has described a method for the quantitative estimation of ammonium urate in canine uroliths using an enzymatic technique. He found that calculi of the urate type contained approximately 30 - 90% ammonium urate mixed with quantities of phosphate and/or oxalates.

3. Spectrographic Analysis.

Spectrographic studies have been made on human uroliths by Mathe and Archambeault (1940) and Thompson, Steadman, Benjamin and Scott (1944), in laboratory animals by Benjamin, Wilson and Leahy (1945) and in dogs by Porter (1963). In human calculi,

evidence/

evidence of unusual elements such as strontium, silicon and boron has been recorded (Mathe and Archambeault, 1940). Spectrographic techniques have also been used for the quantitative analysis of human uroliths by Thompson et al. (loc. cit.).

Porter (1963), using infra-red spectrophotometry as a method of spectrographic analysis, found that the infra-red spectra of salts was unsatisfactory for their detection, but that the method was useful in indicating the composition of urate stones.

4. Paper Chromatographic Analysis.

Organic calculi from dogs have been examined by this technique. With regard to urate calculi, Porter (1963) found that a considerably greater sensitivity was achieved by this method, compared with the murexide test.

In cystine stone detection, the use of two dimensional paper chromatographic techniques described by White, Treacher and Porter (1961) and Treacher (1962) has allowed quantitative analysis of these stones to be made. Using this technique, Treacher (1962) found that cystine calculi invariably contained more than 75% of pure cystine. In 12 cystine stones examined by Treacher, no other amino-acids were found but in all cases, some phosphate and occasionally oxalate were detected.

5. Crystallographic Analysis.

(a) Petrographic Microscopy.

Using this method, Nakano (1923) described the crystallographic features/

features of cystine and the hydrates of calcium oxalate in human calculi. Keyser (1923) applied the method to the examination of experimentally produced oxamide calculi in rabbits. Prien (1941) described the method for the analysis of human uroliths and published a number of papers on this subject over a period of many years (Prien, 1941; Prien and Frondel, 1941; Frondel and Prien, 1942; Prien and Frondel, 1947; Prien, 1949; and Prien, 1963).

The different crystalline constituents of calculi appear to be readily identifiable by petrographic Microscopy, the major disadvantage of the method being that considerable experience is required before a high degree of competence is attained.

(b) X-ray Absorption Analysis (Micro-Radiography).

In 1946 Engstrom described a method of identifying the nature and orientation of calcium salts in thin sections of uroliths by X-ray absorption. This method was later used by Hedenberg, Engfeldt and Engstrom (1953) to study calculi produced experimentally in rats. These authors found that calculi produced in vitamin A deficient rates with infected urine consisted of hydroxylapatite.

(c) X-ray Diffraction Analysis.

Two methods of X-ray diffraction analysis for the determination of the crystal structures in uroliths have been used.

(i) Debye-Scherrer Powder Camera Method.

This method consists of recording on X-ray film, the rays diffracted from powdered calculi. It was first used to study the crystallographic/

crystallographic structure of urinary calculi by Saupe in 1931. Since that time, X-ray diffraction studies of human urinary concrements have been reported by a number of authors, (Ranganathan, 1932; Jensen and Thygesen, 1938; Jensen, 1940; Jensen, 1941a and b; Prien and Frondel, 1947, Brandenberger and Schinz, 1949; Philipsborn, 1953; Carr,1953; and Bieler, Veith, Morriss and Biskind, 1964). Jensen (1941a and b) found that most human stones fell into three categories on the basis of their main crystalline constituents. These categories were:-

(1). Calcium oxalate in the form of Whewellite (CaC_2O_4,H_2O) and Weddellite ($CaC_2O_4,2H_2O$) and mixtures of these together with 'colloidal' apatite.

(2) Alkaline infected stones of struvite, apatite and possibly ammonium urate.

(3) Uric Acid.

Less frequent chief constituents such as Brushite ($CaHPO_4,2H_2O$) and calcium phosphate ($Ca_3(PO_4)_2$) were also found.

Prien and Frondel (1947) combined this method with that of optical crystallography in the study of uroliths from 600 human patients. Their findings were essentially similar to those of Jensen's although cystine was also recorded. In addition to the crystalline compounds found by Jensen, calcium oxalate trihydrate and Whitlockite (anhydrous calcium phosphate) were also observed. Murphy and Pyrah (1962) listed the crystalline compounds isolated from human uroliths, and in addition to the compounds mentioned above, includedsodium acid urate monohydrate, newberyite ($MgHPO_4,3H_2O$), gypsum ($CaSO_4,2H_2O$), Zinc phosphate tetrahydrate

and/

and argonite ($CaCO_3$).

Prien (1963) was of the opinion that two types of apatite occurred in human uroliths. One was hydroxyl-apatite and the other, which they considered to be the 'colloidal apatite' described by Jensen, was carbonate apatite. According to Lagergren (1956), however, apatite is the collective name given to a series of phosphates having the same crystallographic structure and a very similar composition, giving almost identical diffraction patterns, the identification of which is difficult since apatite occurs in the poorly crystallised state which results in broadened diffraction lines. Lagergren suggests that the presence of hydroxylapatite as a crystalline compound in uroliths is undisputed but the existence of carbonate apatite is equivocal. He further suggests that, since the existence of carbonate apatite has not been proven, it is reasonable to regard the apatite in calculi as hydroxylapatite which may exist with surface-bound carbonate.

X-ray diffraction studies on a series of canine uroliths was reported by White, Treacher and Porter (1961). Struvite, Apatite, Weddellite, Whewellite, Cystine, Uric acid and Urate were found. Difficulty in identifying the chief constituent of urate stones was experienced.

Mulvaney et al. (1965) used this method of analysis to confirm the presence of tetracycline as the chief constituent of a canine urolith.

(ii) Quantum-Counting Method.

Recently, the sensitivity of X-ray diffraction methods has been increased by the development of a quantum-counting technique. This

method/

method entails the detection of each diffracted quantum of radiation in a Geiger-Muller counter X-ray diffractometer. The technique is simpler and less time consuming than the previous method. It has been used to identify human calculous constituents by Smothers and Siegel (1954) and by Lagergren (1956). It does not appear to have been used in the analysis of a series of canine uroliths.

Materials and Methods

Calculi from all 55 cases of urolithiasis were chemically analysed and their appearance and physical characteristics recorded. The stones were divided into groups according to their main chemical constituents, as determined by routine qualitative chemical tests (Varley, 1954). In 6 cases, qualitative micro-chemical tests were the only analysis applied. In the remaining 49 cases, quantitative micro-chemical analysis was carried out. In stones composed mainly of magnesium ammonium phosphate or calcium salts, the amounts of calcium, phosphorus, magnesium, ammonia and oxalate were determined. In cystine stones, only the ammonia and phosphorus levels were determined and in urate stones, only the amount of ammonia present was estimated. Three animals (cases 24, 30 and 34) had calculi analysed from 2 anatomical positions in the urinary tract, and in a further 2 cases (cases 37 and 38), calculi from consecutive episodes were quantitatively analysed.

Calculi from 35 cases on which quantitative chemical analysis had been performed, were also examined crystallographically by the Quantum-counting X-ray diffraction technique. The calculi from consecutive clinical episodes in cases 37 and 38 were re-examined

by/

by this method. One urolith (case 5) in this series had 3 visually distinct crystalline layers present and all layers were examined crystallographically.

Methods

Quantitative Micro-Chemical Analysis.

Sampling: Where the calculi were small, a number of complete stones were used as the sample; where large a representative sample of all layers was obtained by sectioning through the centre.

Calcium Magnesium, Phosphorus and Ammonia. Since in some cases the amount of material available was limited, a scheme of analysis in which a range of constituents could be determined on a single weighed sample was preferred. Also such a scheme should reduce sampling variability. Amounts of the order of 100 milligrams were weighted on a STANTON A 43 balance; transferred to 6" x 1" quickfit MF 24/2 test tubes, and digested until colourless by adding 1 ml. of 50% (by volume) sulphuric acid and heating on a hot plate.

In some cases, small amounts of solid potassium persulphate A.R. were added to complete the oxidation. The digest was cooled and quantitatively transferred to 50 ml. graduated flasks, diluted to the mark and well mixed. (Solution A.)

This solution was then used for the estimations of total nitrogen, phosphorus, calcium and magnesium.

Estimation of Calcium.

5 ml. of solution A was pipetted into 15 ml. centrifuge tubes,

made/

made alkaline with 50% NH_4OH and acid with 50% acetic acid, and 1 ml. of 4% ammonium oxalate was added. The mixture was stirred with a fine glass rod which was washed into the tube on its removal, and the tubes were allowed to stand overnight for complete precipitation of calcium. Tubes were centrifuged at 3,000 revs/min. for 15 minutes, washed twice with 5 ml. 2% NH_4OH and allowed to drain. Calcium was titrated with N/50 potassium permanganate as in Methods of Analysis. A.O.A.C. 1960.

Estimation of Magnesium.

The supernatants from the calcium separation were collected in 25 ml. graduated flasks, made slightly acid with acetic acid, diluted to the mark and well mixed. Aliquots were pipetted (2 ml. with triple phosphate calculi; 5 ml. with other types) into centrifuge tubes. 0.5 ml. of 5% ammonium phosphate, containing 5 ml. of concentrated ammonium hydroxide per litre, were added with stirring, followed by 2 drops of concentrated ammonium hydroxide, and left to stand overnight. They were then centrifuged, the supernatant fluid was syphoned off, and the tube washed with 5 ml. 10% ammonium hydroxide. The samples were again centrifuged and the wash-liquid syphoned off. The washing was repeated a second time, and the samples finally washed with 5 ml. of 75% alcohol containing 10 ml. concentrated ammonium hydroxide per litre. The supernatant was again removed by syphoning and the tubes allowed to stand in a warm place until the ammonia had evaporated.

To the residue 1 ml. of molybdate reagent (as used for 'phosphate determination/

determination - see under), 3 ml. of distilled water and 1 ml. of metol reagent (as used for phosphate) was added and the mixture made up to 10 ml. with distilled water. This was then mixed and allowed to stand for 10 minutes and compared, in a UNICAM SP 1400 absorptiometer (set at a wavelength of 680 μ) with a standard solution of magnesium that had been prepared in a similar manner.

Estimation of Total Phosphorus.

Solution A was diluted one in ten and an aliquot, (usually 2 ml.) used for phosphorus estimation by the procedure of Gomori (1942). Aliquots were pipetted into 10 ml. graduated tubes, 1 drop of phenolphthalein added, and sufficient 10% NH_4OH to give a faint pink colour. 1 ml. of ammonium molybdate reagent (7.5 gms. of ammonium molybdate in about 200 ml. distilled water with 100 ml. of 10 N sulphuric acid added, and made up to 400 ml. with distilled water) was added followed by 1 ml. of metol reagent (1 gm. of metol P-Methylaminophenol sulphate dissolved in 100 ml. of 3% sodium metabisulphite). The mixtures were made up to 10 ml. with distilled water, mixed and allowed to stand for 10 minutes, before being compared in an absorptiometer (Unicam S.P. 1400 set at a wavelength of 680 mμ) with a standard phosphate solution containing 0.04 mgm. of phosphorus per 5 ml. of solution.

Estimation of Total Nitrogen.

15 ml. of solution A were pipetted into a Markam's micro-Kjeldahl distillation apparatus, made slightly alkaline with 40% NaOH, and steam distilled for 15 minutes into 10 ml. N/20 sulphuric acid./

acid. Following the distillation, the excess acid present was back-titrated with N/25 NaOH, using methyl red as an indicator.

A blank titration of the N/20 sulphuric acid with N/25 NaOH was also carried out.

Estimation of Oxalate.

Representative samples of approximately 20 mgm. of calculi were weighed into 250 ml. conical pyrex flasks. 30 ml. of 2N H_2SO_4 were added and the mixture brought to the boil to dissolve the calculi. When solution was complete it was titrated with N/25 potassium permanganate.

NOTE This method is only applicable when readily oxidising substances such as cystine and ammonium urate are absent. However, since there does not appear to be appreciable amounts of these substances in oxalate stones, interference with the estimations from this source would be minimal. However, other readily oxidisable organic material, such as proteins, may cause the results to be abnormally high. The presence of cystine and ammonium urate can be suspected when no distinct end point can be reached.

Calculations of the Approximate Percentage of Chemical Constituents of Calculi from the Percentage of Ionic Components.

The approximate composition of magnesium ammonium phosphate stones and the calcium containing stones were calculated as follows:-

From the percentage of ionic components, using the atomic weight of each element, the percentage phosphate (PO_4) ion and ammonium (NH_4) ion were calculated. The amount of magnesium ammonium phosphate hexahydrate was calculated from the level of the magnesium ion. The

phosphate/

phosphate required for magnesium to form magnesium ammonium phosphate was then calculated and the excess phosphate noted. Similarly, the amount of calcium required for the oxalate present to form anhydrous calcium oxalate was calculated, and the excess calcium recorded. The approximate amount of calcium phosphate, calculated as apatite, that could be formed by the excess calcium and phosphate was then determined.

Crystallographic Analysis

Method.

Sampling of the calculi was undertaken in a manner similar to that described for quantitative micro-chemical analysis. The aliquot taken was powdered and a few drops of acetone added. This was then mixed thoroughly and several drops of the suspension placed on a glass slide and spread with a sharp-pointed needle. The acetone was allowed to evaporate leaving the powder evenly distributed over the surface. The slide was then placed in a goniometer and positioned in the path of a parallel monochromatic X-ray beam emitted from a Philip's X-ray Diffraction generator set at 36 Kilovolts and 20 Milliamps. Approximate focusing of the X-ray beam was obtained by using the Bragg-Brentano system Bunn, (1961). The goniometer was set to rotate at a rate of twice the angle of 2 theta (Θ) per minute (Θ donates half the angle between the incident and diffracted rays). Each recording was commenced at a 2 Θ angle of 4^{o} and was terminated at approximately 50^{o}. The beam, after diffraction from the sample, was collected in a scintillation counter, passed through a Philips Discriminator unit (P.W. 4082) to remove extraneous rays, and recorded on a chart recorder (Philips P.W. 1051). Discriminator settings were/

were:-

Contamination, 2; Amplitude, 22 volts; Channel width, 24 volts. The chart recorder settings were:- Rate meter, 4; Time Constant, 2 seconds; Paper speed, 20X (approximately 14 mm/minute), and the scintillation counter voltage was 840.

Theory of Method

The fundamental principle of X-ray diffraction is that when a narrow beam of monochromatic X-rays passes through a crystal or crystalline powder it is diffracted from the atomic planes present. Each crystalline substance consists of a series of parallel atomic planes and the spacial relationship of such planes vary, each pure crystalline material having a characteristic arrangement of these. Consequently when the X-ray beam is diffracted(reflected) from these planes and recorded, a pattern characteristic for each crystalline substance results. When using a Geiger-Muller Counter X-ray Diffractometer, the Diffracted X-rays are collected and transformed into electrical impulses which can be counted directly or, as in this case, transferred to an automatic strip-chart recorder. Using the latter method of recording, when increased numbers of electrical impulses are received by the recorder they are indicated by peaks developing above the base line.

Detailed information regarding the theory of X-ray Diffraction Crystallography is published in the text book "Chemical Crystallography" by Bunn(1961).

Analysis of Data.

The index to the X-ray Powder Data File (1959) records the spacing between/

between the crystal lattice (d spacing) for the major constituents of uroliths, and the relative intensity of the diffractions from each atomic plane for a given crystalline substance. These were converted to 2 Θ angles by referring to "Tables for Conversion of X-ray Diffraction Angles to Interplaner Spacing" (1950). With this information, comparison was made with each X-ray diffraction graph for prominent peaks at these angles, when the peaks (recorded as 2 Θ angles) corresponded to those of a known crystalline substance and were in a similar intensity ratio (i.e. amplitude of the peaks) the presence of the substance was confirmed.

When mixtures of crystals are present, the principle used in identification is to record the 2 Θ angle with strongest intensity and find the crystalline substance from which it results, by reference to the index. The remaining 2 θ angles of this substance then accounts for some of the other peaks. The strongest of the remaining peaks is then used in the same way for identifying the second consituent and the procedure is repeated until each peak is accounted for.

Results.

1. Appearance and Physical Characteristics of Uroliths.

Triple Phosphate Stones. (Fig. 27)

Type 1. Multiple; usually semi-transparent or opalescent on removal from the urinary tract, but becoming more creamy-white and opaque on standing; smooth, or more often, with a slightly roughened surface; usually/

usually small and irregularly shaped with a tendency to be rounded. These uroliths are concentrically laminated and, on cutting, yield a few crystalline flakes rather than a powder.

Type 2. 'Coral' or 'Rough-Stone' Type. Roughened or pitted exterior, large, single, creamy-white coloured, usually rounded with some degree of flattening; on cutting, yields a chalky powder. These uroliths are also concentrically laminated.

Type 3. 'Pyramidal' Type. Tetrahedral shaped with blunt corners; greyish-white in colour, with a smooth surface; usually occur as a few large vesical stones. On cutting these stones show radial striations.

Note. Other types of triple phosphate stones also occur but, in this investigation, were relatively rare and were not classified.

Oxalate Stones.

Type 1. Characterised by the extension of spicules (plate-lile projections) from the surface of the stones; usually dark-yellow in colour but occasionally brown or orange. Almost always multiple and seldom reach a large size. They are heavy and difficult to cut. Their overall shape may be very irregular. On cutting, they reveal concentric laminations.

Type 2. Very dark-yellow, (Khaki) coloured, rounded uroliths with a roughened surface. They are difficult to cut, and when sectioned, are found to be concentrically laminated.

Note. Other types of oxalate cabuli do occur but, in this series, were in insufficient numbers to be classified.

Cystine/

Cystine Stones. (Figs. 28 and 29)

Type 1. Multiple, small, spherical, yellow, smooth calculi, which on cutting, yield a white powder, and are seen to be concentrically laminated. The majority of cystine stones are of this type.

Type 2. 'Mulberry' Type. Smooth, but nodular surface, yellow in colour, but darker than other types of cystine stone. May appear to be an aggregation of Type 1 cystine stones and on cutting reveals radial striations.

Urate Stone.

Urate stones were only present in one patient. They were greyish-white in colour, and concentrically laminated; actually appearing to consist of a number of shells.

2. Results of Chemical and Crystallographic Analysis.

(a) Chemical Analysis.

Chemical analysis of the calculi from the 55 urolithiasis cases revealed that the chief constituents were:- Magnesium ammonium phosphate, calcium oxalate, calcium phosphate, cystine and ammonium urate.

The qualitative micro-chemical analysis on calculi from 6 animals showed the chief constituent to be; calcium oxalate in 3 cases; cystine in two cases, and magnesium ammonium phosphate in one case. Portions of cystine stones from all cases of cystine stone disease were powdered, dissolved in ammonia and, after evaporation, examined microscopically for the presence of cystine crystals.

3. Results of the Determination of the Percentage Ionic Components/

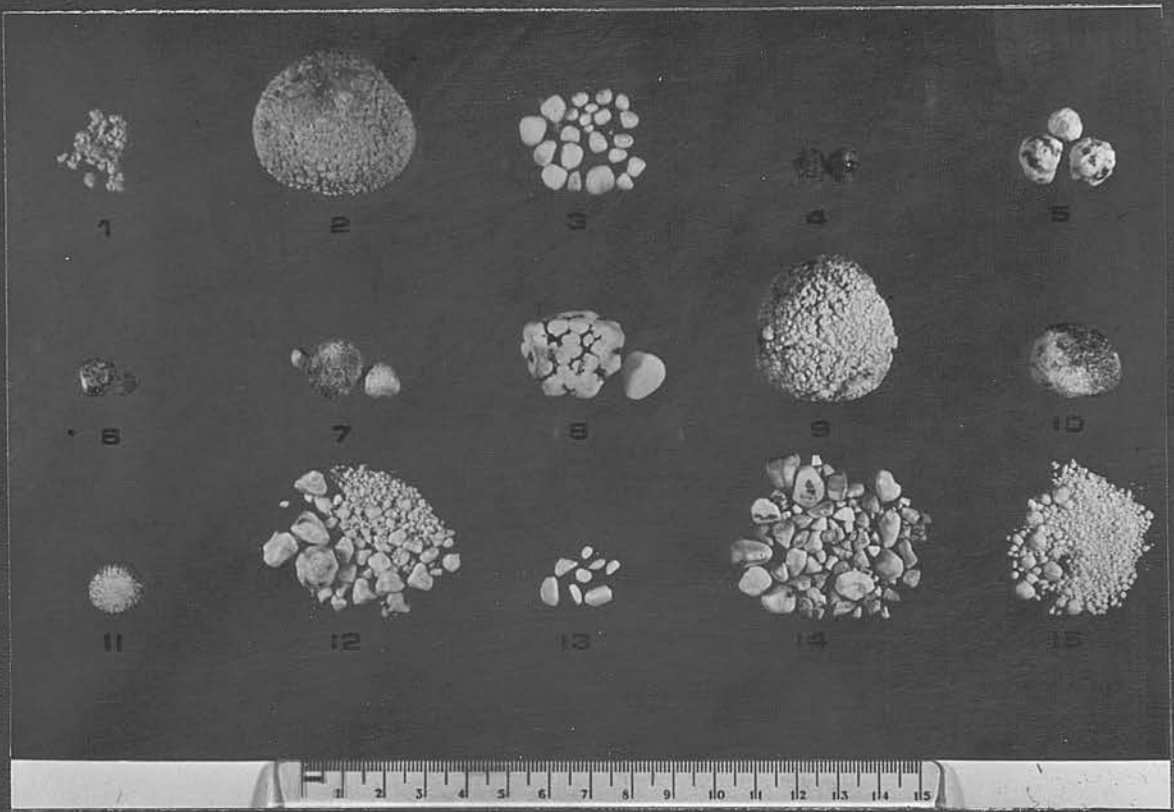
Fig. 27. Calculi Removed from 15 Cases of Struvite Lithiasis Associated with Staphylococcal Urinary Infection. Showing Variation in Types of Struvite Stones occurring in Staphylococcal Infected Urine.

Nos. 5 and 12 are typically Type 1 Struvite Stones.

Nos. 2, 9 and 10 are typically Type 2 Struvite Stones.

No. 3 is a Type 3 Struvite Stone.

Fig. 28. Cystine Stones removed from 6 Cases of Cystine Stone Disease. Showing the Typical Common Type 1 Cystine Stones.


1
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

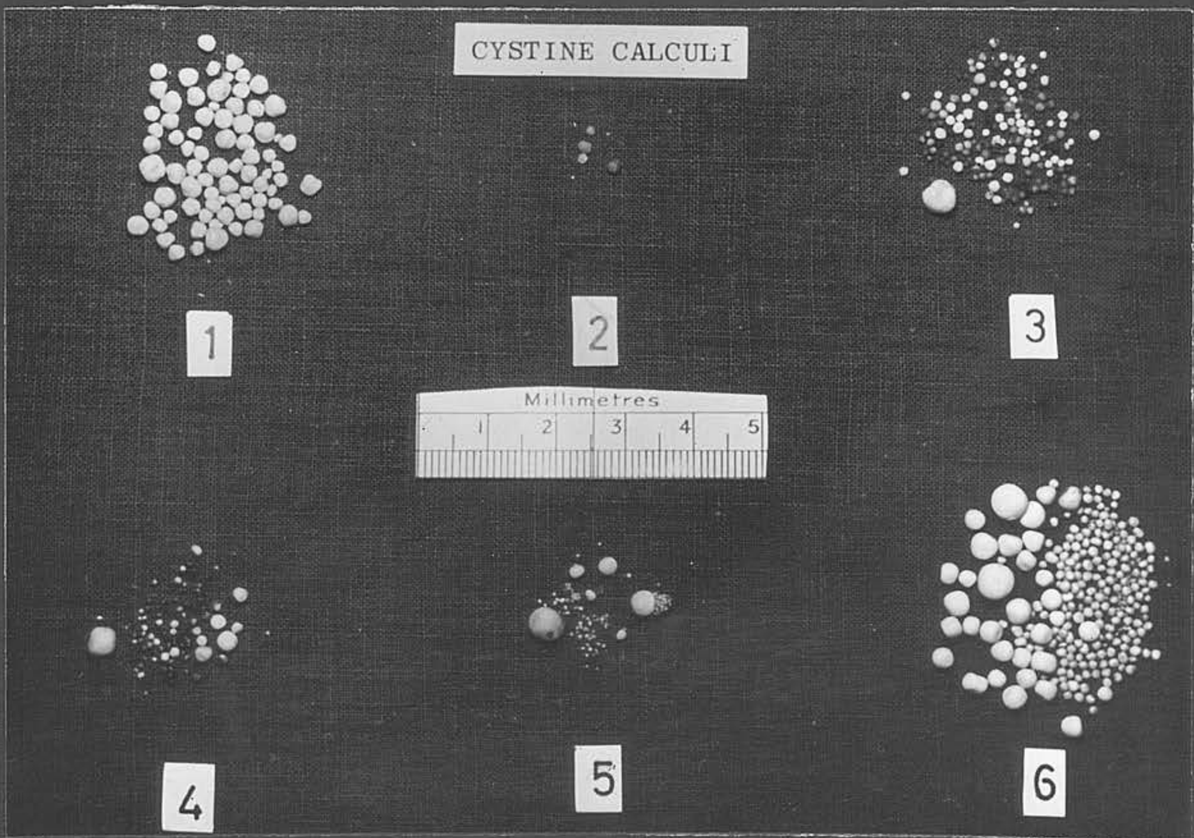
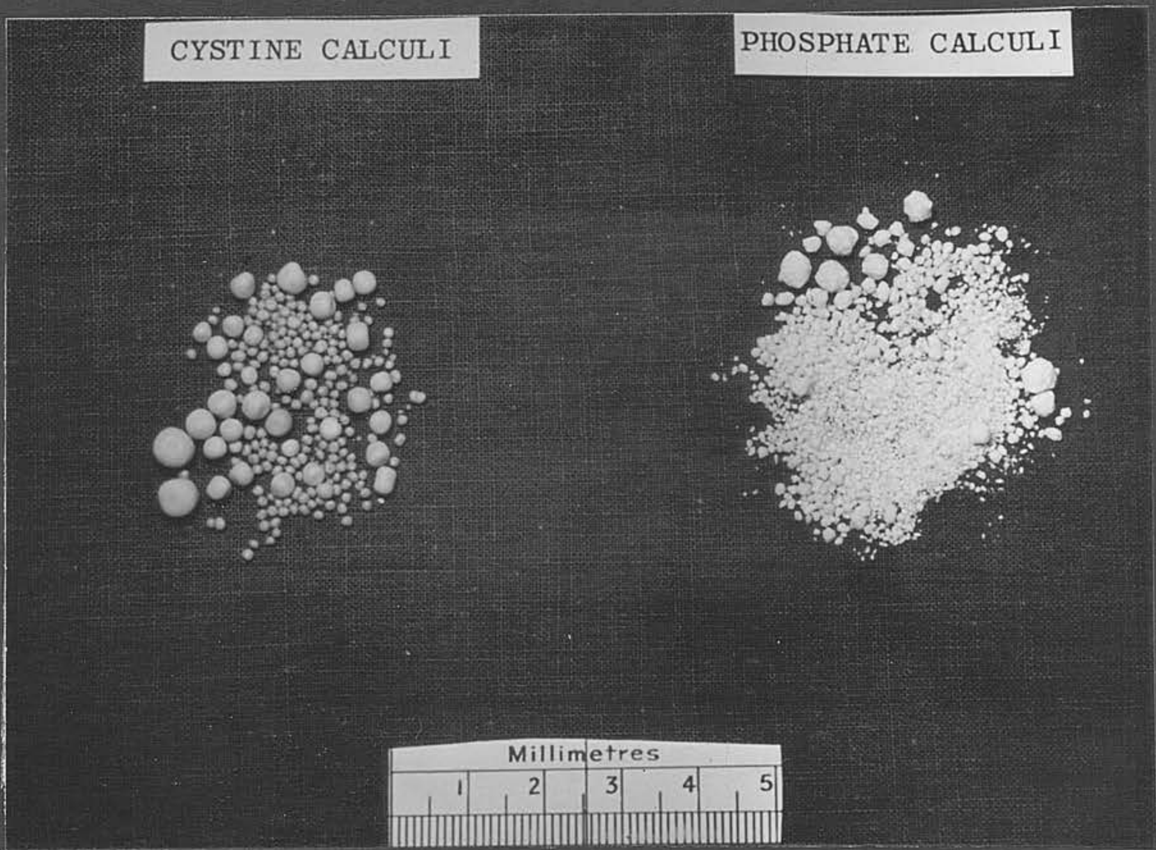

CYSTINE CALCULI
1
2
3
Millimetres
1 2 3 4 5
4
5
6

Fig. 29. Difference Between Multiple Cystine Stones and Multiple Triple Phosphate Stones.

Cystine Stones are Yellow, Smooth surfaced and Spherical. Even very small stones are rounded. Phosphate Stones are creamy-white, with a slightly roughened surface. Small Calculi are often irregular shaped.


CYSTINE CALCULI
PHOSPHATE CALCULI
Millimetres
1
2
3
4
5

3. Results of the Determination of the Percentage Ionic Components of Uroliths.

Tabulated results of the percentage of calcium, phosphorus, magnesium, ammonia and oxalate found in inorganic stones are shown in Tables 44, 45, 46,50 and 51.

With regard to organic uroliths, the ammonia concentration served to distinguish them from the inorganic types and to differentiate them into cystine and urate stones. The ammonia in cases of cystine stones varied from 13.30 to 16.62 grams% whilst in the urate stones was found to be 41.21 grams%. The latter stone gave a positive reaction to the murexide test and this result, together with the extremely high ammonia content found, indicated that the chief constituent of the stone was ammonium urate. Cystine crystals were seen in all cystine stones that were treated with ammonia and, after evaporation, examined microscopically.

Comparison of the variation in the percentage of ionic components in magnesium ammonium phosphate stones and the calcium containing stones (Table 49) shows that distinct differences exist. With regard to the percentage of calcium present in these two types of stones, the lowest amount recorded in the calcium containing stones was 23.77 grams per cent whilst the highest amount recorded in the magnesium ammonium phosphate stones was 10.27 grams per cent. The percentage of magnesium found in each of these stone types was similarly distinctive, the highest amount recorded in the calcium containing stones (1.69 grams per cent) being considerably less than the lowest amount recorded in the magnesium ammonium phosphate type (7.38 grams per cent). An obvious distinction between calcium containing stones and magnesium ammonium phosphate stones can/

TABLE 44

RESULTS OF THE QUANTITATIVE CHEMICAL ANALYSIS OF TRIPLE PHOSPHATE STONES

Case Number	Calcium Ca g%	Phosphorus P g%	Magnesium Mg g%	Ammonia NH_3 g%	Oxalate C_2O_4 g%
1	9.94	12.93	7.50	6.11	2.14
2	2.47	13.06	9.82	7.00	0.58
3	8.71	12.14	8.70	7.86	2.00
4	0.92	12.50	9.70	6.34	0.25
5	5.36	12.34	9.42	6.25	2.50
6	1.39	12.21	9.96	6.92	0.00
8	2.07	13.20	9.79	7.87	0.55
9	2.61	12.27	9.43	6.75	0.00
10	2.83	13.08	9.63	6.48	0.35
11	5.30	9.02	8.47	5.61	11.16
12	2.11	12.60	9.54	6.33	0.00
13	0.55	12.45	9.29	6.88	0.27
14	5.20	13.66	8.96	5.74	0.27
15	10.27	13.31	7.38	4.89	0.74
16	6.04	12.17	8.44	6.33	5.53
17	0.65	12.51	9.66	7.06	0.00
18	6.96	12.84	8.43	5.60	0.51
19	0.19	11.35	9.63	8.59	0.99

N.B. - One Triple Phosphate Stone (Case No. 7) was analysed qualitatively only.

TABLE 45

RESULTS OF QUANTITATIVE CHEMICAL ANALYSIS OF CALCIUM OXALATE STONES

Case Number	Calcium Ca g%	Phosphorus P g%	Magnesium Mg g%	Ammonia NH_3 g%	Oxalate C_2O_4 g%
20	24.85	1.12	0.75	0.93	52.30
21	25.55	1.31	0.11	1.06	52.34
22	26.06	1.99	0.15	0.67	49.92
23	27.38	4.91	0.17	1.18	40.44
24	26.72	0.00	0.32	0.81	53.37
25	24.73	3.50	0.38	1.71	44.95
26	26.72	3.93	0.16	1.24	47.03
27	24.83	0.00	0.58	2.34	55.23
28	24.45	0.00	0.09	1.16	54.35
29	26.10	0.00	0.19	1.60	55.82
30	26.35	0.00	0.00	2.71	57.11
34	26.22	0.00	0.13	1.14	56.22
35	23.77	0.00	0.13	1.23	53.62
36	25.36	0.00	0.16	0.86	55.54
37	24.84	3.90	0.42	2.04	49.51

N.B. - Three Calcium Oxalate Stones (Cases No. 31,32 and 33) were analysed qualitatively only.

TABLE 46

RESULTS OF QUANTITATIVE CHEMICAL ANALYSIS OF CALCIUM PHOSPHATE STONES

Case Number	Calcium g%	Phosphorus g%	Magnesium g%	Ammonia g%	Oxalate g%
38	25.40	16.12	0.62	3.52	3.95
39	26.34	13.21	1.70	1.40	6.42
40	25.64	14.94	1.89	2.44	17.90
41	27.60	15.89	1.69	3.54	14.50

TABLE 47

QUANTITATIVE ESTIMATION OF THE PERCENTAGE AMMONIA IN CYSTINE STONES

Case Number	Ammonia g%
42	15.75
43	14.62
44	13.30
45	15.71
46	15.55
47	14.41
48	14.41
49	15.08
51	16.19
52	15.24
53	16.36

Phosphorus estimations on the above stones revealed traces in 44, 45, 48, 51 and 52 but all were less than 0.2%. Case numbers 50 and 54 were qualitatively analysed only.

TABLE 48

THEORETICAL COMPOSITION OF CALCULI

Constituent	Phosphorus g%	Calcium g%	Magnesium g%	Ammonia g%	Oxalate g%	Water of Crystallisation g%
Struvite $MgNH_4PO_4, 6H_2O$	12.64		9.91	6.92		44.03
Apatite $Ca_{10}(PO_4)_6(OH)_2$	18.53	39.83				
Whewellite $CaC_2O_4 \cdot H_2O$		27.4			60.20	12.40
Weddellite $CaC_2O_4, 2H_2O$		24.40			53.70	21.90
Cystine				14.17		
Ammonium Urate				47.53		
Brushite $CaHPO_4, 2H_2O$	18.02	23.26				20.97

TABLE 49

COMPONENT VARIATIONS IN CALCULI COMPARED WITH THE THEORETICAL PERCENTAGES EXPECTED IN STONES

COMPOSED ENTIRELY OF THE CHIEF CONSTITUENT

Chemical Type of Stone	Number of Cases Analysed	Calcium g%		Phosphorus g%		Magnesium g%		Ammonia g%		Oxalate g%	
		Range	Theoretical	Range	Theoretical	Range	Theoretical	Range	Theoretical	Range	Theoretical
Triple Phosphate	18	0.19 - 10.27	0.00	9.02 - 13.66	12.64	7.38 - 9.96	9.91	4.89 - 8.59	6.92	0.00 - 11.16	0.00
Calcium Oxalate	15	23.77 - 27.38	24.40 - 27.40	0.00 - 4.91	0.00	0.00 - 0.75	0.00	0.67 - 2.71	0.00	40.44 - 57.11	53.70 - 60.20
Calcium Phosphate	4	25.40 - 27.60	23.26 - 39.83	13.21 - 16.12	18.02 - 18.53	0.62 - 1.69	0.00	1.40 - 3.54	0.00	3.95 - 17.90	0.00
Cystine	12			0.00 - 0.20	0.00			13.30 - 16.62	14.17		
Ammonium Urate	1							41.21	47.53		

TABLE 50

RESULTS OF QUANTITATIVE CHEMICAL ANALYSIS OF STONES TAKEN FROM DIFFERENT ANATOMICAL SITES FROM THREE DOGS

Case Number and Anatomical Position	Calcium g%	Phosphorus g%	Magnesium g%	Ammonia g%	Oxalate g%
24 Urethra	26.72	0.00	0.32	0.81	53.37
24 Bladder	25.68	1.30	0.20	0.00	54.17
30 Urethra	26.35	0.00	0.00	2.71	57.11
30 Bladder	24.80	0.00	0.00	1.26	54.11
34 Urethra	26.22	0.00	0.13	1.14	56.22
34 Bladder	26.62	0.00	0.00	1.93	55.56

TABLE 51

CHEMICAL ANALYSIS OF STONES FROM CONSECUTIVE EPISODES OF TWO PATIENTS

Case Number and Date of episode	Calcium g%	Phosphorus g%	Magnesium g%	Ammonia g%	Oxalate g%
37 1-9-64	24.84	3.90	0.42	2.04	49.51
37 18-3-65	22.56	16.24	0.06	1.32	1.92
38 7-8-64	25.40	16.12	0.62	3.52	3.95
38 14-5-65	30.80	6.09	1.26	5.86	39.48

can therefore be made on the basis of these two estimations. The quantitative test for oxalates differentiates oxalate stones from the other varieties; oxalate stones are characterised by levels higher than 40 grams per cent whilst the highest level recorded in the other stone types (17.90 grams per cent) is less than half the amount present in the oxalates.

The percentage ammonia present in each of the different stone types is also characteristic for each. Little ammonia (0.67 - 3.54 grams per cent) is found in the calcium containing stones; between 4 and 10 grams per cent is present in magnesium ammonium phosphate stones. These amounts are distinctly less than that present in the organic stones.

The two types of calcium containing stones (calcium oxalate and calcium phosphate) can be distinguished on the percentage of calcium and oxalate present in each. Confirmatory evidence, however, is provided by the determination of the total phosphorus content; the phosphorus content of calcium phosphate stones is greater than 13 grams per cent whereas the content in oxalate stones did not exceed 5 grams per cent. The total phosphorus content of magnesium ammonium phosphate stones varied from 9.02 to 13.66 grams per cent.

The results of the quantitative chemical analysis of stones removed from two anatomical positions of the urinary tract in 3 dogs (Table 50) indicated that little variation between the composition of stones from each site occurred. The 1.55 grams per cent calcium difference in stones taken from case 30 being the greatest disparity recorded. These results suggest that the variation due to analysis of different oxalate stones removed at the one clinical episode is slight, and that the quantitative

determinations/

determinations on these stones are repeatable.

The percentage of ionic components in stones taken from consecutive episodes of urolithiasis in two animals showed gross chemical changes with respect to the anion. (Table 51). In both animals, and on both occasions, a high calcium content was observed. The chief anionic component in the stones, taken at the initial episode from one animal (Case 37), was oxalate, whilst the stones removed at the repeated episode were high in phosphate. In the second animal (Case 38), the initial stones contained a high proportion of phosphorus, but the stones taken from the subsequent episode were high in oxalate.

Results of Calculations of the Percentage of Chemical Constituents of Calculi from the Ionic Components.

The results of the estimations of the chemical constituents of the inorganic stones are shown in Tables 52, 53, 54, 55 and 56. The stones with magnesium ammonium phosphate as the chief constituent were estimated to contain between 74 and 99 per cent of this compound (Table 52). Small percentages of calcium oxalate and calcium phosphate were estimated to be present in most stones of this type.

The estimated percentage of calcium oxalate, present in the stones with this compound as the chief chemical constituent, varied from 59 - 83 per cent. This percentage was calculated as the anhydrous form. Since this compound is known to occur in its hydrated forms in canine uroliths (White, Treacher and Porter, 1961), the actual percentage of hydrated oxalate from theoretical calculations would be at least 70% in these stones. Magnesium ammonium phosphate was estimated to be present in only very small amounts, but in 5 of the 15 oxalate stones analysed quantitatively, more than 10% of a calcium

phosphate/

TABLE 52

APPROXIMATE PERCENTAGE OF THE CHEMICAL CONSTITUENTS OF CALCULI ESTIMATED FROM THE PERCENTAGE OF IONIC COMPOTENTS.

TRIPLE PHOSPHATE STONES

Case Number	Estimated % $MgNH_4PO_4 6H_2O$	Estimated % Ca_2O_4 (Anhydrous)	Estimated % Calcium Phosphate (Apatite)	Total	% Impurities and Water of Crystallisation
1	76	3	18	97	3
2	99	0.8	3	102.8	0
3	88	3	6	97	3
4	98	0.4	0.7	99.1	0.9
5	95	3	2	100	0
6	99	0	0	99	1
8	98	0.8	3	101.8	0
9	95	0	1	96	4
10	97	0.5	4	101.5	0
11	85	16	0	101	0
12	96	0	2	98	2
13	93	0.4	3	96.4	3.6
14	90	0.4	12	102.4	0
15	74	1	21	96	4
16	85	8	8	101	0
17	97	0	1	98	2
18	85	0.7	11	96.7	3.3
19	97	1.5	0	98.5	1.5

TABLE 53

APPROXIMATE PERCENTAGE OF THE CHEMICAL CONSTITUENTS OF CALCULI ESTIMATED FROM THE PERCENTAGE OF IONIC COMPONENTS

CALCIUM OXALATE STONES

Case Number	Estimated % $MgNH_4PO_4 6H_2O$	Estimated % CaC_2O_4 (Anhydrous)	Estimated % Calcium Phosphate (Apatite)	Total	% Impurities and Water of Crystallisation
20	8	76	1	85	15
21	1	76	6	83	17
22	2	73	10	85	15
23	2	59	25	86	14
24	3	78	0	81	19
25	4	65	16	85	15
26	2	68	20	90	10
27	6	80	0	86	14
28	1	79	0	80	20
29	2	81	0	83	17
30	0	83	0	83	17
34	1	82	0	83	17
35	1	78	0	79	21
36	2	81	0	83	17
37	4	72	18	94	6

TABLE 54

APPROXIMATE PERCENTAGE OF THE CHEMICAL CONSTITUENTS OF CALCULI ESTIMATED FROM THE PERCENTAGE OF IONIC COMPONENTS

CALCIUM PHOSPHATE STONES

Case Number	Estimated % $MgNH_4PO_4.6H_2O$	Estimated % CaC_2O_4 Anhydrous	Estimated % Apatite	Estimated % $CaHPO_4$	Total	% Impurities and Water of Crystallisation
38	6	6	83	-	95	5
39	17	9	60	-	86	14
40	19	26	-	55	100	-
41	17	21	-	60	98	2

TABLE 55

ESTIMATED APPROXIMATE PERCENTAGE OF THE CHEMICAL CONSTITUENTS IN THE CALCULI IN TABLE 51.

Case Number and Date of episode	Estimated % $MgNH_4PO_4.6H_2O$	Estimated % CaC_2O_4 Anhydrous	Estimated % Apatite	Total	% Impurities and Water of Crystallisation	Classified Type of Stone
37 1-9-64	4	72	18	94	6	Oxalate
37 18-3-65	1	3	87	91	9	Phosphate
38 7-8-64	6	6	83	95	5	Phosphate
38 14-5-65	13	57	24	94	6	Oxalate

TABLE 56

ESTIMATED APPROXIMATE PERCENTAGES OF THE CHEMICAL CONSTITUENTS IN TABLE 50.

Case Number and Anatomical Position	Estimated % $MgNH_4PO_4.6H_2O$	Estimated % CaC_2O_4 Anydrous	Estimated % Apatite	Total	% Impurities and Water of Crystallisation
24 Urethra	3	78	0	81	19
24 Bladder	2	79	6	87	13
30 Urethra	0	83	0	83	17
30 Bladder	0	79	0	79	21
34 Urethra	1	82	0	83	17
34 Bladder	0	81	0	81	19

phosphate compound was estimated to be present.

Some difficulty in the estimations of the percentage chemical constituents of calcium phosphate stones was encountered. This difficulty may have been due to the mixed nature of the stones and to the several distinct crystalline or colloidal forms of calcium phosphate that may be present in uroliths. The calcium phosphate was estimated as apatite ($Ca_{10}(PO_4)_6)OH_2)$) in two stones and as calcium acid phosphate ($CaHPO_4$) in the remainder (Table 54). In the latter stones (both of which were associated with primary hyper-parathyroidism) significant quantities of magnesium ammonium phosphate and calcium oxalate were also present.

(b) Crystallographic Analysis.

Eight distinct X-ray diffraction patterns were observed. The characteristic major, approximate 2 θ angle peaks (expressed in degrees) of each type are included in the appendix, and are discussed below.

Type 1. Stones from 12 dogs, that had magnesium ammonium phosphate as their chief constituent on quantitative micro-chemical analysis, had this type of pattern. The urine of 11 of these 12 cases was infected, 10 infections being due to urease-positive staphylococci.

A remarkable similarity in the diffraction pattern of almost all cases was apparent. (Figs. 30a and b). The major constituent appeared to be Struvite ($Mg\ NH_4PO_4,\ 6H_2O$). Chemical analysis suggested the presence of calcium oxalate. Weddellite, the dihydrate form of this compound, would account for the peaks present at 14.2

The/

The major Weddellite peak , according to the "Index to the X-ray Powder Data File" (1959), is at 32.2; it is possible that this peak is included in the Struvite peak of 31.8. It is of interest to note that in many of the mixed oxalate stones, and in the stones containing mostly Weddellite, the 14.2 peak has been of greater intensity than the 32.2 peak. The presence of Whewellite (the monohydrate form of calcium oxalate) was also deduced by the peaks at 14.9 (possibly the result of both Struvite and Whewellite peaks at approximately this angle) and 30.1. Further evidence for the presence of Whewellite was indicated by the presence of minor peaks at 38.1. No peak was observed at 24.4 which, according to the "Index to the X-ray Powder Data File" (1959), is the position of one of the major Whewellite peaks. However, in the stones consisting mainly of Whewellite, the peak present at this angle was of lower intensity than the peaks at 14.9, 30.1 and 38.1. The presence of Apatite in these Struvite stones was suspected by the presence of a strong peak at 31.8 and peaks at 32.2 and 32.9. The intensity of the peak at 31.8 was possibly due to the presence of both weak Struvite and strong Apatite peaks at this point.

It seems, therefore, that this type of stone had Struvite as its major crystalline constituent, and Whewellite, Weddellite and possibly Apatite as minor constituents. Two major peaks at approximately 16.4 and 21.4 are unaccounted for.

Type 2. **Seven** of 11 stones, chemically analysed as calcium oxalate, were of this type.

Peaks at 14.2, 20.0, 28.8, 32.2 and 37.3 indicating the presence of Weddellite, and further peaks at 14.9, 24.3, 30.0 and 38.2 being

evidence /

evidence of the presence of Whewellite. Minor peaks of these two substances were also present. These stones were therefore considered to be mixtures of Weddellite and Whewellite. The constant presence of a peak at 31.7 may have indicated that small amounts of Apatite were present in these stones. (Figs. 31a and b).

Type 3. One of the 11 stones chemically analysed as calcium oxalate were of this type. The presence of major peaks at 14.2, 20.0, 22.6 and 32.2 indicating that Weddellite was the chief constituent. Minor peaks of this substance were also present. (Fig. 32).

Type 4. Three of the 11 stones chemically analysed as calcium oxalate was found to have this pattern. The presence and intensity of peaks at 14.9, 24.3, 30.0 and 38.2 indicated that the monohydrate of calcium oxalate (Whewellite) was the main crystalline constituent of this stone. Further peaks at 15.2, 23.4, 30.7, 31.3, 35.9, 43.4 and 45.8 corresponded closely to the weaker-intensity peaks of Whewellite. (Fig. 33).

Type 5. Three of the 4 stones, chemically analysed as calcium phosphate, were of this type. Two of these cases were known to be associated with primary hyperparathyroidism; the other occurring in urine infected with a urease-positive staphylococci.

Diffuse peaks ranging from 31.0 to 33.1 are consistent with the presence of apatite. According to Jensen (1941a and b), the high degree of dispersion of colloidal apatite causes this diffuse, but characteristic pattern. Other peaks at 14.8 and 30.0 indicated the presence of Whewellite. Minor peak at 15.8, 20.7, 30.5 and 33.3 indicated that some Struvite was also present. Further peaks at 14.2,

28/

28.9, 32.2 and 38.2 may have been due to Weddellite impurities. It seems likely, therefore, that the chief constituent of these stones was Apatite and that small amounts of Whewellite, Struvite and Weddellite were present. Some variation in the minor constituents of these stones was observed. (Fig. 34).

Type 6. The major peaks in this pattern corresponded closely to those recorded in the "Index to the X-ray Powder Data File" (1959) for secondary calcium ortho phosphate hydrate ($CaHPO_4$, $2H_2O$). Smaller peaks described were also present and confirmed the presence of this compound. Two obvious peaks at 34.1 and 34.4 may have indicated the presence of minor amounts of another substance. (Fig. 35).

Type 7. Stones from 7 animals were found to be of this type. The major peaks present were similar to those recorded for cystine in the "Index to the X-ray Powder Data File"(1959). Only one peak at 9.4, constantly present in each recording, did not appear in this reference file. (Figs. 36a and b).

Type 8. The single stone removed from a young Dalmatian dog, and analysed chemically to be ammonium urate, was of this type. The peaks recorded, however, do not correspond closely in the reference file. (Fig. 37).

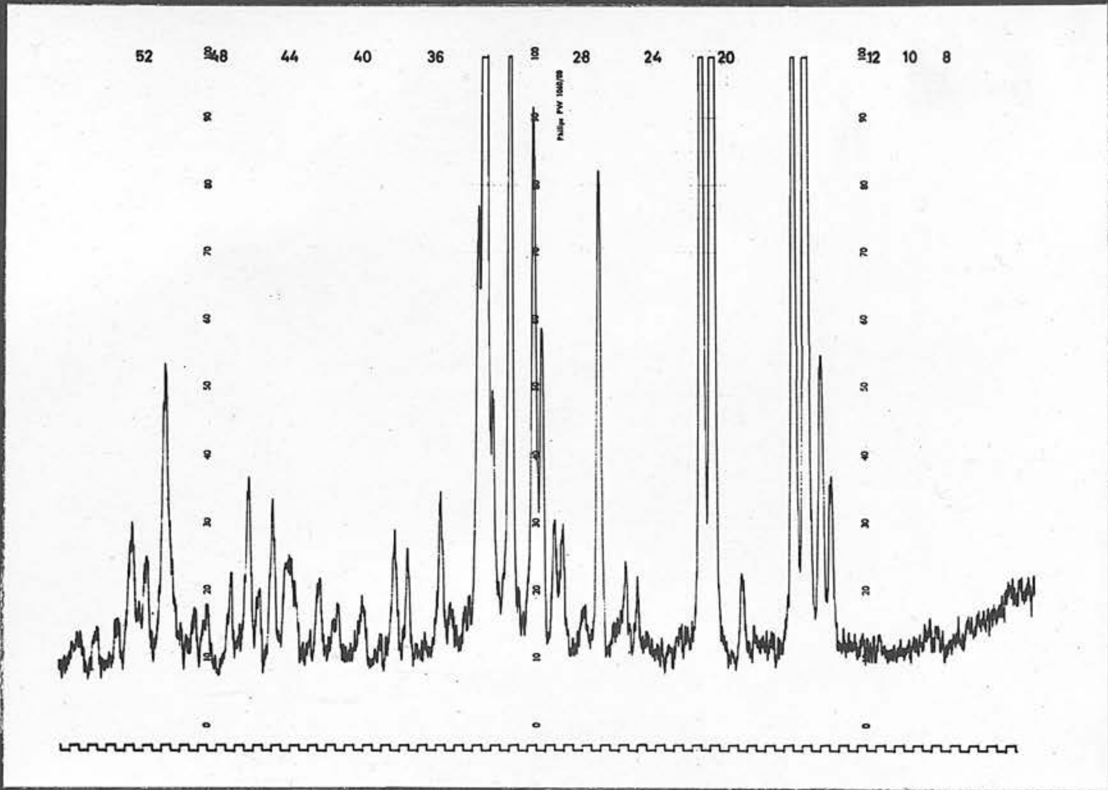
Results of X-ray Diffraction Crystallography on Stones Removed at Consecutive Clinical Episodes from two Animals.

Case 37. Analysis of Stone removed at Initial Episodes (1/9/64):-

This/

Fig. 30a. X-ray Diffraction Pattern of a Struvite Stone.

Fig. 30b. X-ray Diffraction Pattern of a Struvite Stone. Comparison between this Stone and the above Demonstrates the close similarity in position and relative intensity of the Diffraction Peaks in these Stones.


52
48
44
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12
10
8

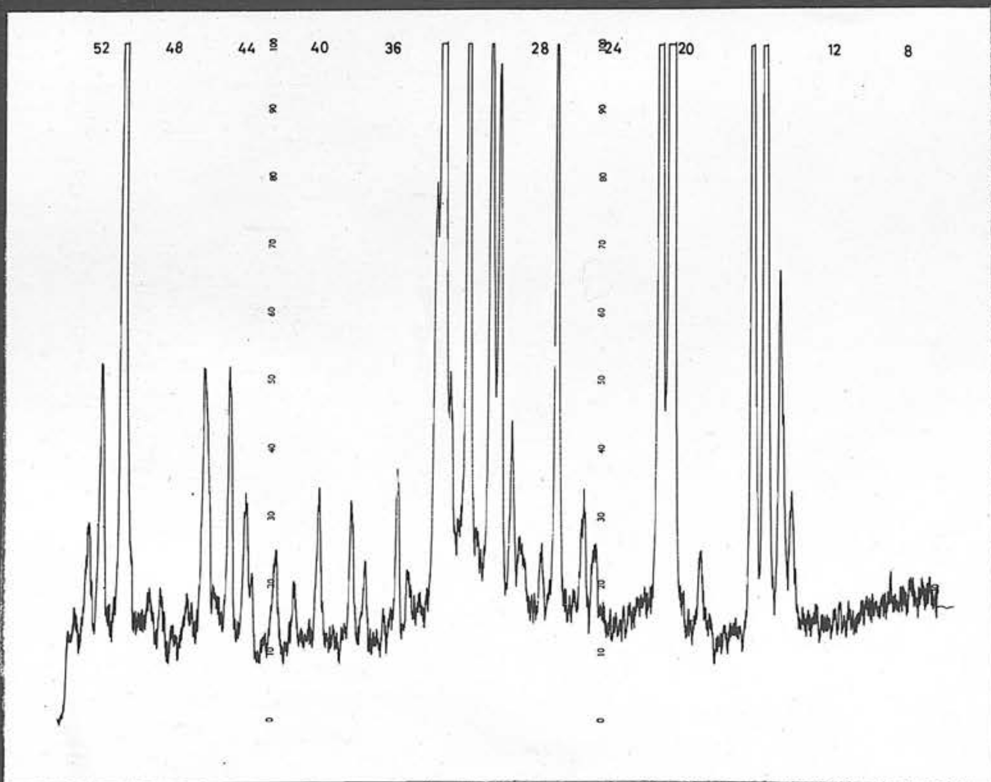
52
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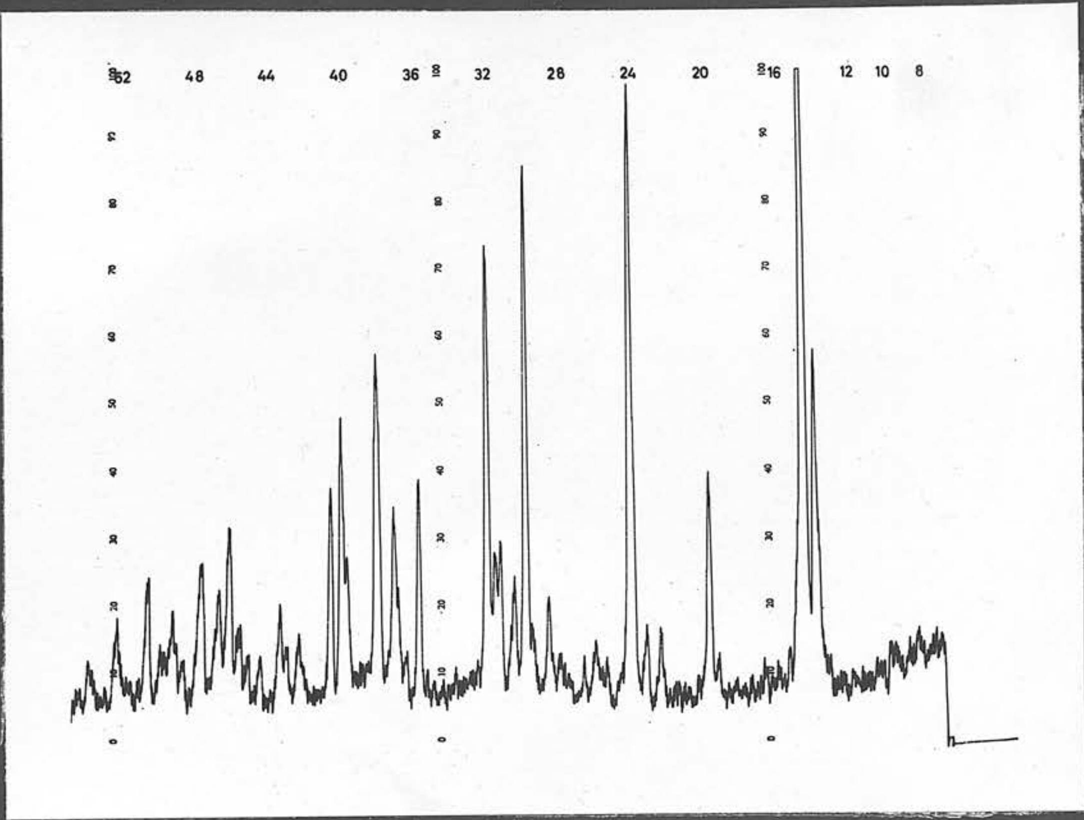
Fig. 31a X-ray Diffraction Pattern of a Mixed Whewellite and Weddellite Stone.

Fig. 31b. X-ray Diffraction Pattern of a Mixed Whewellite and Weddellite Stone. Comparison between this Stone and the above demonstrates the close similarity in position and relative intensity of the Diffraction Peak in these Stones.


52 48 44 40 36 32 28 24 20 16 12 10 8

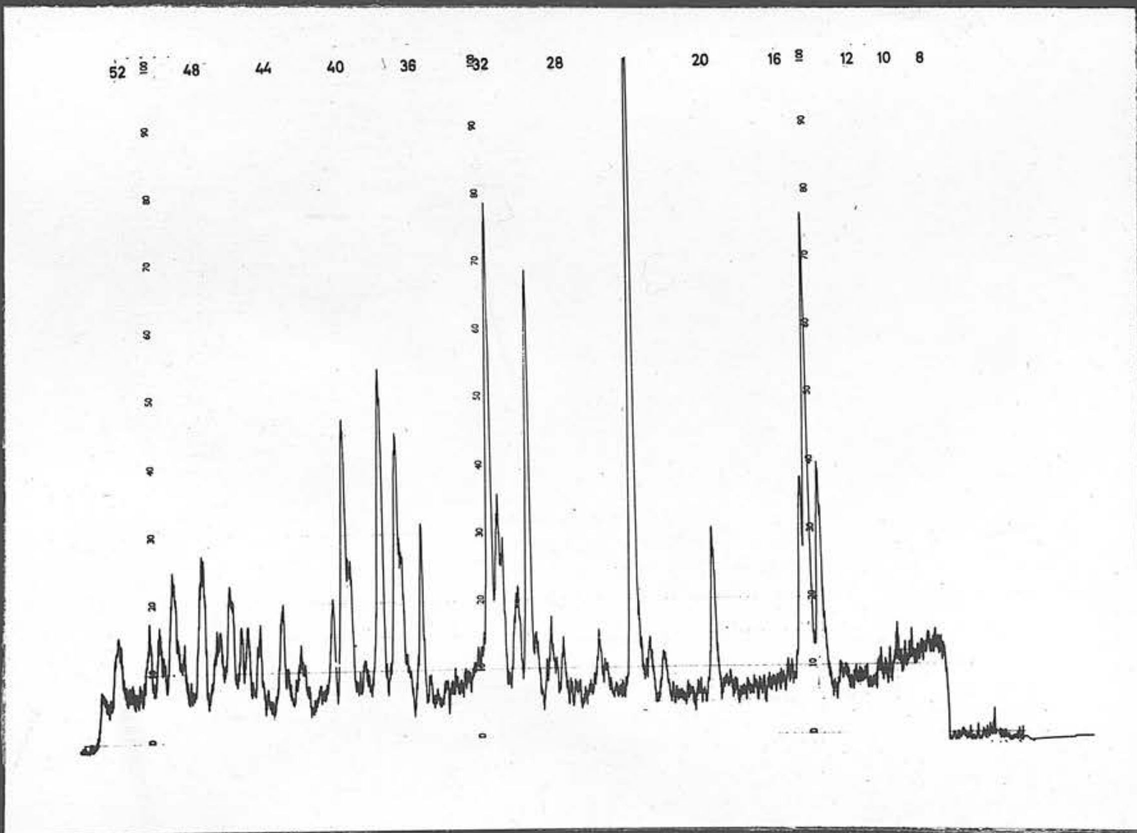
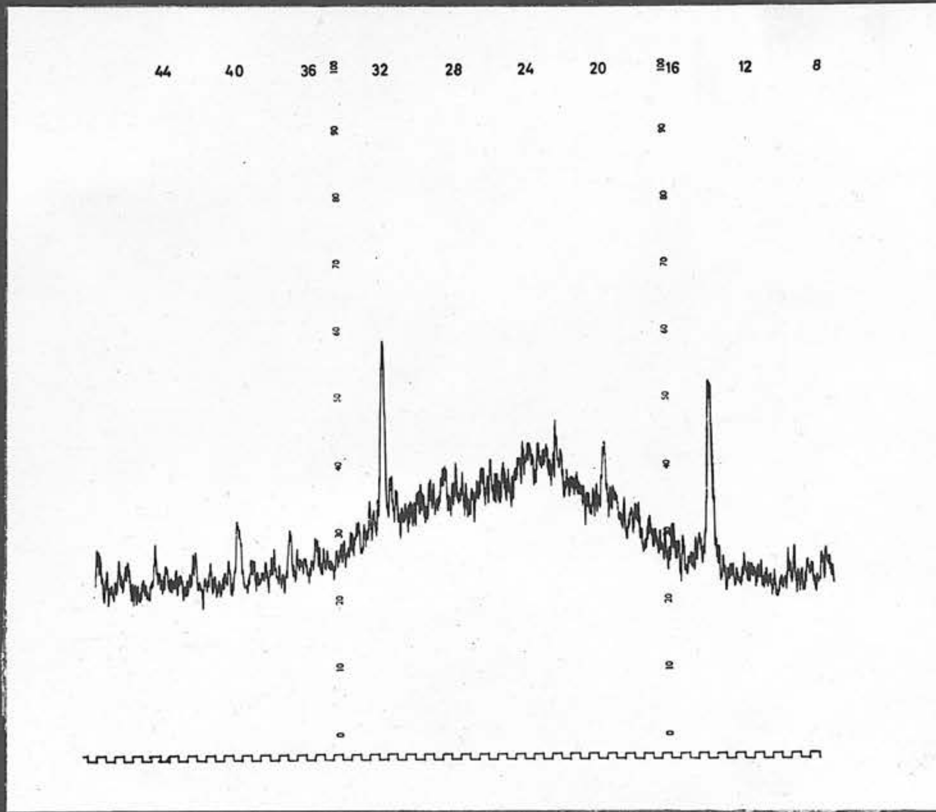

52 48 44 40 36 32 28 20 16 12 10 8

Fig. 32. X-ray Diffraction Pattern of a Weddellite Stone.

Fig. 33. X-ray Diffraction Pattern of a Predominantly Whewellite Stone.


44 40 36 32 28 24 20 16 12 8

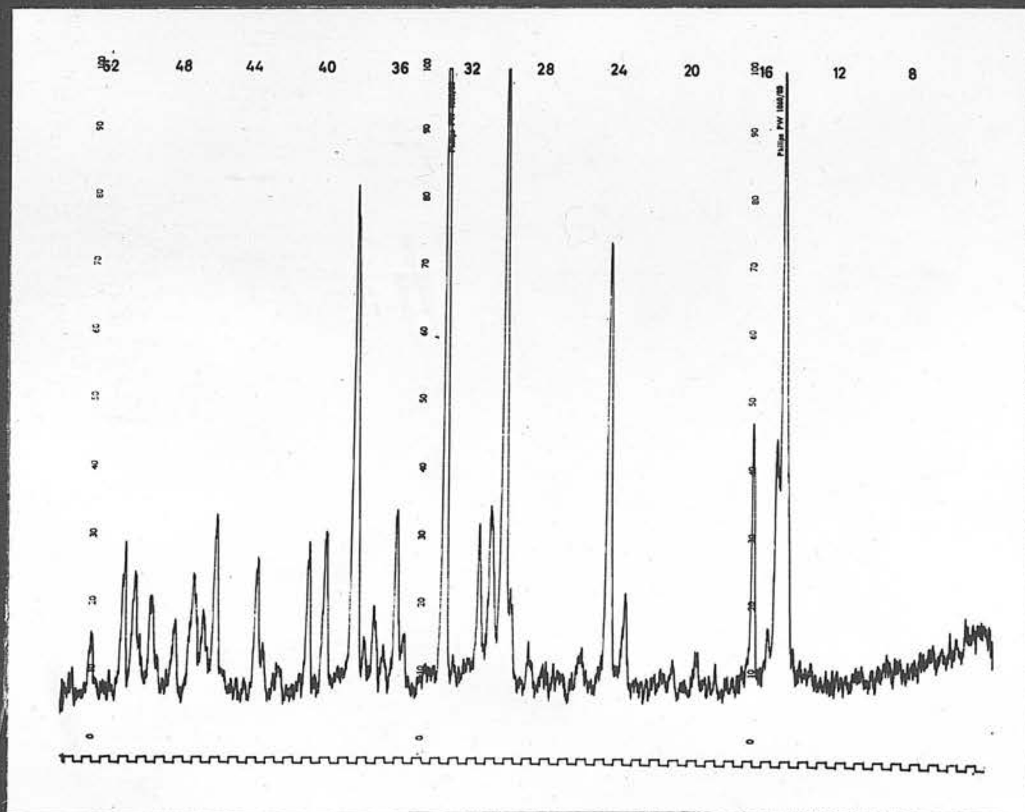
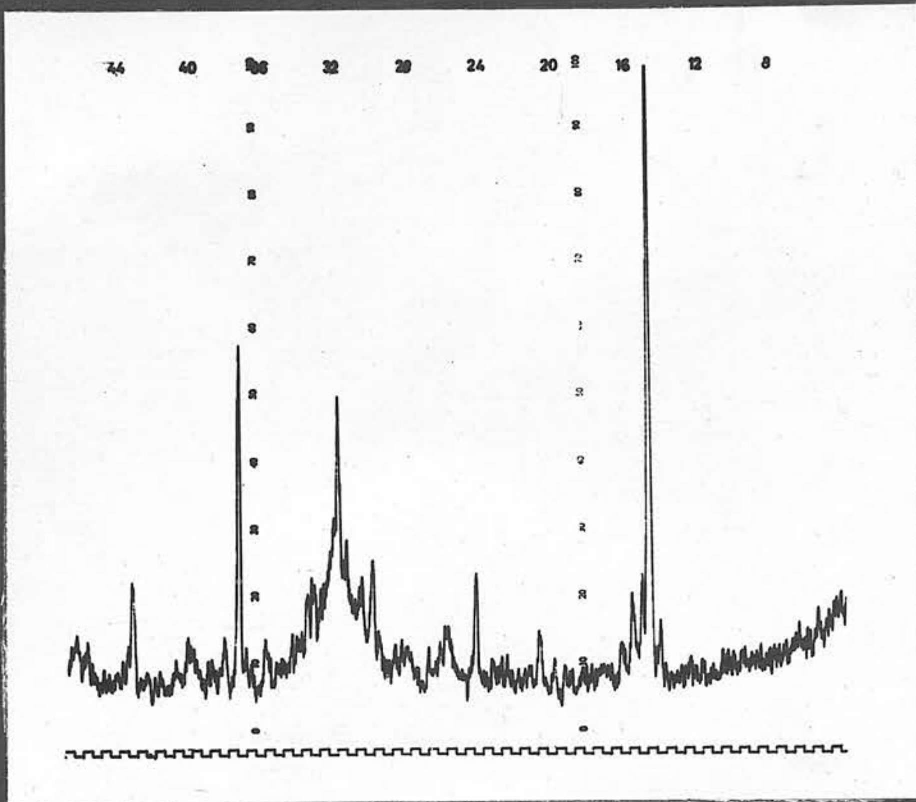

52 48 44 40 36 32 28 24 20 16 12 8

Fig. 34. X-ray Diffraction Pattern of a Predominantly Apatite Stone occurring in a case of Primary Hyperparathyroidism.

Fig. 35. X-ray Diffraction Pattern of a Secondary Calcium Ortho Phosphate Stone.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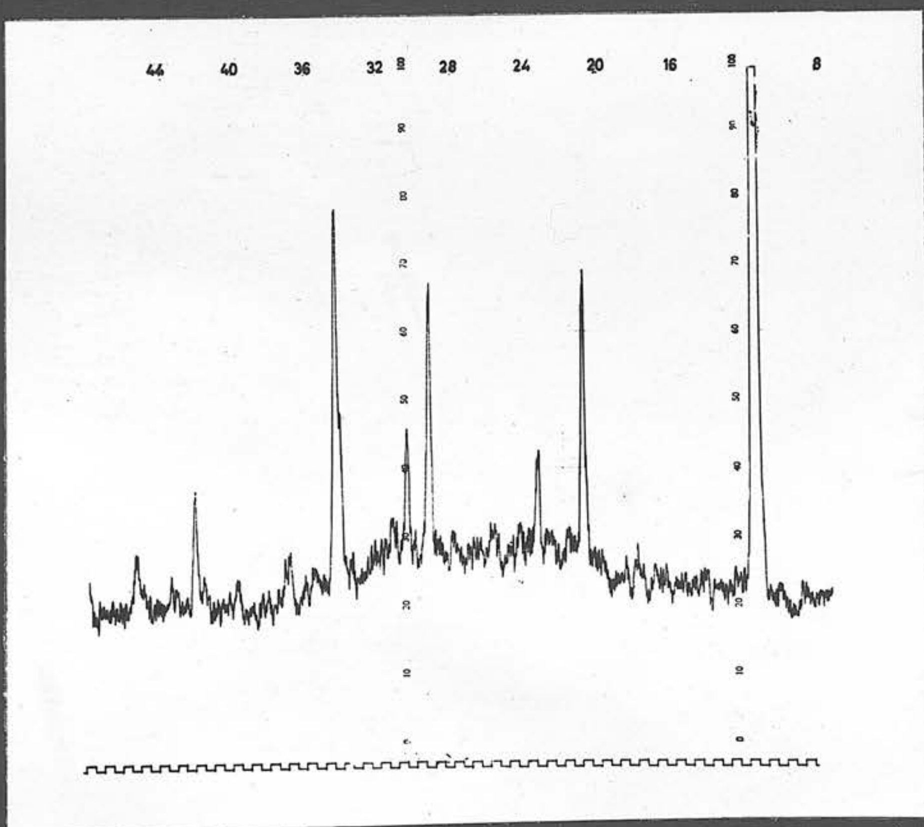
44
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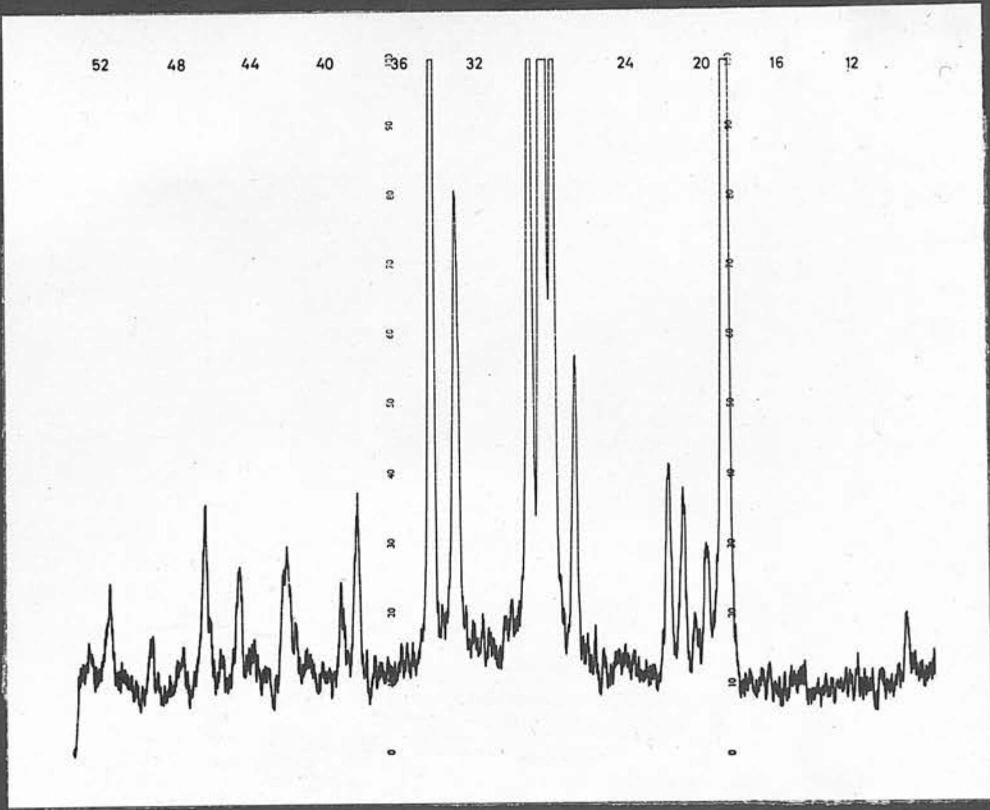
Fig. 36a X-ray Diffraction Pattern of a Cystine Stone.

Fig. 36b. X-ray Diffraction Pattern of a Cystine Stone. Comparison between this Stone and the Above demonstrates the close similarity in position and relative intensity of the Diffraction Peaks in these Stones.


52
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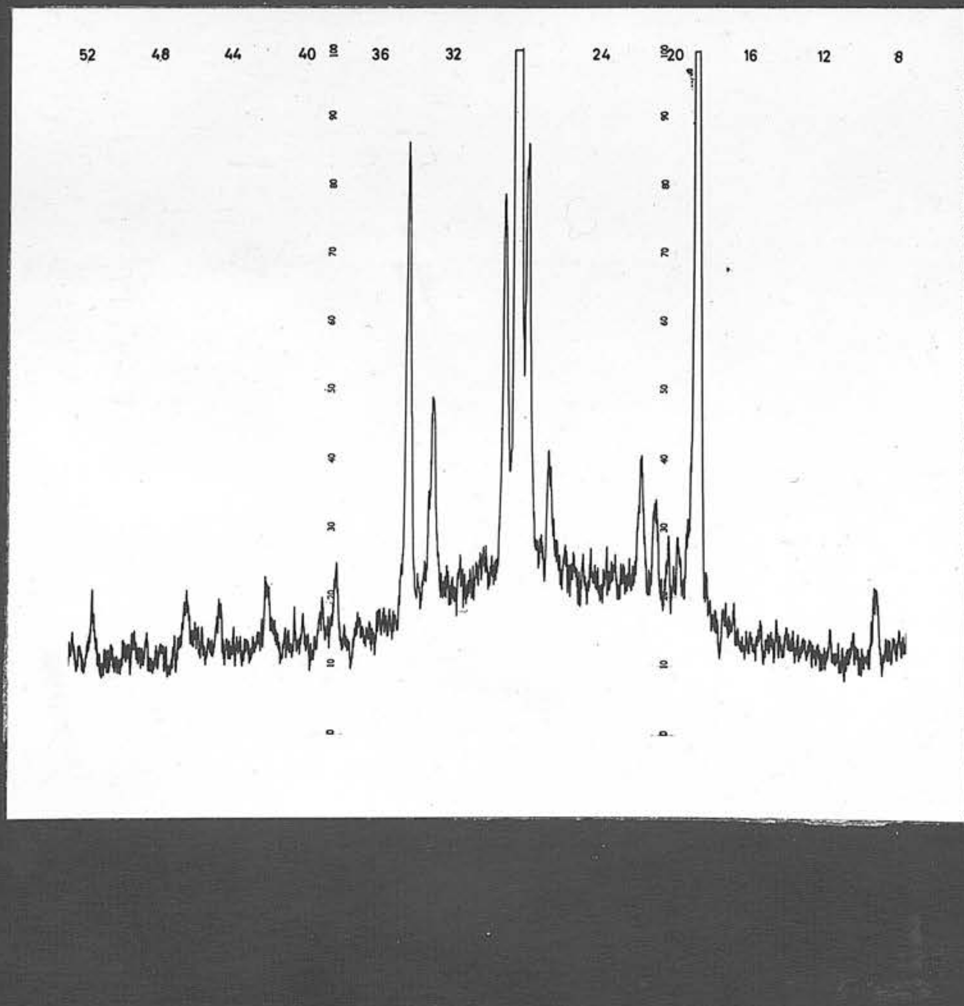
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Fig. 37. X-ray Diffraction Pattern of a Urate Stone.

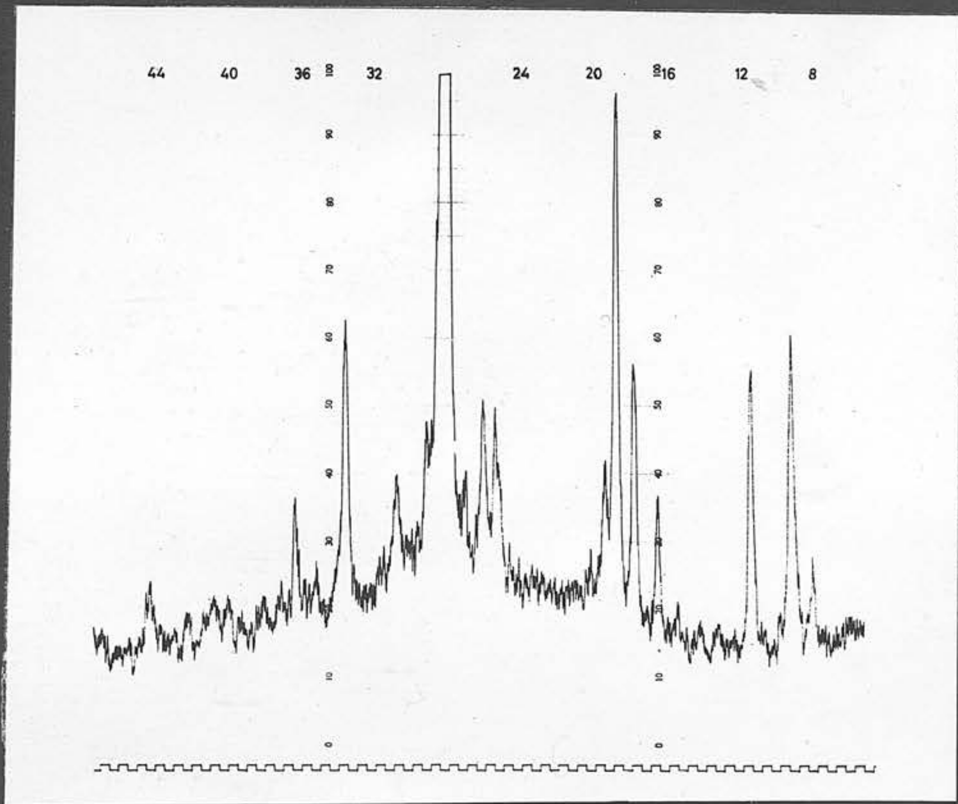
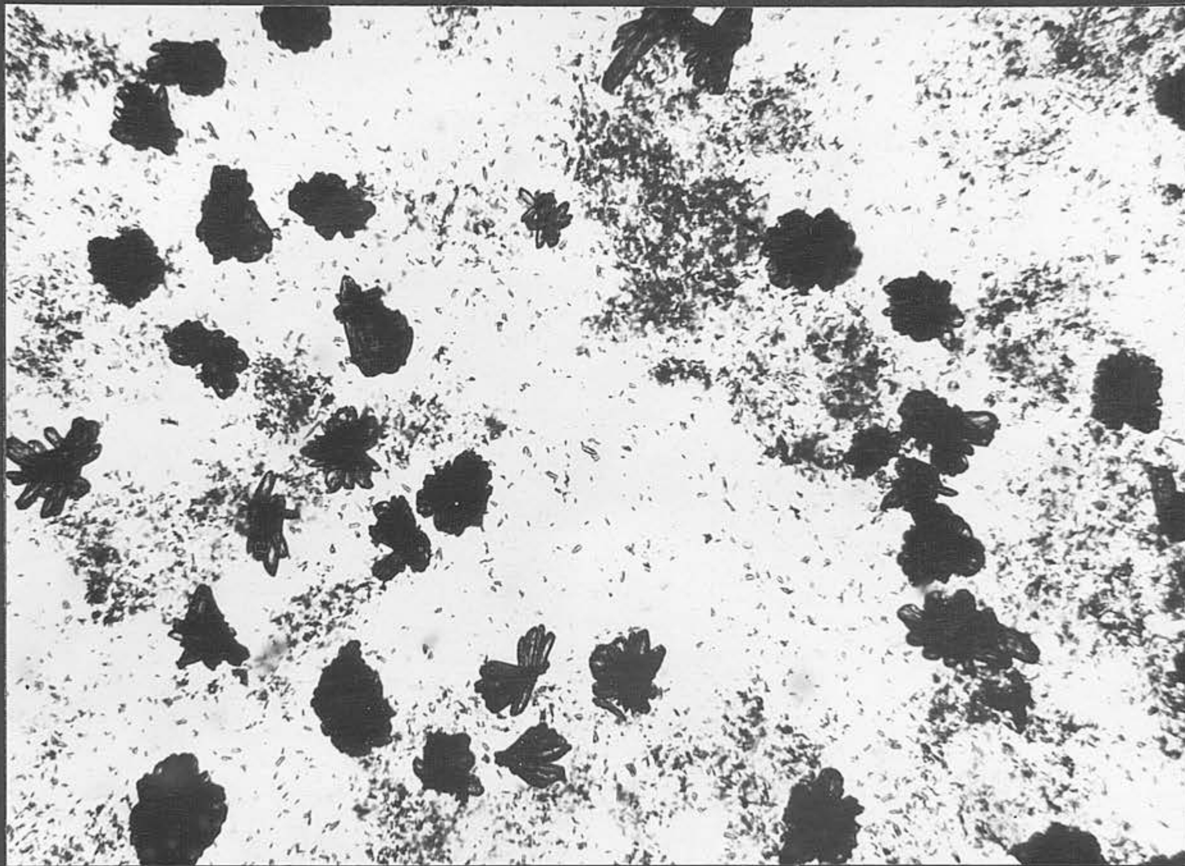
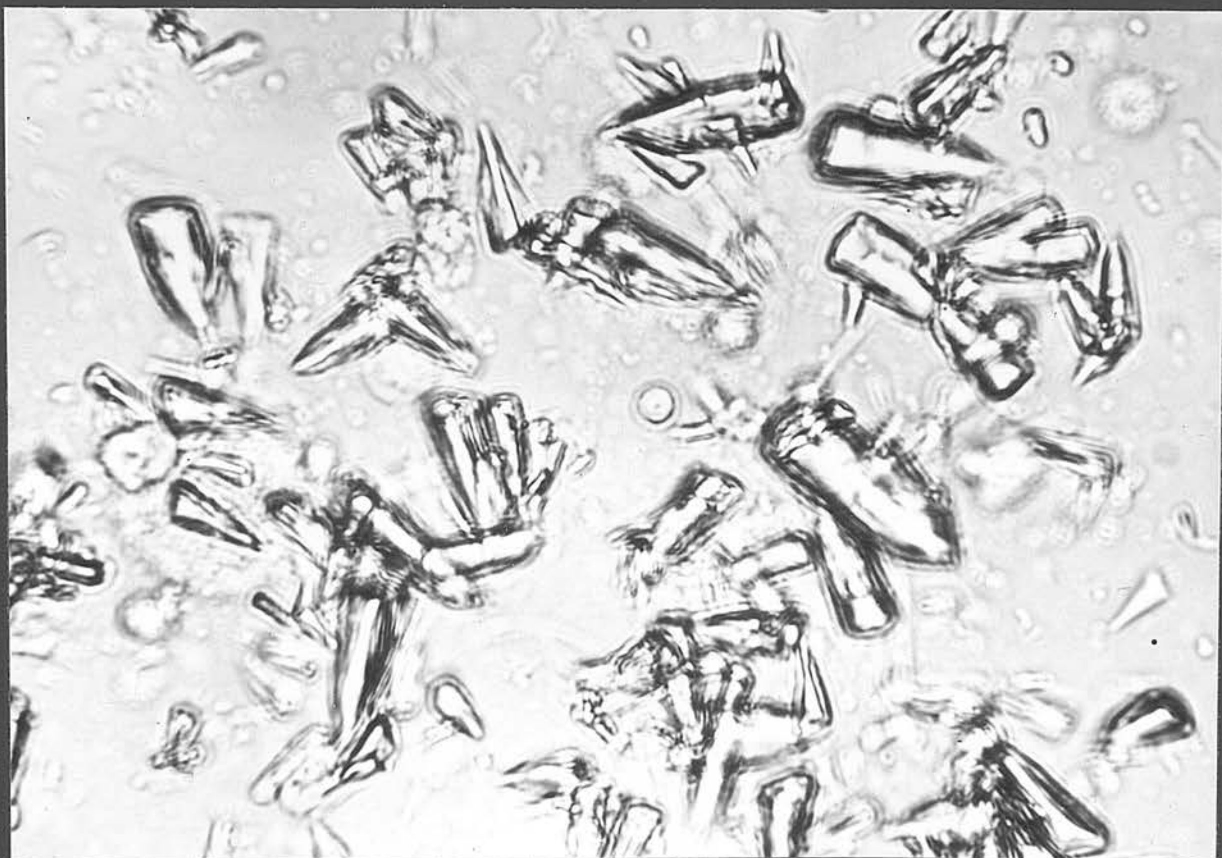

44
40
36
32
24
20
16
12
8

Fig. 38a. Photomicrograph of the Urinary Sediment in a Dog with Secondary Calcium Ortho Phosphate Stone. (x200) Showing Rosetted Cluster of needle-like Crystals.

Fig. 38b. Photomicrograph of the Urinary Sediment in a dog with Secondary Calcium Ortho Phosphate Stone. (x900) Showing the needle-like form of the Crystals present.





This urolith had, as its chief constituent, Whewellite; a small amount of Weddellite also being present.

Analysis of Stones removed at the Subsequent Episode (18/3/65):-

Secondary calcium ortho phosphate hydrate was found to be the chief constituent. Two peaks, at approximately 34.2 and 34.4 in the diffraction pattern, were unaccounted for.

Case 38 Analysis of Stones Removed at Initial Episode (2/8/64):-

Secondary calcium ortho phosphate hydrate was again found to be the chief constituent.

Analysis of Stones Removed at the Subsequent Episode (12/5/65):-

The chief crystalline constituent of this stone was Weddellite.

Clinical Notes on these Two Animals.

A number of clinical similarities in these two animals were observed and thought worthy of recording.

Both were small, young, male adults which were affected by very rapidly forming calcium-containing uroliths. Neither had bacteria detected in urine samples taken on several occasions during each episode. The pH in both cases remained acid throughout. The diet was similar to that most commonly fed to dogs in this area; no large amounts of vegetables, vitamins or mineral supplements were administered. The calculi were, in both cases, very small; in case 38, crystalline material was observed in both the bladder and kidneys after the second episode. The appearance and physical characteristics of the stones were different at each episode and for this reason particular atention was paid to their analysis. Unusual shaped crystals (Figs. 38a and b) were also seen constantly in the urinary sediment of case 38.

One animal was destroyed on the owners request at the second episode; the other (case 38) remains alive and clinically asymptomatic 1 year after the last clinical episode, although radiographic evidence of calculous material in the bladder and kidneys remain. The blood calcium levels of both cases were within normal limits.

A rational explanation for this type of syndrome is that it may possibly result from a type of 'idiopathic hypercalciuria' similar to that described in humans.

(c) Comparison between the Results of Chemical and Crystallographic Analysis.

A comparison of the results of these 2 methods of analysis is shown in Table 57. There was complete agreement with regard to the nature of the chief constituent in both methods. Some considerable variation in minor constituents was observed; this variation was considered to be due to the fact that whilst minute quantities of substances can be detected by the quantitative micro-chemical method, the crystallographic method is relatively insensitive to small amounts (less than 5%) of impurities, especially when these impurities consist of substances such as Apatite that are associated with poorly defined diffraction patterns.

Discussion.

1. Appearance and Physical Characteristics of Uroliths.

The simplest method of determination of the chief constituent of stones/

TABLE 57

A COMPARISON BETWEEN THE RESULTS OF QUANTITATIVE MICRO-CHEMICAL ANALYSIS AND X-RAY DIFFRACTION CRYSTALLOGRAPHY

Case No,	Quantitative Chemical Analysis		X-ray Diffraction Crystallography	
	Major Constituent	Minor Constituents	Major Constituent	Minor Constituents
2	99% Magn. Amm. Phos.	0.8% Calcium Oxalate 3% Calcium Phosphate	Struvite	Weddellite Whewellite and possibly Apatite
4	98% Magn. Amm. Phos.	0.4% Calcium Oxalate 0.7% Calcium Phosphate	Struvite	Weddellite Whewellite and possibly Apatite
5	95% Magn. Amm. Phos.	3% Calcium Oxalate 2% Calcium Phosphate	Struvite	Weddellite Whewellite and possibly Apatite
6	99% Magn. Amm. Phos.	Nil	Struvite	Weddellite Whewellite and possibly Apatite.
8	98% Magn. Amm. Phos.	0.8% Calcium Oxalate	Struvite	Weddellite, Whewellite and possibly Apatite
9	95% Magn. Amm. Phos.	1% Calcium Phosphate	Struvite	Weddellite, Whewellite and possibly Apatite.
11	85% Magn. Amm. Phos.	16% Calcium Oxalate	Struvite	Weddellite, Whewellite and possibly Apatite.

Case No.	Quantitative Chemical Analysis		X-ray Diffraction Crystallography	
	Major Constituent	Minor Constituents	Major Constituent	Minor Constituent
12	96% Magn. Amm. Phos.	2% Calcium Phosphate	Struvite	Weddellite, Whewellite and possibly Apatite
13	93% Magn. Amm. Phos.	0.4% Calcium Oxalate 3% Calcium Phosphate	Struvite	Weddellite, Whewellite and possibly Apatite
15	74% Magn. Amm. Phos.	1% Calcium Oxalate 21% Calcium Phosphate	Struvite	Weddellite, Whewellite and possibly Apatite.
18	85% Magn. Amm. Phos.	0.7% Calcium Oxalate 11% Calcium Phosphate	Struvite	Whewellite
19	97% Magn. Amm. Phos.	1.5% Calcium Oxalate	Struvite	Weddellite, Whewellite and possibly Apatite
20	76% Calcium Oxalate	8% Magn. Amm. Phos. 1% Calcium Phosphate	Whewellite	--------
21	76% Calcium Oxalate	6% Calcium Phosphate 1% Magn. Amm. Phos.	Whewellite	Weddellite
22	73% Calcium Oxalate	10% Calcium Phosphate 2% Magn. Amm. Phos.	Whewellite and Weddellite	Possibly Apatite
23	59% Calcium Oxalate	25% Calcium Phosphate 2% Magn. Amm. Phos.	Whewellite and Weddellite	Possibly Apatite
25	65% Calcium Oxalate	16% Calcium Phosphate 4% Magn. Amm. Phos.	Whewellite and Weddellite	Possibly Apatite
26	68% Calcium Oxalate	20% Calcium Phosphate 2% Magn. Amm. Phos.	Whewellite and Weddellite	Possibly Apatite

Case No.	Quantitative Chemical Analysis		X-ray Diffraction Crystallography	
	Major Constituent	Minor Constituents	Major Constituent	Minor Constituents
30	83% Calcium Oxalate	--------	Whewellite and Weddellite	Possibly Apatite
34	81% Calcium Oxalate	--------	Whewellite and Weddellite	Possibly Apatite
35	78% Calcium Oxalate	1.31% Magn. Amm. Phos.	Weddellite	Whewellite
36	81% Calcium Oxalate	2% Magn. Amm. Phos.	Whewellite and Weddellite	Possibly Apatite
37	72% Calcium Oxalate	18% Calcium Phosphate 4% Magn. Amm. Phos.	Whewellite	Weddellite and Possibly Apatite.
38	83% Calcium Phosphate	6% Magn. Amm. Phos. 6% Calcium Oxalate	Sec. calcium Ortho Phosphate Hydrate	---------
39	60% Calcium Phosphate	17% Magn. Amm. Phos.	Apatite	Whewellite and Struvite
40	55% Calcium Phosphate	26% Calcium Oxalate	Apatite	Whewellite, Weddellite and Struvite
41	60% Calcium Phosphate	21% Calcium Oxalate 17% Magn. Amm. Phos.	Apatite	Whewellite
42	Cystine		Cystine	
43	Cystine		Cystine	
44	Cystine	Trace of Phosphate	Cystine	
46	Cystine		Cystine	
47	Cystine		Cystine	
48	Cystine	Trace of Phosphate	Cystine	

Case No.	Quantitative Chemical Analysis		X-ray Diffraction Crystallography	
	Major Constituent	Minor Constituents	Major Constituent	Minor Constituents
49	Cystine		Cystine	
55	Ammonium Urate		Pattern not recorded in "Index"	
37 Second Episode	87% Calcium Phosphate	2% Calcium Oxalate 1% Magn. Amm. Phos.	Sec. Calcium Ortho phosphate hydrate	Unidentified Compound
38 Second Episode	57% Calcium Oxalate	24% Calcium Phosphate 13% Magn. Amm. Phos.	Weddellite	--------

stones is by visual appraisal and observations on their physical characteristics. The predominant constituent of most stones can be readily and correctly determined in this manner. In the present series, 3 major types of triple phosphate stones are described. Most triple phosphate stones conformed to one or another of these types. Oxalate stones, when characterised by spicules extending from their surface, are easily distinguishable. However, a second type of oxalate stone, unassociated with obvious spicule formation, has been observed and recorded. The various forms of cystine stones as previously described by Treacher (1962) were observed and relatively easily identified.

2. <u>Chemical and Crystallographic Analysis.</u>

(a) <u>Chemical Analysis.</u>

The quantitative chemical analysis of uroliths was selected in preference to qualitative chemical analysis, as the former method was thought to be the more accurate. In the latter type of analysis, the sensitivity of each test varies, and unless confirmatory tests are performed in every case, some inaccuracies may ensure. The quantitative method, however, allows one to calculate the approximate percentage of the suspected chemical constituents within reasonably narrow limits. Calculation of percentage composition is based on the fundamental assumption thatthe major chemical substances present in the stones exist in known, usually hydrated, crystalline forms. This assumption was substantiated by the results of theXray diffraction crystallographic analysis. The results of the quantitative chemical analysis/

analysis are in close agreement with previously published work of Verstraete, van der Stock, and Mattheeuws (1964). In Magnesium Ammonium Phosphate stones the percentages of magnesium, phosphorus and ammonium were found to be close to the expected calculated concentration (Table 49). Calcium containing impurities were indicated by the presence of up to approximately 10% calcium ion. In most cases these were apparently in the form of calcium phosphate, although in one animal (Case 11) a significantly high proportion of calcium oxalate was present. The total calculated percentages of constituents of these stones in some cases added to slightly more than 100%. This could be explained by the possible presence of small amounts of magnesium phosphate, orcalcium phosphate in several different crystalline forms such as calcium hydrogen phosphate dihydrate, or tricalcium phosphate. The occasional recording of an ammonia concentration slightly in excess of the theoretical amount is possibly indicative of the presence of protein material in the stones.

In the calcium oxalate stones, only small amounts of magnesium were found. The maximum concentration indicating that none of these stones contained more than 10% Magnesium Ammonium Phosphate and, in most cases, levels of this compound did not exceed 5%. The total phosphorus concentration in a few oxalate uroliths was quite high. It seems likely that these are the stones that are usually reported as mixed calcium oxalate and phosphate stones. The low magnesium concentration in these cases indicates that the phosphate exists mainly as a calcium phosphate compound and not as magnesium ammonium phosphate. In a number of oxalate stones no phosphate was found.

Since/

Since the test for phosphorus is extremely sensitive, it seems possible that some oxalate stones consisted of almost pure calcium oxalate. It has been recorded that calcium oxalate exists, in canine uroliths, in both the dihydrate and monohydrate form (White, Treacher and Porter, 1961). Since the actual crystalline form present in each of the stones analysed was not known at the time of analysis, the total percentage of calcium oxalate in the stones was calculated as the anhydrous salt to give a range of values which varied from 59 to 83%. It was later shown, that for the most part, these stones existed as a mixture of the dihydrate and monohydrate form of calcium oxalate, and since the water of crystallisation content of these compounds varies from 12.4 grams. per cent (monohydrate) to 21.90 grams. per cent (dihydrate), the actual percentage of hydrated calcium oxalate in these stones was probably not less than 70%, and in most cases was considerably more than this figure.

The greatest difficulty in the estimation of the approximate percentage of components from the ratio of ionic constituents of uroliths was experienced with the calcium phosphate variety.

The reasons for this may have been due to the various calcium phosphate compounds existing in uroliths, or, to the mixed nature of the constituents. Significant concentrations of both magnesium and oxalate occurred in the two cases (40 and 41) associated with primary hyperparathyroidism. In one case (38), lesser amounts of both these impurities were found, whilst in another (39) only magnesium ammonium phosphate was apparently present in amounts exceeding 10%.

Because/

Because of the unusual ratio of ionic constituents in case 40 and 41, the calcium phosphate compound was thought to be calcium, ortho phosphate and the percentage of calcium phosphate present in these stones was calculated using the chemical formula for this substance. Later crystallographic analysis, however, indicated that the chief calcium containing substance present in these stones was apatite.

Complete quantitative analysis was not carried out in the organic stones. The estimation of the ammonia concentration sufficing to differentiate them from the inorganic type. The percentage phosphorus in cystine stones, however, was determined, and in no case was it found to be above 0.20 grams. per cent, and in approximately half the stones analysed no phosphorus was found. This observation concurs with the view of Verstraete, van der Stock and Mattheeuws (1964) who were of the opinion that cystine stones contained practically no inorganic impurities, but is at variance with the opinion of Treacher (1962) who stated that cystine stones invariably contained some phosphate.

Quantitative chemical tests are not frequently performed on canine uroliths, probably because a complete analysis by this method is time consuming. However, the methods used involve routine tests that are generally available in most biochemical laboratories, and the results obtained allow one to state the approximate nature and amount of the chief constituentpresent, with a reasonable degree of accuracy. Qualitative chemical tests, on the other hand, may give little indication as to which constituent present in stones is the predominant/

predominant one. For example, in the case of the calcium containing stones, qualitative analysis may merely result in the knowledge that both calcium phosphate and calcium oxalate are present and the particular calcium salt predominating may not be known. It was mainly due to this disadvantage of the qualitative chemical analysis that the more accurate quantitative type of analysis was used in this study.

(b) Crystallographic Analysis.

In canine uroliths, the crystallographic analysis of White, Treacher and Porter (1961) has given some indication as to the nature of the chemical formula of the chief constituents and has shown that these are almost identical in nature to the crystalline substances that occur in human urinary stones.

On the basis of the crystalline form of the major constituent of the stones examined by the quantum-counting method of X-ray diffraction crystallography in this investigation, 8 different X-ray diffraction patterns were recorded. The relationship of these to the chief chemical constituent is shown in Table 58.

All triple phosphate stones had Struvite ($MgNH_4PO_4, 6H_2O$) as their major crystalline constituent, and in each stone of this type analysed some form of calcium oxalate was also present. In most cases the minor constituents were Weddellite, ($CaC_2O_4, 2H_2O$), Whewellite (CaC_2O_4, H_2O), and possibly Apatite. The presence of Apatite in small amounts in these stones was difficult to detect using/

<u>TABLE 58</u>

RELATIONSHIP BETWEEN THE CRYSTALLINE AND
<u>CHEMICAL COMPONENTS OF CANINE UROLITHS</u>

<table>
<tr><th></th><th>Chief crystalline constituents</th><th>Chief chemical constituents</th></tr>
<tr><td>1</td><td>Struvite</td><td>Magnesium, Ammonium, Phosphate</td></tr>
<tr><td>2</td><td>Whewellite and Weddellite Mixture</td><td rowspan="3">Calcium Oxalate</td></tr>
<tr><td>3</td><td>Whewellite</td></tr>
<tr><td>4</td><td>Weddellite</td></tr>
<tr><td>5</td><td>Apatite</td><td rowspan="2">Calcium Phosphate</td></tr>
<tr><td>6</td><td>Sec.Calcium Ortho Phosphate</td></tr>
<tr><td>7</td><td>Cystine</td><td>Cystine</td></tr>
<tr><td>8</td><td>Ammonium Urate</td><td>Pattern not recorded in the "Index to the X-ray Powder Data File" (1959).</td></tr>
</table>

using the X-ray diffraction method, as the intensity of the peaks produced by Apatite is not as great as that produced by the calcium salts or Struvite.

A notable repetitive irregularity in all struvite stones was the presence of 2 unaccountable major peaks at 2 Θ angles of approximately 16.4° and 21.4°. Corresponding peaks were not recorded in the 'Index to the X-ray Powder Data File' (1959). Several reasons may account for this disparity. Firstly, some form of ionic crystalline substitution may have occurred in the conditions under which these stones were formed, thus causing a change in the crystalline structure. Secondly, the crystalline water content may have been slightly different from pure struvite and thirdly, it seems possible that failure to record these two peaks in the Powder Data File may have been due to less acurate techniques of detection available when the file was compiled. The establishment of the data on Struvite in this Index was probably undertaken using the powder photograph technique, the resolution of which may not have been sufficiently acute to distinguish between these 2 peaks and the other strong Struvite peaks that occur in close proximity.

Most oxalate stones apparently consisted of a mixture of Whewellite and Weddellite with possibly some small amounts of Apatite. Occasionally, however, an oxalate stone was either predominently Whewellite or Weddellite with minor amounts of the alternative form as impurities. One stone apparently consisted of Whewellite only.

Of the four calcium phosphate stones, three were found to have Apatite as their chief constituent; the other consisted mainly of secondary/

secondary calcium ortho phosphate hydrate ($CaHPO_4, 2H_2O$). The presence of this compound in canine uroliths has not previously been reported but its presence was suspected by Verstraete, van der Stock, and Mattheeuws (1964).

In the Apatite stones, some oxalate crystals and struvite crystals were usually present.

Cystine stones did not contain detectable amounts of other crystalline substances. With regard to the urate stone, an unusual pattern, which did not correspond closely with any in the Powder Data File, was recorded.

In 2 animals that suffered recurrent attacks of calcium containing lithiasis, the chemical analysis and the diffraction patterns indicated that a change in formula of the major constituents had occurred. This phenomenon has not been previously reported in the dog. White (1944) analysed calculi from repeated episodes of urolithiasis in 8 animals, and found that, in each case, the same type of stones recurred. Whilst this probably occurs as a general rule, the aforementioned results indicate that, at least in some cases of calcium containing stone, the anionic content of the predominant constituent may vary between episodes. The variations occurred between calcium oxalate and secondary calcium ortho phosphate, both of which seem likely to be deposited in acid urine.

It is postulated that in these two animals a possible hypercalciuria was occurring. Such Hypercalciuria in the dog may possibly result from interference with the active renal tubular transport-mechanism for calcium (Chen/

(Chen and Newmann, 1955) in much the same way as the defect in active renal tubular transport of cystine is instrumental in cystine stone formation.

In one case of Struvite lithiasis, in which 3 visually distinct crystalline layers were present, analysis of all 3 layers by the diffraction technique did not indicate any change in the crystalline form present between each. Prien (1963) is of the opinion that many Struvite stones formed in human patients have different chemical compounds as their nucleus, indicating that the nucleus of these stones antedated the urea-splitting infection that is considered necessary for the formation of Struvite by this author. Lagregren (1956), however, showed that the composition of human Struvite stones was, for the most Part, either homogeneous throughout or regularly laminated with Apatite. Murphy and Pyrah (1962) are also of the opinion that Struvite stones with a distinct nucleus are in the minority. Observations on Struvite stones in the present series, also suggest that a crystalline nucleus of a different macroscopic nature than the external layers is relatively uncommon in canine uroliths of this type.

The quantum counting technique is a simple, accurate method of X-ray diffraction analysis and it has, for the most part, superceded the older, more tedious, photographic technique.

The use of the newer method requires less effort or attention than chemical analysis, and can be used on much smaller stones than is necessary for a complete chemical analysis. Since many of the urethral stones in dogs are of this type, its application to canine uroliths should/

should receive closer attention. A complete analysis, together with a permanent record of each stone analysed, can be obtained within half an hour. Its major disadvantage is the intricate and expensive apparatus required. However, with increasing demand for these machines in Universities and Commercial enterprises, they should be much more readily available to Veterinarians in the future.

(c) Comparison Between Chemical and Crystallographic Analysis.

A comparison of the results of quantitative chemical analysis and X-ray diffraction analysis were of interest. The X-ray diffraction confirmed the major constituent found by chemical means in every case. Some differences in the minor constituents, however, were noted. Most of these differences were insignificant, but in some cases when the minor constituents, according to the results of the quantitative chemical analysis, exceeded 10% they were not observed in the X-ray diffraction patterns. This may have been due to sampling error, but it is more likely to result from the relative insensitivity of the X-ray diffraction technique to minor amounts of certain substances, the crystalline structure of which results in poorly defined diffraction patterns.

GENERAL CONCLUSIONS

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Part 1. Incidence.

The incidence of urolithiasis in the population of dogs presented at the Small Animal Clinic of the Royal (Dick) School of Veterinary Studies during the years 1964-65 was lower than that recorded by other authors and is approximately one fifth that of the previously recorded incidence in the British Isles (White, Treacher and Porter, 1961). Similarly the incidence of the different chemical types of stone in this investigation significantly varied from that of a summary of previously recorded surveys. An approximately equal incidence of triple phosphate and calcium oxalate calculi being encountered in the present series. Cystine stone formation was also frequently observed, thus confirming the opinion of White, Treacher and Porter (1961) who considered cystine calculi to be a common cause of urolithiasis in the male dog in the United Kingdom.

The onset of signs of the initial episode of urolithiasis was found to occur significantly more often in dogs 6 years of age or older than in dogs less than 6 years of age. One of the most obvious reasons for this being that older dogs were found to be significantly more prone to oxalate lithiasis than their younger counterparts. Cystine Stones are more commonly observed in younger animals but it is not known whether this is due to a true susceptibility of younger animals to cystine stone disease or whether it is merely a reflexion of the greater number of young dogs at risk.

The/

The age distribution of the onset of clinical signs of the initial episode of urolithiasis shows a tendency towards a bimodial form.

Although more males than females were affected with urolithiasis, the total clinic population also contained a preponderance of male animals and the difference in incidence between the sexes was not statistically significant.

Statistical analysis also revealed that sex probably influenced the occurrence of cystine stone disease and oxalate stone formation in this survey. Cystine stones occurred only in males and this fact was considered additional evidence that the disease is confined only to this sex. The figures for sex incidence of urolithiasis were not in accord with those reported by Krook and Arwedsson (1956) but are similar to those of White, Treacher and Porter (1961).

Breed incidence studies indicated that the Cairn Terrier, Border Terrier, Dachshund and Corgi were prone to the disorder whilst the mongrel was comparatively resistant. A possible increased susceptibility of Corgis and Dachshunds to triple phosphate lithiasis recorded in the present investigation concurs with the findings of White (1963) who reported the common occurrence of this type of stone in these breeds.

The incidence of the position of lodgement of stones in the different anatomical sites of the urinary tract was also found to be significantly different from that of a summary of previously recorded series. Urethral Calculi were usually accompanied by uroliths in more anterior portions of the urinary tract and were usually/

usually due to either cystine or oxalate lithiasis. Magnesium ammonium phosphate was the chief constituent of most uroliths in patients in which only the vesical site was involved.

Part 2. Aetiologic Factors.

Quantitative bacteriology using the serial dilution (colony-counting technique) was found to be a useful method of distinguishing between infected and contaminated, fresh, canine urine samples. Similarly the examination of a gram-smear of the fresh uncentrifuged urine is an efficient practical test for the detection of significant bacteriuria in dog's urine.

An intimate relationship between urinary staphylococcal infection and canine triple phosphate lithiasis exists. The majority of canine triple phosphate calculi form in conjunction with infected urine, occasionally however, no bacteria can be isolated from the urine of dogs forming this type of stone. Urinary infection is present in some cases of oxalate lithiasis but rarely occurs in cystine stone disease.

Urolithiasis in the bitch is usually associated with a concurrent urinary infection but such infections are relatively infrequently observed in male dogs with this disorder.

Infection was not found in most cases of reforming cystine and oxalate calculi, thus adding further evidence that infection does not play an important role in the formation of the majority of these uroliths. Two cases of reforming triple phosphate lithiasis were encountered and in both, urinary infection was observed. This was considered additional evidence that urinary infection plays some part in/

in the aetiology of this stone type.

The introduction of infection during the normal surgical procedures for the removal of uroliths from the lower urinary tract is an infrequent occurrence in animals in which reasonable aseptic precautions are taken and which receive penicillin and streptomycin therapy for a limited post-operative period.

Canine cystine stone disease usually occurs in young adult male dogs and is manifested by urethral obstruction. Occasionally however, the initial clinical episode of cystine stone disease occurs in aged dogs. Cystine stones are radio-opaque but may not be seen on plain lateral radiography taken with the animal in lateral recumbancy in some cases with vesicular or urethral stones. Pure cystine is virtually radio-translucent.

Cystine crystals may not be seen in the urine of a significant number of cystine stone diseased animals and is thus an inefficient means of diagnosing this condition. The test of Brand, Harrison and Biloon (1930) will detect most cases of cystinuria. An accurate diagnosis can be achieved using electrophoretic detection of the typical amino-aciduria associated with this anomaly.

Cystine stone disease is known to be a serious condition in man which results in a substantially reduced life expectancy. The case records in this investigation show that the condition has similarly grave consequences in the dog and it appears to be the most serious of the common forms of urolithiasis in this animal.

The majority of dogs surviving the first clinical episode of cystine stone disease have a recurrent episode within 18 months.

Occasional/

Occasional cases however may survive for longer periods without clinical or radiographic signs of reformation.

Most cases of urolithiasis have serum calcium levels which lie within the accepted normal limits, occasionally however a raised serum calcium level is observed in these animals and is indicative of parathyroid dysfunction.

Most cases of urolithiasis have normal magnesium and inorganic phosphorus levels but a significant proportion of cases, when presented for treatment, have chemical evidence of uraemia. The clinical rubber-jaw syndrome in dogs may in some cases be related to the primary rather than the secondary form of hyperparathyroidism. Distinguishing features between the two conditions being that, in the former, bilateral nephrolithiasis and raised serum calcium levels are found.

Primary hyperparathyroidism was observed in two cases of bilateral nephrolithiasis, but was not seen in animals with calculi lodged in the lower urinary tract. Parathyroid dysfunction, as assessed byabnormal serum calcium levels, did not appear to play an important part in the formation of the majority of uroliths.

Most cases of urolithiasis were not associated with grossly abnormal diets and dogs forming different chemical types of uroliths were receiving similar dietary regimes. Oxalate lithiasis did not appear to be related to the feeding of diets with a high oxalate content, and phosphate lithiasis did not seem to be related tothe ingestion of an urinary alkalinising diet.

A statistically significant difference existed between the urinary pH associated with the different chemical types of uroliths. Phosphate calculi were found in alkaline urine and oxalate calculi in

acid/

acid urine. The mean urinary pH in cases of cystine lithiasis was approximately neutral. Alkaline pH in phosphate stones was for the most part related to the presence of urinary infection with urea-splitting organisms, but occasionally occurred in association with parathyroid dysfunction. Retention of urine for short periods did not lead to alkalinisation.

Micturating urography as described in this thesis, was a valuable aid in the differential diagnosis of lower urinary tract conditions, some of which had not previously been recorded in the dog.

A significant proportion of animals with urinary lithiasis had concomitant urinary tract anomalies such as prostatic enlargement, urethral abnormalities, bladder neck obstruction and vesico-ureteral reflux. The latter condition, which has only rarely been reported in dogs, was found to be a common phenomenon in some urinary conditions. It was frequently observed in infected urolithiasis cases in the bitch and in urinary infections unassociated with calculi formation.

Passage of the vesical contents into the prostate gland during micturition was also commonly observed in the dog.

Part 3. Analysis of Calculi.

The appearance and physical characteristics of many uroliths indicated the nature of the chief chemical constituent present, but difficulty was experienced in classifying some stones in this manner.

Quantitative micro-chemical analysis of urinary calculi is an accurate method of determining the chemical constituents and allows one to state precisely the nature of the predominating chemical substance present. Results of this type of analysis in the present investigation/

investigation are in accordance with the recently published work of Verstraete, van der Stock and Mattheeuws (1964). The main chemical constituents were; magnesium ammonium phosphate, calcium oxalate, cystine, calcium phosphate and ammonium urate.

Crystallographic analysis of calculi using the quantum counting technique is a rapid, reliable and relatively simple method of determining the chief crystalline constituents of canine uroliths which proved to be Struvite, Whewellite, Weddellite, Apatite, Secondary Calcium Ortho Phosphate, Cystine and Urate. The calculi found in cases with urinary infection due to urease-positive staphylococci usually had Struvite as the chief predominating crystalline constituent and calcium forming in the presence of primary hyperparathyroidism were composed mainly of Apatite. Calcium oxalate stones for the most part consisted of mixtures of both Whewellite and Weddellite but occasionally one or the other of these hydrates predominated. Calcium phosphate existed in canine uroliths as both Apatite and Secondary Calcium Ortho Phosphate.

Contrary to general opinion, the nature of the chief constituents may not be identical in consecutive episodes of the condition in the same animal. In 2 cases of rapidly forming calcium-containing uroliths, the chief constituents in the initial and subsequent episodes were different, an interchange between calcium oxalate and calcium ortho phosphate having occurred.

The results of the chemical and crystallographic methods of analysis were in close agreement in relation to the major components of uroliths but some variation between the two methods was noted in the detection of minor constituents.

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APPENDIX

TABLE 1

CHI SQUARE CALCULATIONS

COMPARISON OF THE INCIDENCE OF THE DIFFERENT CHEMICAL STONE TYPES IN PREVIOUSLY RECORDED SERIES WITH THIS SERIES

Null Hypothesis:- The distribution of the different chemical stone types in this series is similar to that of a summary of previously recorded series.

Main Chemical Constituent		Edinburgh Series No. of Cases	Other Series No. of Cases	TOTAL
Phosphate	Observed	23	541	564
	Expected	(35.4)	(528.6)	
Oxalate	Observed	18	133	151
	Expected	(9.5)	(141.5)	
Cystine	Observed	13	106	119
	Expected	(7.5)	(111.5)	
Urate and Others	Observed	1	40	41
	Expected	(2.6)	(38.4)	
	TOTAL	55	820	875

X^2 values 4.34 + 0.29: 7.61 + 0.51: 4.03 + 0.27: 0.98 + 0.07:

Total X^2 value 18.10.
X^2 value when 3 d.f. present and P = 0.01 is 13.34 (Snedecor, 1956).

Conclusion: This series differs significantly in the comparative incidence of the Different Chemical Stone Types from a summary of previous work and the discrepancies are mainly associated with an excess of oxalate and cystine stones and a reduction in phosphate stones.

TABLE 2

CHI SQUARE CALCULATIONS

AGE INCIDENCE OF THE ONSET OF CLINICAL SIGNS OF UROLITHIASIS IN THE CLINIC POPULATION.

Null Hypothesis:- If age did not influence the onset of urolithiasis, the age distribution of affected animals would be similar to the age distribution of the total population.

AGE	OBSERVED	EXPECTED	% AGE DISTRIBUTION IN TOTAL POPULATION
Under 6 years old	21	(29.2)	71.1
6 years and older	20	(11.8)	28.9
TOTAL	41		100.0

X^2 value 2.30 + 5.70 = 8.0.

X^2 value = 6.63 when 1 d.f. is present and P = 0.01 (Snedecor, 1956)

Conclusion: Urolithiasis occurs significantly more often in animals 6 years old and older than in younger dogs.

TABLE 3

CHI SQUARE CALCULATIONS

RELATIONSHIP BETWEEN AGE AND CHEMICAL TYPE OF STONE

Null Hypothesis:- The age distribution of each chemical stone type is similar

Age in Years	TRIPLE PHOSPHATE		CALCIUM OXALATE		CYSTINE		CALCIUM PHOSPHATE		URATE		TOTAL
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	
<6	10	(9.7)	5	(9.2)	11	(6.6)	1	(2.0)	1	(0.5)	28
6+	9	(9.3)	13	(8.8)	2	(6.4)	3	(2.0)	0	(0.5)	27
TOTAL	19	(19)	18	(18)	13	(13)	4	(4)	1	(1)	55

Total X^2 value = 11.9

X^2 value when 4 d.f. present and $P = 0.025$ is 11.14 (Snedecor, 1956)

Conclusion: The effect of age is not equally distributed throughout the different chemical types of stone, and from inspection this variation is mostly in the oxalate and cystine stones.

TABLE 4

CHI SQUARE CALCULATIONS

RELATIONSHIP OF AGE AND THE DIFFERENT CHEMICAL TYPES OF STONES OCCURRING IN THE CLINIC POPULATION

Null Hypothesis:- If age has no effect on the incidence of urolithiasis, the age incidence of this disease should be distributed in the same proportion as the age distribution of the total clinic population.

AGE		Triple Phosphate Cases	Oxalate Cases	Cystine Cases	Calcium Phosphate Cases	Total Urolithiasis Cases	% Distribution of total clinic Population
Under 6 Years	Observed	7	5	8	1	21	71.1
	Expected	(9.2)	(10.6)	(6.4)	(3.0)	(29.2)	
6 years and older	Observed	6	10	1	3	20	28.9
	Expected	(3.8)	(4.4)	(2.6)	(1.0)	(11.8)	
	TOTAL	13	15	9	4	41	100

X^2 value with Yate's correction: Triple Phosphate cases 0.31 + 0.77 = 1.08; Oxalate cases 2.46 + 5.91 = 8.37; Cystine cases 0.20 + 0.47 = 0.67.

X^2 value when 1 d.f. is present and P = 0.01 = 6.63 (Snedecor, 1956)

Conclusion: Oxalate stones occur significantly more often in dogs 6 years of age and older.

TABLE 5

CHI SQUARE CALCULATIONS

THE INFLUENCE OF SEX ON UROLITHIASIS

Null Hypothesis:- The sex incidence of canine urolothiasis is distributed similarly to the distribution of the sexes in the total clinic population.

		MALES	FEMALES	TOTAL
Urolithiasis Patients	Observed	30	11	41
	Expected	(24.4)	(16.6)	
Percentage of Total Population		59.4	40.6	100

X^2 value 1.29 + 1.89 = 3.18.

The value X^2 when 1 d.f. is present and P = 0.05 is 3.84 (Snedecor, 1956)

Conclusion:- The difference in sex incidence of clinic urolithiasis in this series is not statistically significant.

TABLE 6

CHI SQUARE CALCULATIONS

THE INFLUENCE OF SEX ON THE CHEMICAL TYPES OF STONE.

Null Hypothesis:- The distribution of the sexes in each of the chemically distinct types of stones is similar to that in the total clinic population.

CHIEF CHEMICAL CONSTITUENT		MALES	FEMALES	TOTAL
Triple Phosphate	Observed	4	9	13
	Expected	(7.7)	(5.3)	
Calcium Oxalate	Observed	14	1	15
	Expected	(8.9)	(6.1)	
Cystine	Observed	9	0	9
	Expected	(5.3)	(3.7)	
Calcium Phosphate	Observed	3	1	4
	Expected	(2.4)	(1.6)	
TOTAL	Observed	30	11	41
	Expected	(24.4)	(16.6)	
PERCENTAGE DISTRIBUTION OF THE SEXES IN THE CLINIC POPULATION		59.4	40.6	100

Since the numbers in each group were small and since only one degree of freedom was present in the comparison of each chemical stone type and the total population, a Yate's correction was applied.

X^2 values with Yate's correction: Triple Phosphate Patients 1.32 + 1.93 = 3.26; Calcium Oxalate Patients 2.37 + 3.47 = 5.84; Cystine Patients 1.93 + 2.76 = 4.69; Calcium Phosphate Patients 0.15 + 0.22 = 0.37. The X^2 value when 1 d.f. present and when P = 0.05 is 3.84 and when P = 0.01 is 6.63 (Snedecor, 1956).

Conclusion: 1. The clinical occurrence of both cystine stone disease and oxalate stone disease is probably influenced by sex.

TABLE 7

CHI SQUARE CALCULATIONS

BREED INCIDENCE OF UROLITHIASIS

Null Hypothesis:- If all breeds are equally susceptable to urolithiasis, the incidence in each breed is similar to the incidence in the total population.

BREED	Number of animals affected		Number of animals not affected		Total X^2 Value with Yate's Correction
	Observed	Expected	Observed	Expected	
Cairn Terrier	10	(1.8)	438	(445.2)	33.03
Corgie	4	(1.1)	259	(261.9)	6.18
Miniature Poodle	5	(4.1)	991	(991.9)	0.04
Dachshund	4	(0.9)	226	(229.1)	7.53
Border Terrier	3	(0.5)	131	(133.5)	8.03
Border Collie	3	(3.3)	801	(800.7)	-
Mongrel	1	(9.3)	2,226	(2258.3)	6.57
Greyhound	0	(1.4)	340	(338.6)	0.58
Alsatian	0	(2.5)	618	(615.5)	1.61
Labrador	0	(2.8)	679	676.2	2.13

X^2 value for 1 d.f. when $P = 0.05$ is 3.84 and for when $P = 0.01$ is 6.63 (Snedecor, 1956).

Conclusions:

1. In this series an increased incidence of urolithiasis occurred in the Cairn Terrier, the Dachshund and the Border Terrier.
2. The increased incidence in the Welsh Corgi was probably significant.
3. Mongrels had a decreased susceptibility to urolithiasis which is probably significant.

TABLE 8

.CHI SQUARE CALCULATIONS

BREED INCIDENCE OF THE CHEMICAL TYPES OF STONE

Null Hypothesis:- Breeds are equally susceptible to the different chemical types of stones and the proportion of each breed affected with each type will be similar to the proportion of the breed in the total population.

BREED	Chemical Type of Stone	Number of affected animals		Number of unaffected animals		Total X^2 value with Yate's correction
		Observed	Expected	Observed	Expected	
Welsh Corgi	Triple Phosphate	3	(0.34)	260	(262.66)	13.72
Dachshund	Triple Phosphate	3	(0.30)	227	(229.7)	16.15
Cairn Terrier	Triple Phosphate	3	(0.58)	444	(446.42)	6.37
Cairn Terrier	Oxalate	4	(0.67)	443	(446.33)	11.72
Cairn Terrier	Cystine	3	(0.40)	444	(446.6)	11.04
Miniature Poodle	Cystine	3	(3.90)	993	(992.1)	0.04.

X^2 value for 1 d.f. when P = 0.05 is 3.84 and when P = 0.01 is 6.63 (Snedecor, 1956)

Conclusions:

1. The Welsh Corgi and Dachshund in this population had a significantly increased incidence of Triple Phosphate stones.
2. The Cairn Terriers in this population had a significantly increased incidence of Oxalate and Cystine stones and probably had an increased incidence of Triple Phosphate stones.
3. In this population the incidence of cystine stones in Miniature Poodles was not significantly different from other Breeds.

TABLE 9

CHI SQUARE CALCULATIONS

THE INCIDENCE OF LOWER URINARY TRACT CALCULI:

Comparison of this series with a summary of previously recorded Series

Null Hypothesis:- The distribution of stones in the different anatomical positions of the Lower Urinary Tract in this series is similar to that of a summary of previous recorded series.

		Urethral Stones Only	Urethral and Vesical Stones	Vesical Stones Only	TOTAL
SUMMARY OF PREVIOUS SERIES	Observed	158	77	368	603
	Expected	(154.0)	(91.2)	(354.7)	
THIS SERIES	Observed	10	23	19	52
	Expected	(13.3)	(7.90)	(30.6)	
		168	100	387	655

X^2 value for previous series 0.1 + 2.61 + 0.48 = 3.10.

X^2 value for this series 0.9 + 9.9 + 7.1 = 17.90.

Total X^2 value is 20.0

X^2 value for P = 0.01 when 2 d.f. are present is 9.21 (Snedecor, 1956)

Conclusion: A significant difference in the distribution of the anatomical positions of lodgement of calculi between this series and a summary of previously recorded series exists.

TABLE 10

CHI - SQUARE CALCULATIONS

RELATIONSHIP OF THE FREQUENCY OF OCCURRENCE OF INFECTION WITH THE DIFFERENT CHEMICAL TYPES OF STONE

Null Hypothesis:- Infection occurs with equal frequency in each of the chemically distinct types of calculi

Chemical Stone Type		Number of infected cases	Number of cases not infected	Total
Triple Phosphate	Observed Expected	15 (7.0)	1 (9.0)	16
Calcium Oxalate	Observed Expected	6 (7.5)	11 (9.5)	17
Cystine	Observed Expected	0 (5.7)	13 (7.3)	13
Calcium Phosphate	Observed Expected	1 (1.3)	2 (1.7)	3
Urate	Observed Expected	0 (0.4)	1 (0.6)	1
Total		22	28	50*

* The 5 cases associated with recent antibacterial therapy were not included in this calculation.

Total X^2 value = 27.73 X^2 value for 4 d.f. when P = 0.01 is 13.28 (Snedecor, 1956)

Conclusion:- In this series the incidence of infection is unequally distributed throughout the different chemically distinct types of calculi.

TABLE 11

CHI - SQUARE CALCULATIONS

RELATIONSHIP OF THE FREQUENCY OF OCCURRENCE OF STAPHYLOCOCCI WITH THE DIFFERENT CHEMICAL TYPES OF STONE

Null Hypothesis:- **Staphylococci occur with equal frequency in all chemical types of calculi.**

Chemical Stone Type		Number of cases infected with Staphylococci	Number of cases not infected with Staphylococci	Total
Triple Phosphate	Observed Expected	13 (5.4)	3 (10.6)	16
Calcium Oxalate	Observed Expected	3 (5.8)	14 (11.2)	17
Cystine	Observed Expected	0 (4.4)	13 (8.6)	13
Calcium Phosphate	Observed Expected	1 (1.0)	2 (2.0)	3
Urate	Observed Expected	0 (0.4)	1 (0.6)	1
Total		17	33	50*

* **The 5 cases associated with recent antibacterial therapy were not included in this calculation.**

Total X^2 value = 25.51 X^2 **value for 4 d.f. when P = 0.01 is** 13.28 (Snedecor, 1956)

Conclusion:- **In this series a significant difference in the frequency of occurrence of staphylococci** with the different chemical types of stone existed.

TABLE 12

CHI - SQUARE CALCULATIONS

RELATIONSHIP BETWEEN THE INCIDENCE OF STAPHYLOCOCCI IN TRIPLE PHOSPHATE LITHIASIS AND IN OTHER CHEMICAL TYPES OF STONE

Null Hypothesis:- Staphylococci occur with equal frequency in triple phosphate and other chemical stone types.

Chemical Stone Type		Number of cases with Staphylococci	Number of Cases in which Staphylococci were not isolated	Total
Triple Phosphate	Observed	13	3	16
	Expected	(5.4)	(10.6)	
Other Chemical Types of Stone	Observed	4	30	34
	Expected	(11.6)	(22.4)	
Total		17	33	50*

* The 5 cases associated with recent antibacterial therapy were not included in this calculation.

X^2 value with Yates' correction

$9.33 + 4.76 = 14.09$

$4.35 + 2.25 = 6.60$ Total X^2 value = 20.69

X^2 value at 1 d.f. when P = 0.01 is 6.63 (Snedecor, 1956)

Conclusion:- In this series staphylococci occurred significantly more often with triple phosphate stones than with the other chemical stone types.

TABLE 13

CHI-SQUARE CALCULATIONS

RELATIONSHIP OF INFECTION TO SEX IN CASES OF UROLITHIASIS

Null Hypothesis:- Urinary Infection in cases of urolithiasis occurs with equal frequency in both sexes.

		MALES	FEMALES	TOTAL
INFECTED	Observed	8	14	22
	Expected	(15.8)	(6.2)	
NOT INFECTED	Observed	28	0	28
	Expected	(20.2)	(7.8)	
TOTAL		36	14	50*

* The 5 cases treated with antibacterial therapy prior to sampling were not included.

X^2 value with Yates' correction

3.37 + 8.59
2.64 + 6.83

Total X^2 value 21.43

X^2 value for 1 d.f. when P = 0.01 is 6.63 (Snedecor, 1956)

Conclusion:- A significant difference in the distribution of infection in the sexes in urolithiasis was present in this series. The difference was due to a higher incidence in females than males.

TABLE 14

CHI - SQUARE CALCULATIONS

RELATIONSHIP OF THE FREQUENCY OF OCCURRENCE OF STAPHYLOCOCCI IN UROLITHIC AND NON-UROLITHIC URINARY TRACT INFECTIONS

Null Hypothesis:- **Staphylococci occur in the same frequency with respect to other bacteria in infected cases of urolithiasis as in urinary infections unassociated with stones.**

Urinary Disorder		Staphylococci	Bacteria other than Staphylococci	Total
Infected cases of Urolithiasis	Observed	17	5	22
	Expected	(10.8)	(11.2)	
Urinary infections unassociated with urolithiasis	Observed	4	17	21
	Expected	(10.2)	(10.8)	
Total		21	22	43

X^2 value with Yates' correction

$3.01 + 2.90 = 5.91$

$3.18 + 3.10 = 6.19$ Total X^2 value = 12.10

X^2 value for 1 d.f. when P = 0.01 is 6.63 (Snedecor,1956)

Conclusion:- **In this series staphylococci occurred more often in comparison with other bacteria in association with urolithiasis than in urinary tract infections unassociated with calculi formation.**

TABLE 15

CHI-SQUARE CALCULATIONS

THE ASSOCIATION OF STAPHYLOCOCCI WITH STONES OTHER THAN TRIPLE PHOSPHATE

Null Hypothesis:- **The distribution of staphylococci in infected non-triple phosphate stones is similar to the distribution in urinary infections unassociated with urolithiasis.**

		No. of cases in which staphylococci were isolated	No. of infected stones in which no staphylococci were isolated	Total
Non-triple phosphate urolithiasis associated with infection	Observed	4	3	7
	Expected	(2.0)	(5.0)	
Urinary tract infections unassociated with urolithiasis	Observed	4	17	21
	Expected	(6.0)	(15.0)	
Total		8	20	28

Result: X^2 **value with Yates' correction**

$$1.13 + 0.45 = 1.58$$
$$0.38 + 0.15 = 0.53$$

Total X^2 **value = 2.11**

X^2 **value for 1 d.f. when P = 0.05 is 3.84 (Snedecor, 1956)**

Conclusion:- **The apparent difference in frequency of occurrence of Staphylococci in patients with infected stones other than triple phosphate from that in urinary tract infections unassociated with calculous disorders in this series is not statistically significant.**

TABLE 16

CHI - SQUARE CALCULATIONS

MORTALITY RATE ASSOCIATED WITH OPERATIONS ON CYSTINE STONE DISEASED ANIMALS

Null Hypothesis:- The mortality rate of animals on which operations were performed for Cystine Stone disease is similar to the mortality rate of animals on which operations were performed for uroliths composed chiefly of other chemical constituents.

		No. of cases died	No. of cases survived	TOTAL
CYSTINE	Observed	6	6	12
	Expected	(2)	(10)	
OTHER CHEMICAL TYPES	Observed	2	34	36
	Expected	(6)	(30)	
		8	40	48

X^2 value with Yates correction 7.78 + 1.55; 2.58 + 0.52; Total X^2 value 12.43.

X^2 value when P = 0.01 and 1 d.f. present is 6.84 (Snedecor, 1956).

Conclusion:- A statistically significant difference between animals on which operations were performed for the removal of cystine stones and animals on which operations were performed for the removal of other chemical types of stone existed in this series and this was mainly due to the greater number of deaths occurring in cystine stone diseased patients.

TABLE 17

CHI SQUARE CALCULATION

RELATIONSHIP OF VESICO-URETERAL REFLUX TO SEX IN UROLITHIASIS

Null Hypothesis: Vesico-ureteral reflux occurs with equal frequencyin both sexes in cases of urolithiasis.

		Reflux Prescent	Reflux Absent	Total
Males	Observed	0	11	11
	Expected	(2.6)	(8.4)	
Females	Observed	5	5	10
	Expected	(2.4)	(7.6)	
TOTAL		5	16	21

X^2 value with Yates' correction

1.70 + 0.53

1.84 + 0.58

Total X^2 value 4.65

X^2 value 1 d.f. when P = 0.05 is 3.84 (Snedecor,1956).

Conclusion: The difference in frequency of occurrence of vesico-ureteral reflux in the sexes in cases of urolithiasis is probably significant ($P < 0.05$).

TABLE 18

CHI SQUARE CALCULATION

RELATIONSHIP OF VESICO-URETERAL REFLUX AND SEX IN MISCELLANEOUS URINARY DISORDERS

Null Hypothesis: Vesico-ureteral reflux occurs with equal frequency in both sexes with urinary disorders.

		Reflux Present	Reflux Absent	Total
Females	Observed	7	7	14
	Expected	(4.3)	(9.7)	
Males	Observed	4	18	22
	Expected	(6.7)	(15.3)	
	TOTAL	11	25	36

X^2 value with Yates' correction

1.12 + 0.50

Total X^2 value 2.66

0.72 + 0.32

X^2 value when 1 d.f. is present and when P = 0.05 is 3.84 (Snedecor, 1956)

Conclusion: The difference in distribution of vesico-ureteral reflux in the sexes with urinary disorders in this series is not statistically significant.

TABLE 19

CHI SQUARE CALCULATION

RELATIONSHIP OF VESICO-URETERAL REFLUX AND INFECTION IN UROLITHIASIS.

Null Hypothesis:- Vesico-ureteral reflux occurs with equal frequency in infected and non-infected cases of urolithiasis.

		Reflux Present	No Reflux	Total
Infected Cases	Observed	4	7	11
	Expected	(2.2)	(8.8)	
Non-infected Cases	Observed	0	9	9
	Expected	(1.8)	(7.2)	
	TOTAL	4	16	20*

*The animal that received antibacterial therapy prior to examination was not included.

X^2 value with Yates' correction.

0.77 + 0.19

Total X^2 value = 2.13

0.94 + 0.23

X^2 value for 1 d.f. when P = 0.05 is 3.84 (Snedecor, 1956).

Conclusion: The difference in frequency of occurrence of vesico-ureteral reflux in infected and non-infected cases of urolithiasis recorded was not statistically significant.

TABLE 20

CHI SQUARE CALCULATION

RELATIONSHIP OF VESICO-URETERAL REFLUX TO TRIPLE PHOSPHATE LITHIASIS

Null Hypothesis:- Vesico-ureteral reflux occurs with equal frequency in triple phosphate patients and in patients suffering from other chemical stone types.

		Reflux Present	Reflux Absent	Total
Triple Phosphate	Observed	4	6	10
	Expected	(2.4)	(7.6)	
Other Chemical Stone Types	Observed	1	10	11
	Expected	(2.6)	(8.4)	
	TOTAL	5	16	21

X^2 value with Yates' correction

0.50 + 0.16

0.47 + 0.14 Total X^2 value 1.27

X^2 value for 1 d.f. when P = 0.05 is 3.84 (Snedecor, 1956)

Conclusion: The difference in frequency of occurrence of vesico ureteral reflux in triple phosphate lithiasis compared with that in the other stone types was not statistically significant in these observations.

TABLE 21

CHI SQUARE CALCULATION

RELATIONSHIPS OF VESICO-URETERAL REFLUX AND URINARY INFECTION IN MISCELLANEOUS URINARY DISORDERS

Null Hypothesis:- Vesico-ureteral reflux occurs with equal frequency in infected and uninfected urinary disorders.

		Reflux Present	Reflux absent	Total
Infected	Observed	5	7	12
	Expected	(3.7)	(8.3)	
Not infected	Observed	6	18	24
	Expected	(7.3)	(16.7)	
	TOTAL	11	25	36

X^2 value with Yates' correction

0.17 + 0.08

0.09 + 0.04

Total X^2 value 0.38

X^2 value when 1 d.f. present and when P = 0.05 is 3.84 (Snedecor, 1956).

Conclusion: The difference in frequency of occurrence of vesico-ureteral reflux in infected and non-infected urinary disorders in these observations is not statistically significant.

TABLE 22

COMPARISON OF RESULTS OF GRAM SMEAR EXAMINATION OF FRESH UNCENTRIFUGED URINE WITH THAT OF THE COLONY-COUNTING TECHNIQUE: 139 OBSERVATIONS

No.	Colony Count Organisms/ml. urine	Results of Gram Smear Examinations
1	45	Negative
2	0	Negative
3	0	Negative
4	39.6 x 10^6	Gram Negative Bacilli Present
5	0	Negative
6	0	Negative
7	3 x 10^6	Negative
8	170	Negative
9	6.2 x 10^6	Gram Negative Bacilli
10	0	Negative
11	750 x 10^6	Gram Negative Bacilli
12	37.8 x 10^6	Gram Negative Bacilli
13	30	Negative
14	0	Negative
15	19.15 x 10^6	Gram Negative Bacilli
16	50 x 10^6	Gram Negative Bacilli
17	10	Negative
18	31.5 x 10^6	Gram Negative Bacilli
19	750 x 10^6	Gram Negative Bacilli
20	0	Negative
21	0	Negative
22	30	Negative
23	0	Negative
24	10	Negative
25	90	Negative
26	40 x 10^6	Gram Positive Cocci
27	0	Negative
28	0	Negative

29/

29	1,400	Negative
30	10	Negative
31	0	Negative
32	10	Negative
33	0	Negative
34	10.3×10^6	Gram Negative Bacilli
35	10	Negative
36	35.1×10^6	Gram Negative Bacilli
37	1070	Negative
38	20,000	Negative
39	40	Negative
40	40×10^6	Gram Negative Bacilli
41	750×10^6	Gram Negative Bacilli
42	200	Negative
43	10	Negative
44	750×10^6	Gram Positive Cocci
45	20	Negative
46	27×10^6	Gram Negative Bacilli
47	10	Negative
48	0	Negative
49	0	Negative
50	7.6×10^6	Gram Negative Bacilli
51	13.5×10^6	Gram Positive Cocci
52	750×10^6	Gram Positive Cocci
53	150×10^6	Gram Positive Cocci
54	1550	Negative
55	0	Negative
56	0	Negative
57	0	Negative
58	42.1×10^6	Gram Positive Cocci
59	20	Negative
60	0	Negative
61	750×10^6	Gram Negative Bacilli
62	0	Negative
63	40	Negative

64/

64	38.4×10^6	Gram Positive Cocci
65	750×10^6	Gram Negative Bacilli
66	400,000	Negative
67	0	Negative
68	0	Negative
69	580	Negative
70	18.9×10^6	Gram Positive Cocci
71	125,000	Negative
72	0	Negative
73	78,000	Negative
74	0	Negative
75	0	Negative
76	0	Negative
77	30	Negative
78	0	Gram Negative Bacilli
79	0	Negative
80	0	Negative
81	0	Negative
82	10	Negative
83	0	Negative
84	20	Negative
85	10	Negative
86	4.4×10^6	Gram Negative Bacilli
87	20.95×10^6	Gram Negative Bacilli
88	40×10^5	Gram Negative Bacilli
89	750×10^6	Gram Positive Cocci
90	0	Negative
91	28.95×10^5	Gram Positive Cocci
92	0	Negative
93	0	Negative
94	275,000	Negative
95	40.8×10^6	Gram Positive Cocci Gram Negative Bacilli
96	10	Negative
97	0	Negative
98	12.1×10^6	Gram Negative Bacilli
99/		

99	750×10^6	Gram Positive Cocci
100	10	Negative
101	0	Negative
102	10	Negative
103	750×10^6	Gram Positive Cocci
104	0	Negative
105	22,500	Negative
106	90	Negative
107	0	Negative
108	0	Negative
109	750×10^6	Gram Negative Bacilli
110	42,500	Gram Negative Bacilli
111	4.77×10^6	Gram Negative Bacilli
112	10.6×10^6	Gram Positive Cocci
113	10	Gram Positive Cocci
114	750×10^6	Gram Positive Cocci
115	750×10^6	Gram Negative Bacilli
116	30	Negative
117	750×10^6	Gram Negative Bacilli Gram Positive Cocci
118	750×10^6	Gram Positive Cocci
119	40	Negative
120	10	Negative
121	10	Negative
122	35	Negative
123	10	Negative
124	0	Negative
125	0	Negative
126	430	Negative
127	15	Negative
128	750×10^6	Gram Positive Cocci
129	750×10^6	Gram Negative Bacilli
130	0	Gram Negative Bacilli Gram Positive Cocci
131	750×10^6	Gram Negative Bacilli

132/

132	750×10^6	Gram Negative Bacilli
133	10	Negative
134	36	Negative
135	20	Negative
136	350	Negative
137	10	Negative
138	0	Negative
139	43.5×10^6	Gram Positive Cocci

TABLE 23

SENSITIVITY TESTING ON ORGANISMS ISOLATED FROM URINARY UROLITHIASIS CASES

	Streptomycin		Neomycin		Furadantin		Chloromycetin		Bacitracin		Polymixin		Tetracycline		Penicillin		Erythromycin	
	S*	R*	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Staphylococci (14 cases)	11	3	12	2	11	3	12	2	11	3	8	6	13	1	11	3	13	1
Str. faecalis (8 cases)	1	7	3	5	8	0	7	1	6	2	1	7	7	1	7	1	6	2
E. coli (5 cases)	2	3	5	0	5	0	4	1	0	5	5	0	3	2	0	5	0	5

S* Sensitive

R* Resistent

TABLE 24

TWO θ ANGLES (EXPRESSED IN DEGREES) IN THE DIFFRACTION PATTERNS OF CANINE UROLITHS

	2 θ Angles of High Intensity	2 θ Angles of Lower Intensity	2 θ Angles Present in most stones
Type 1.	15.8, 16.4, 20.8, 21.4, 31.8, 33.2.	14.9, 27.0, 30.1, 30.5, 33.5.	14.2, 19.1, 25.1, 25.6, 29.0, 29.5, 32.8, 35.2, 35.7, 37.5, 38.2, 40.1, 42.3, 43.7, 43.9, 44.9, 45.7, 46.3, 50.4, 50.5, 50.8, 51.8, 52.6.
Type 2.	14.2, 14.9, 24.3, 30.1, 32.2.	20.0, 22.6, 28.8, 30.7, 31.7, 35.9, 37.3, 38.2, 40.2.	15.2, 19.6, 23.0, 26.2, 28.1, 31.4, 39.8, 40.7, 42.5, 43.5, 44.6, 45.8, 46.3, 47.1, 47.8.
Type 3.	14.2, 32.2.	20.0, 22.6, 37.3, 40.1.	
Type 4.	14.9, 30.1.	15.2, 16.5, 23.4, 30.7, 31.3, 33.3, 35.9, 38.2, 40.6, 43.4, 45.8, 50.7.	
Type 5.	14.8, Plus a Diffuse Group of Peaks Between 31.0 and 33.0.	25.8, 30.0, 35.8, 38.2.	
Type 6.	11.6, 20.9, 29.3.	23.3, 30.5, 31.3, 34.2, 34.4, 35.5, 36.8, 39.7, 41.5, 42.0, 43.0, 45.2, 47.9, 49.0, 50.2.	
Type 7.	18.8, 28.6.	9.4, 26.8, 28.0, 29.2, 33.1, 38.4.	19.9, 21.8, 34.4, 42.2, 44.6, 46.2, 49.2.
Type 8.	28.3.	9.4, 11.6, 17.9, 18.9, 25.7.	

TABLE 25

X-RAY DIFFRACTION ANGLES OF POSSIBLE CRYSTALLINE CONSTITUENTS OF CANINE UROLITHS*

Crystalline Compound	2θ Angle of Main Peaks (Degrees)	Ratio of Intensity of Peaks
STRUVITE	14.75	28
$MgNH_4PO_4 \cdot 6H_2O$	15.8	32
	18.9	8
	20.7	100
	22.65	16
	26.6	8
	27.0	20
	29.45	8
	30.5	50
	31.8	24
	33.3	50
	43.7	8
	46.0	12
	50.4	12
	52.55	12
WEDDELLITE	14.2	90
$CaC_2O_4 \cdot 2H_2O$	15.0	10
	19.9	70
	22.6	40
	24.2	60
	26.5	10
	28.9	50
	29.95	20
	32.2	100
	35.9	10
	37.3	60
	38.3	50
	40.2	80
	41.0	20
	43.7	10
	44.6	20
WHEWELLITE	14.9	100
$CaC_2O_4 \cdot H_2O$	15.2	5
	19.6	5
	23.6	5
	24.4	90
	28.7	5
	30.1	50
	30.7	10
	31.5	10
	35.9	20
	36.85	20
	38.1	80
	43.5	10
	45.8	5
	46.4	5
	47.05	5
	48.1	5

Crystalline Compound	2θ Angle of Main Peaks (Degrees)	Ratio of Intensity of Peaks
APATITE	10.8	11
$Ca_5(PO_4)_3$	16.85	5
(OH)	21.8	9
	22.9	9
	25.9	40
Basic	28.1	11
Calcium	29.0	17
Phosphate	31.8	100
or	32.2	60
Hydroxyla-	32.9	60
patite	34.05	25
	35.5	5
	39.2	7
	39.8	20
	42.0	9
	43.8	7
	45.3	5
	46.7	30
	48.1	15
	48.6	5
	49.5	40
	50.5	20
	51.3	11
	52.1	15
	53.15	20
SEC. CALCIUM	11.7	100
ORTHO	20.9	100
PHOSPHATE	23.4	7
HYDRATE	29.25	75
$CaHPO_4 \cdot 2H_2O$	30.5	50
	31.3	9
	33.5	5
	35.1	5
	35.6	5
	36.9	15
	37.1	15
	39.7	5
	39.85	30
	41.55	20
	42.0	17
	43.0	7
	43.4	9
	44.8	5
	45.3	9
	48.4	13
	50.1	20

Crystalline Compound	2θ Angle of Main Peaks (Degrees)	Ratio of Intensity of Peaks
CYSTINE	18.7	100
$SCH_2CH(NH_2)COOH$	19.75	30
	20.05	30
	21.9	30
	26.8	30
	28.2	90
	28.7	90
	29.25	20
	33.0	80
	34.3	70
	38.4	60
	39.1	20
	42.2	50
	44.6	40
	46.3	60
	47.8	10
	49.2	10
	50.7	10
	51.6	60
	52.9	10
WHITLOCKITE	10.85	11
Calcium	13.6	15
Orthophosphate	17.0	20
$Ca_3(PO_4)_2$	21.9	15
	25.8	25
	26.5	9
	27.8	55
	29.65	15
	31.0	100
	32.45	20
	33.0	9
	35.6	11
	37.3	9
	39.8	9
	41.1	13
	41.7	11
	44.5	9
	47.0	20
	48.0	15
	48.4	13
	49.8	11
BRUSHITE	21.1	80
$CaHPO_4.2H_2O$	29.55	80
	34.5	100
URIC ACID	13.2	90
	15.5	60
	17.8	90
	22.7	90
	27.0	30
	27.7	80
	28.6	100
	30.8	70

Crystalline Compound	2θ Angle of Main Peaks (Degrees)	Ratio of Intensity of Peaks
AMMONIUM	10.2	30
ACID	13.2	90
URATE	19.2	20
	25.85	100
	28.5	40
	29.65	60
	34.9	20
	35.9	20
	36.95	20
	50.4	30
XANTHINE	10.00	10
	13.05	10
	16.75	10
	19.5	10
	24.85	10
	26.85	100
	31.6	10
	35.9	10
	39.1	10
CARBONATE	21.75	20
APATITE**	22.8	20
	25.9	80
	28.0	10
	29.0	20
	31.7	100
	33.0	90
	34.2	50
	35.6	10
	40.0	60
	42.2	10
	43.9	10
	45.3	10
	47.3	70
	48.1	30
	49.8	70
	50.7	40
	51.6	40
	52.2	40
	56.0	20

Crystalline Compound	2θ Angle of Main Peaks (Degrees)	Ratio of Intensity of Peaks	Crystalline Compound	2θ Angle of Main Peaks (Degrees)	Ratio of Intensity of Peaks
URIC ACID	31.8 34.6	40 60			

* Calculated from the "Index to the X-ray Powder Data File" (1959) using the "Tables for Conversion of X-ray Diffraction Angles to Interplaner Spacing" (1950).

** Recorded from Prien & Frondel (1947).

"CORK-SCREW URETHRA" IN THE DOG.

Introduction. The term "Cork-Screw Urethra" has been used here to indicate a condition similar to that of the same name described in children (Smellie, Hobson, Edwards and Normand, 1964).

Subject. 18 month old, Male, Black Miniature Poodle.

History. The dog was presented at the clinic because of constant nocturia which had been noticed since birth. During the past 6 months the urine had been passed without force, so that it ran vertically from the external urethral meatus to the ground. The animal's appetite was apparently normal and no vomiting or increased water intake had been observed.

Clinical Examination. The animal was noticeably thin (4.9 Kgms). No abnormalities of temperature, heart rate, pulse rate or respiratory rate were noticed and the mucous membranes were normal in colour. Rectal and abdominal palpation did not reveal any abnormalities. The dog adopted a squatting posture to urinate and the urinary stream, which appeared to be of normal diameter, was passed as previously described. No recognisable signs of pain were seen during micturition, nor was there any apparent prolongation of the time taken to urinate.

Clinical Laboratory Examination. A blood sample was tested for the determination of the blood urea concentration and a urine sample was collected for urinalysis, urinary sediment examination and bacteriology.

Blood Urea Level. The level of blood urea (22.2 mgms.%) was within normal limits.

Urinalysis/

Urinalysis. The urine was a cloudy yellow colour, acid (pH 6.4) in reaction and with a specific gravity of 1.050. A trace of protein and some bile pigments were present but no bile salts, indican, blood, sugars, ketones or cystine were detected.

Urinary Sediment. A very little deposit resulted when 10 ml. of urine was centrifuged for 5 minutes in a M.S.E. Minor Centrifuge. The deposit was mostly composed of epithelial cells, with some hyaline casts, amorphous crystals and sperm also present.

A trypsin digest test was performed on each of 5 consecutive days and was negative on each occasion. A number of undigested muscle fibres in the faeces were a constant finding.

Urine Bacteriology. No bacteria were isolated on quantitative bacteriology (colony counting technique).

Radiography. Plain lateral and ventro-dorsal rays, taken with the animal in lateral recumbancy, failed to reveal any abnormalities.

A pneumocystogram performed with the urethral catheter still in position showed an unusual position of the urethral entrance into the bladder (Fig. 40a).

Micturating urography indicated that, during micturition, an unusual twisting of the proximal urethra occurred. A slight dilation of the urethra immediately posterior to the twist was present and a sudden diminition of the size of the urethral lumen was seen towards the anterior end. The ventro-dorsal radiographs showed a defect in urethral filling in the area of the prostatic urethra (Fig. 40c). Radiographs taken immediately following micturition revealed some retention of the Radio-Opaque material (Fig. 40d). A repeat micturating cystogram/

cystogram was taken after an interval of one week and the urethral twisting was still obvious (Fig. 40c).

Diagnosis.

Two significant facts emerged from the examinations performed. The first being that a urethral abnormality involving the proximal position of the urethra was present. The second being that, the constant failure to detect trypsin in the faeces, together with the presence of undigested food particles seen in repeated samples, indicated a pancreatic deficiency. A diagnosis of pancreatic deficiency together with a structural urethral abnormality was therefore made. Euthanasia was requested by the owner.

Post Mortem. The intra-pelvic portion of the urethra in this animal was of greater length (4½cms. - measured from the posterior border of the prostate gland to the level of the ischial arch) than expected in an animal of this size. The bladder was small (2½ cms. x 2¼ cms. x 2¼ cms.) and thick walled. The ureters appeared to be of normal diameter and no obvious renal defects were observed. The prostate was also small (2 cms. x 1 cm. x 2 cms.) and macroscopically normal. No abnormalities of the other body systems were seen. No histopathology was performed.

Discussion. The aetiology of this hitherto unrecorded urethral condition is obscure. The elongated intra-pelvic urethra seen on post mortem and the abnormal position of the bladder neck observed in the pneumocystogram together with the history suggest that an anatomical developmental anomaly may have been responsible for its occurrence. It is not known whether any importance can be attached to the presence of a co-existing pancreatic deficiency, but it seems unlikely that the two conditions are intimately connected.

FOLDING OF THE VENTRAL URETHRAL WALL INTO THE LUMEN OF THE PROXIMAL URETHRA.

Subject. A 4 year old female Welsh Corgi.

History. The animal was presented to the clinic with signs of dysuria which, according to the owner, had been noticed over the previous 8 months. Haematuria, persistent nocturia and increased frequency of urination during the day were also features of the condition. Immediately prior to the onset of urinary signs, the animal had shown evidence of pain in the back and had temporarily lost the full use of its hind limbs. The back pain and posterior paresis gradually improved and were not apparent after a period of several weeks.

Clinical Examination. The animal was in good condition (10.6 kgms.). On clinical examination no signs of illness were observed. However on micturition, the urine had a strong ammoniacal odour and was seen to be sanguinous in colour and contained blood clots. Following urination the bitch continued to squat and strain for a considerable period.

Clinical laboratory Examination. A blood sample was taken for the determination of the blood urea concentration and a urine sample was collected for urinalysis, urinary sediment examination, and bacteriology.

Blood Urea. The blood urea level (27.3 mgms.%) was found to be within normal limits.

Urinalysis. The urine was highly alkaline (pH 7.9). The specific gravity was 1.032 and both protein and blood were present. No bile salts or pigments, indican, sugar, ketones or cystine were found.

Urinary/

Urinary Sediment. After centrifuging 10 cc of urine for 5 minutes in an M.S.E. Minor Centrifuge, a heavy brown cellular deposit (0.2cc) was observed. On microscopic examination this was seen to be mainly composed of red blood cells. However numerous pleomorphic bacilli, leucocytes and epithelial cells were also distinguishable. No casts or crystals were seen.

Bacteriology. Quantitative bacteriological examination revealed that approximately 4.4×10^6 Proteus Mirabilis organisms per ml. of urine were present. These organisms were sensitive to Streptomyzin and Neomycin but resistent to Furadantin, Penicillin, Chloramphenicol, Bacitracin, Polymixin, Erythromycin and Tetracycline.

Radiographic Examination. No abnormalities of the Urinary Tract were found on plain lateral and Ventro-Dorsal rays taken with the animal in lateral recumbancy. However, an exostosis (spondylosis) involving the ventral borders of the vertebral bodies L4 - L5 and at the Lumbo-sacral junction was noted. Evidence of calcification of the intervertebral discs L3 - L4 and L4 - L5 were also obvious. On micturating urography, bilateral vesico-ureteral reflux was observed. A filling defect was present in the proximal urethra and appeared to extend, from the ventral urethral outline, upwards and forward into the lumen of the urethra for more than half of its width (Fig. 40a). A repeat micturating urogram taken two days later revealed a similar picture (Fig. 40b).

Diagnosis. A Proteus urinary infection complicated by bilateral vesico-ureteral reflux and a proximal urethral defect was diagnosed.

Treatment/

Treatment. In the case presented here it seemed advisable to attempt to remove both the urethral defect and the urinary infection. Streptomycin therapy was immediately instituted and after 3 days the colour of the urine returned to normal. An operation was then performed in an endeavour to determine the exact nature of the urethral abnormality and, if possible to remove it. At this time it was not certain whether the defect was a urethral valve, or whether it was a folding of the ventral wall of the urethra into its lumen, both of which are recognised conditions in human urology.

The animal was prepared as for a cystotomy operation and a ventral mid-line abdominal incision was made immediately in front of the pubis. A urethral catheter was placed in position and the urine drained from the bladder. After exposure of the ventral bladder neck area a longitudinal incision into the ventral mid-line of the urethra over the area of the defect was made. After removal of the urethral catheter a careful search of the mucosal surface of the urethra and bladder-neck was carried out but no anatomical defect was seen and it was therefore considered that the defect was probably due to a folding of the urethral wall occurring during the act of micturition. The catheter was replaced in position and the urethral incision closed with a double row of medium chromic catgut using the Czerny-Lembert technique. The abdominal wound was closed in the usual manner.

A micturating urogram taken 1 week post-operatively showed that the urethral defect was no longer present, nor was the abnormality at the bladder-neck observable, the bilateral vesico-ureteral reflux however, remained, and the lumen of the urethra was apparently narrowed, probably as a result of the operative procedure.

Antibiotic/

Antibiotic therapy was continued for one month post-operatively and, at that time, according to the owner, no signs of urinary dysfunction were obvious.

<u>Discussion</u>. Urethral defects are commonly encountered in human urology but rarely diagnosed in dogs. In the case recorded here a urethral defect causing obstruction to the urinary outflow, as evidenced by a dilated portion of the urethra immediately anterior to the defect, was observed. The occurrence of a concurrent proteus infection is of interest in the light of the recently reported association between proteus urinary infections and structural urinary tract abnormalities in man (McGeachie, 1966). The bilateral vesico-ureteral reflux in this dog seems likely to be related to the infection, although the increased vesical pressure during micturition that is likely to be present in an animal with vesical outflow obstruction may also play a part in causing incompetency of the uretero-vesical valve.

The radiographic picture of the urethral defect in this case is similar in many respects to that found in urethral valvular obstruction in children (Kjellberg, Ericsson and Rudhe, 1957). However these valves are congenital in origin, and lie in the area of the verumontanum. It seems unlikely that valvular defects such as these would be associated with a clinical syndrom of sudden onset in an adult dog. The complete lack of a valvular flap within the urethra and the disappearance of the obstructive lesion following operative intervention suggests that the radiographic defect seen in repeated pre-operative micturating urographs was due to a folding of the ventral urethral wall occurring during micturition.

The/

The aetiology of such a defect is unknown. In this animal however, the posterior paresis observed immediately prior to the onset of signs of urinary dysfunction may indicate that some de-arrangement of the nervous control of this portion of the urethra was implicated.

References.

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McGEACHIE, J. (1966). Quantitative Bacteriology of Urinary Infection. Brit. J. Urol., 38, 3, 294-301.

Fig. 39a. Lateral Pneumocystogram in a Case of "Cork-Screw Urethra". The position of the Urethral Catheter indicates the abnormal position of the Bladder Neck.

Fig. 39b Lateral Micturating Urogram in the same Animal. Showing (a) Twisting of the Intrapelvic Urethra (b) Slight dilation of the Urethra Posterior to the twist. (c) "Intussusception-Like" appearance at the Bladder end of the Defect.


Fig. 39c. Ventro-Dorsal Micturating Urogram in the same Animal. Showing a Defect in Urethral filling and the twisting form of the Pelvic Urethra.

Fig. 39d Lateral Radiograph taken immediately following Micturating Urography in the same animal. Showing Retention of the Radio-Opaque Material.

Fig. 39e A repeat Lateral Micturating Urogram in the same Animal. Showing continued presence of the Defect.

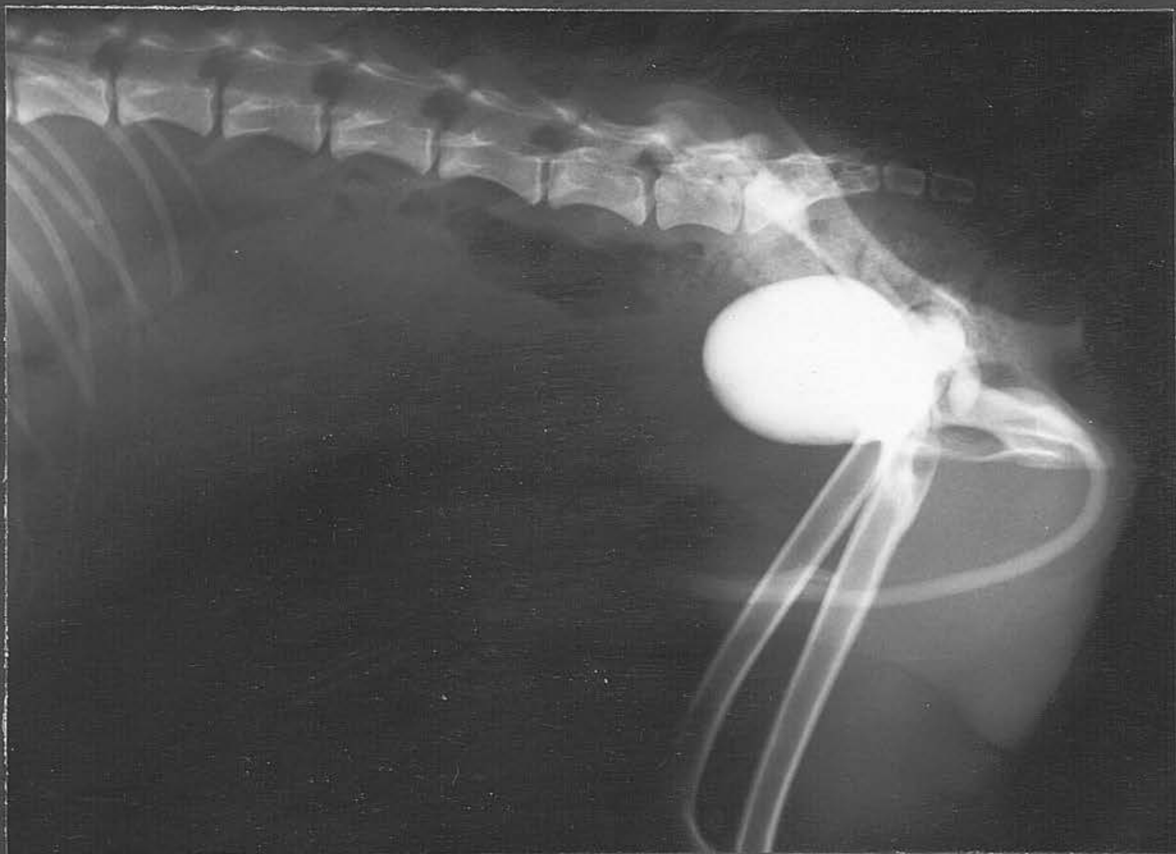

Fig. 40a Lateral Micturating Urogram in a dog with a Urethral Abnormality. Showing (a) Bilateral Vesico-Ureteral Reflux (b) A filling Defect of the Proximal Urethra (c) A Defect encircling the bladder neck area.

Note: The appearance of the Urethral Defect is consistent with the Diagnosis of a Folding of the Ventral Urethral Wall into its Lumen - A condition known to exist in Humans.

Fig. 40b. A Repeat Micturating Urogram of the above Animal. Showing similar defects.

Fig. 40c. Post-Operative Micturating urogram in the same Animal as 40a and 40b. Showing a narrowed Urethral Lumen and Bilateral Vesico-Ureteral Reflux.

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Urolithiasis in the Dog—V Treatment and Prophylaxis

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INTRODUCTION

THE BASIC pre-requisite for adequate treatment of urinary lithiasis in the dog is a thorough examination of the entire urinary tract and an investigation into the metabolic processes that are responsible for the formation of the chief calculous constituents. Because the techniques of investigating the metabolic processes have not yet been generally applied to the clinical disease in the dog, the main aetiological factors are still unknown and the treatment of urinary lithiasis is consequently restricted to the surgical removal of the calculi and to empirical measures to prevent their reformation.

Since the biochemistry of the most common calculous disorders is not known and since there are virtually no long-term results of the comparative value of the different methods of therapy in the dog, the methods of treatment in current use lack evidence to support their value. As is usual in such circumstances there are many explanations of and treatments for those diseases we do not understand.

My remarks here will be limited to discussing the methods of treatment and prophylaxis with which I am familiar. These remarks are based on data derived from the examination of fifty clinical cases of urolithiasis in the dog. I intend to divide the paper into two parts, firstly, the removal of the calculi from the urinary tract and secondly, the prophylaxis of further calculous epidoses.

THE REMOVAL OF CALCULI FROM THE URINARY TRACT

In cases of urolithiasis the accepted principle of surgical treatment is the removal of all calculi from the urinary tract. Therefore in every case a thorough radiological examination of the entire system must be carried out. To stress this point, in thirty-three consecutive cases of urethral calculi in dogs presented for treatment, twenty-four had radiographically demonstrable calculi in the more anterior portions of the urinary tract.

The operative procedures for removal of stones are urethrotomy, cystotomy, nephrectomy, pelviolithotomy and nephrolithotomy. Since renal calculi are seldom encountered in clinical practice, the last three of these operations are rarely performed. The first two however are common operations and although time does not permit a full description of these procedures, a few points will be considered.

Urethrotomy

The operation of urethrotomy is well described in the veterinary text-books and in cases of a single stone occurring in a young non-uraemic animal the procedure is simple and easily performed.

However in cases of obstruction with constant vomiting and other uraemic signs present, especial attention should be paid to the establishment of normal fluid and electrolyte levels, either immediately before or during the operation.

Antibiotics may be used prior to and following the operations.

General anaesthesia is preferred but in uraemic animals care should be taken in the use of the longer acting barbiturates. In these cases unless an inhalation anesthetic apparatus is available it is probably better to use local or spinal techniques.

Immediately the stones are removed a catheter should be passed into the bladder in order to ensure patency of the entire urethra and to drain the accumulated urine from the bladder.

To prevent the urethra becoming obstructed with blood clots, etc., the catheter should be clamped and left in position until the animal recovers from the anesthetic. It should be noted, however, that the longer the catheter is left in position the greater the likelihood of introducing infection into the bladder. The urethrotomy wound is left open and allowed to heal.

Cystotomy

In cases in which single, or large multiple stones are present, removal of these presents little difficulty. Only when numerous small stones are present is it difficult to remove them all from the bladder.

During the operation small calculi tend to become lodged in the bladder neck or prostatic urethra. To prevent this occurring and to ensure the complete removal of all bladder calculi the following procedure is recommended: a catheter is placed in the urethra prior to the operation and the bladder drained of urine. The catheter is then connected to a large bottle of sterile physiological saline with a "drip-set" or flutter-valve connection. The bottle of saline is then positioned a few feet above the level of the animal—usually on a fluid-therapy stand. By these means a threefold advantage is gained, firstly, the catheter acts as a barrier to the passage into the pelvic urethra of any small calculi that may be present in the bladder; secondly, a source of fluid is available for thorough flushing of the bladder for the removal of small calculous material; thirdly, the bladder may be filled with saline after the cystotomy incision is completed so that the effectiveness of the bladder closure can be tested before the laparotomy incision is closed. Thorough washing of the bladder is effected by a constant flow of saline through the urethral catheter into the bladder from where it is removed together with any calculous material present by a suction apparatus.

On radiological examination, calculi may be found at various different anatomical sites of the urinary tract. The more usual locations occurred in the following order of frequency:

1. urethral and vesical stones;
2. vesical stones only;
3. urethral stones only;
4. renal stones only, and
5. renal and vesical, or renal, vesical and urethral stones.

In the Small Animal Clinic at the Royal (Dick) School of Veterinary Studies where radiological examination of the entire urinary tract is routine practice in cases of urolithiasis, in forty-seven (94 per cent) of the fifty cases the calculi were urethral, vesicular or both, i.e. only the lower urinary tract was involved. Of these patients twenty-three (46 per cent) had calculi in the urethra and bladder; fifteen (30 per cent) had calculi only in the bladder; while in nine cases (18 per cent) the calculi were restricted to the urethra.

1. *Removal of calculi from patients with urethral and vesical stones*

These cases are frequently of an emergency nature as urinary outflow is usually obstructed, consequently the obstruction must be dealt with initially.

Following this initial urethrotomy the animal is observed carefully during the post-operative period for signs of further obstruction. Then some 8 days subsequent to the first operation the animal is again X-rayed and in most instances calculi are still present in the bladder. In these cases a laparocystotomy is performed. On occasions, in reasonably healthy dogs, when the vesical calculi are large a urethrotomy and a cystotomy may be performed at one operating session.

2. *Removal of vesical stones*

The removal of these stones is undertaken by cystotomy. In bitches, with vesical stones, urinary tract infection is common. The organisms most commonly identified are *staphylococci*. Treatment with the appropriate antibiotic (as determined by sensitivity tests) immediately prior to operating and for the first few post-operative days is indicated.

3. *Removal of urethral stones*

The procedure for the removal of urethral calculi has already been discussed. I would merely emphasize again that in the majority of cases presented with urethral stones further stones in the anterior portions of the urinary tract should be suspected.

With regard to the role of infection in cases of urethral stones, only six of thirty-three urethral obstructions in this series were found to be infected.

4. *Removal of renal stones*

Operations for the removal of kidney stones are well described in the literature (Archibald *et al.*, 1957; Archibald, 1965), and I do not propose to describe them here. The operative techniques which have been described were apparently selected because of the ease of the operative procedure rather than on any evidence that one is superior to another, by being associated with a more prolonged survival or a reduced recurrence rate.

Nephrectomy may only be performed when unilateral kidney involvement is present because success depends on the adequate functioning of the remaining kidney. A number of successful cases of nephrectomy for unilateral nephrolithiasis have been recorded (Überreiter, 1929; Freiermuth, 1944; Lindsay and Bruce, 1955; Whitford, 1964; Pearson *et al.*, 1965), but only one successful operation for bilateral nephrolithiasis could be found in the literature (Chernesky and Cawley, 1966).

5. *Removal of concurrently occurring renal and vesical stones or renal vesical and urethral stones*

The removal of any obstructing stones is usually urgent and should be given first consideration. In cases of renal and vesical stones the calculi may be removed by a one stage operation if the renal stones affect only one kindey.

THE PROPHYLAXIS OF FURTHER CALCULOUS EPISODES

For the purpose of prophylaxis, urinary calculi are divided into four main groups according to their major chemical compound. They occur in the following order of frequency:

1. phosphate stones;
2. cystine stones;
3. oxalate stones, and
4. urate stones.

1. *Prophylaxis of phosphate stones*

The majority of phosphate calculi in dogs have Struvite (triple phosphate) as their major constituent (White *et al.*, 1961) and there is some evidence that this type of calculi is usually associated with urinary tract infections due to urea-splitting organisms (Brodey, 1955). Consequently it is important to eradicate these organisms from the urinary tract by appropriate antibacterial therapy.

In human urology the eradication of infection and the maintenance of this state in the urinary tract is extremely difficult; it is not generally realised in the veterinary profession that the task of clearing and keeping the urinary tract of the dog free from organisms once a urinary infection has been established seems to be equally difficult.

Treatment with the appropriate antibiotic for prolonged periods may be necessary. Such treatment however may result in a change in the species of organisms present and although urolithiasis may be prevented for a time, urinary tract infection may continue.

Another procedure that has been advocated for the prevention of phosphate stones in dogs is to change the pH of the urine from alkaline to acid by the use of acid-ash diets and by the use of urinary acidifiers (e.g. ammonium chloride).

The theory supporting these procedures is based on the biochemical fact that the solubility of triple phosphate increases with increasing acidity. However the value of these measures in the prophylaxis of canine phosphate stones has still to be established.

2. *Prophylaxis of cystine stones*

The procedures used for prevention of further episodes of cystine stones are based on three biochemical facts, namely:

(a) that the solubility of cystine increases with pH especially above 7·6;
(b) that the urinary cystine is derived from the sulphur containing amino-acids, methionine and cysteine in the food, and
(c) the greater the volume of fluid passing through the urinary apparatus the lower will be the concentration of cystine in the urine.

Some veterinary authors (Harper, 1960; Brodey, 1964) are of the opinion that canine cystine stone disease can be prevented by reduction of dietary protein. As yet no recorded evidence to support these opinions has been found in the literature.

In man, a reduction of the urinary cystine level can be attained by reducing dietary protein intake but it is seldom possible to keep cystine levels sufficiently low to prevent stone formation (Collins *et al.*, 1965).

Treacher (1962) in his report on the biochemistry of this condition recommended treatment by the administration of 1 g sodium bicarbonate per 5 kg body weight three times daily in order to maintain the pH in excess of 7·6. He considered that this therapy had a two-fold advantage namely, it rendered the urine alkaline and the animals drank more water which also promoted solubility of cystine. However, there are several disadvantages to this method of prophylaxis. Some dogs will not readily eat food to which sodium bicarbonate has been added and in these cases bicarbonate tablets have to be administered which, when carried out over long periods, becomes tedious and inconvenient. Furthermore alkalinization only achieves constant under-saturation of the urine in a proportion of cases which does not include those animals excreting large amounts of cystine. Nevertheless it seems likely that effective alkalinization of the urine may benefit a proportion of these cases.

Other methods recently favoured in the therapy of the condition in man, such as the administration of penicillamine and the anabolic steroids are largely precluded from general use in veterinary practice due to their high cost. The use and value of all these methods of prophylaxis have not yet been critically assessed in the canine species.

3. *Prophylaxis of oxalate stones*

From a study of the literature there appear to have been no biochemical investigations on the clinical condition of canine oxalate urolithiasis. Consequently, the aetiological factors associated with this type of stone are unknown and the establishment of rational prophylactic procedures are difficult.

Some authors advocate restriction of foods rich in oxalates but in the dog it seems unlikely that the important source of urinary oxalate is derived from exogenous substances. This is therefore of questionable value. Similarly since pH in the physiological range would appear to have little influence on oxalate solubility the adjustment of urinary pH by certain diets or drugs would appear to be of limited value.

In man, many different regimes are employed in oxalate calculi prophylaxis. These are based on a comprehensive assessment of the underlying metabolic factors pertinent to each case. In the dog the careful monitoring of the effects of these different prophylactic procedures to assess their value has not yet been recorded. The prophylactic programmes adopted are largely based on opinion rather than on fact.

In the treatment of this and other types of calcium containing stones in humans a number of compounds are recorded as being of some value namely, orthophosphates, acetysalicylic acid, bendrofluazide, citrates, magnesium, aluminium hydroxide, etc., but the fact that at the present no single agent appears to be consistently effective or even obviously superior to any other in preventing recurrences indicates the measure of their value.

In two dogs in this series with bilateral renal stones of this type a diagnosis of primary hyperparathyroidism was made on clinical and radiological signs and biochemical findings.

Both the animals were destroyed at the request of the owners and the diagnosis was confirmed on post-mortem examination. In cases such as these, exposure of the parathyroid glands and removal of any that may appear to be enlarged is recommended. In man where this procedure is adopted routinely, dissolution of the renal calculi occasionally occurs. Thus surgical removal of renal stones need only be carried out if the calculi persist.

4. *Prophylaxis of urate stones*

Most veterinarians who have recorded their experiences in treating urate calculi have advocated the use of low purine diets in the prophylaxis of this type of stone. The purpose of the treatment is to lower urinary uric-acid levels. However it has been shown experimentally that dietary purine levels have little or no effect on urine uric acid concentrations (Benedict, 1915–16; Young *et al.*, 1938).

Dr. Porter (1963) in his thesis on some biochemical aspects of urate-stone disease found that such stones occurred in alkaline urines accompanied by high ammonium levels and he suggested that the administration of 2 g sodium bicarbonate twice daily would stabilize the ammonium-ion concentration at low levels. He also postulated that the raised ammonium levels were due to the presence of urea-splitting organisms. If this is correct the administration of appropriate antibiotic treatment is indicated and if successful the treatment by bicarbonate would appear to be unnecessary. In the patients with urate calculi studied in the present investigation, no urea-splitting organisms have been found and the urine of these animals on initial examination has been acid. However the administration of bicarbonate may still prove effective and certainly merits trial.

General Prophylactic Procedures

A number of general procedures for the prophylaxis of canine urinary calculi have been recommended. These include ensuring that affected animals have free access to water, adequate exercise, frequent opportunity to micturate and have a balanced diet.

Compounds such as hyaluronidase, vitamin A and various other vitamins have been recommended but their value in preventing recurrence of calculi is not established.

In conclusion may I stress two points first, the division of urinary calculi into four main chemical types is arbitrary and merely manifests our ignorance of the aetiological factors involved in calculi formation; second, that the treatment of calculi by surgical removal and by attempting to influence the solubility, concentration, precipitation or aggregation of the calculous constituents in the urine, we treat merely a condition or state which fairly certainly may result from a number of quite distinct disorders.

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