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STUDIES BY SCANNING ELECTRON MICROSCOPE AND X-RAYS MICROANALYZER ON URINE SEDIMENTS OF HEALTHY, AFFECTED BY VARIOUS PATHOLOGIES AND CANCER DOGS

Riassunto — *Studi con SEM e MICRO-X di sedimenti di urine di cani sani, affetti da patologie varie e da tumori.* Esami clinici e di laboratorio hanno permesso di suddividere i cani in 3 categorie: sani, affetti da patologie varie e da tumori. Gli studi morfologici e chimici effettuati con SEM e MICRO-X su microcristalli dei sedimenti urinari dei cani dei 3 gruppi forniscono nuove informazioni sui processi degenerativi dei soggetti affetti da cancro. Le analisi permettono confronti e correlazioni. Questi studi, sui sedimenti urinari dei cani affetti da cancro, sono un importante e nuovo contributo alla ricerca.

Summary — By clinical and laboratory tests we have subdivide the dogs into three groups: healthy individuals, individuals affected by various pathologies and cancer affected individuals. Following morphological and chemical studies affected by SEM X-Ray Microanalyzer on urine microcrystals of dogs from the three groups provide new information about degenerative processes of cancer affected subjects. These results make comparisons and collations possible.

Definite evaluations may be done in the morphological and chemical field of urine sediments in cancer affected dogs resulting in an important contribution to this type of research.

Key words — Urinary sediments dogs - Scanning electron microscopy.

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Introduction

This study on dog urine sediments was executed by SEM and electronic microanalyzer, to discover, in cancer cases, analogies and/or differences in the degenerative processes of such sediments as already tested in men (Altman, 1979).

Since 1979 observations of human urine sediments of presumably healthy individuals showed remarkable morphological and chemical differences (Grazzini and al., 1980) with the ones of individuals affected with neoplasia. The chemical equilibriums which cause the crystallization in healthy individuals are enough complex but regular in their ionic contitutions, while in the individuals affected with neoplasia these equilibriums and consequently the crystallizations change so much that they transform themselves in a complete degeneration of the compound (Grazzini and al., 1984).

This information has been given by the study of the analysis executed by SEM and Micro-X using particular methodologies already proved with human beings and now used with animals. With this study we want to note the existing phenomena in both healthy or sick or affected with neoplasia dog's crystallury in order to integrate and relate the preceding study with human urine sediments. To date, the crystallury had a limited symptomatological importance and we directed our research on this part of the sediment.

In this part there are macro and micro-crystals: we prevalently analyzed micro-crystals as already done with human urine sediments, as their morphology and their chemical composition provide important information in the study of metabolism.

The presence of certain crystals (chlorure, carbonate, sulphate etc.), their formation and evolution, can be related to specific conditions in the individual both healthy or sick.

The experimental results permitted us to formulate some hypothesis.

Materials and methods

Among domestic mammals, dogs have been chosen for the following reasons:

a) as resulting from data reported in Tables 1 and 2, the chemical composition, physical properties, electrolytic proportions of urine are well known and defined;

TABLE 1 - *Physical properties and Chemical composition of dog urine.*

Freezing point lowering	1.573 - 3.638 C°	
Specific gravity	1.025 (1.016 - 1.060)	
Volume	20 - 100	ml/kg body wt.
Ca	1 - 3	mg/100 ml
Mg	1.7 - 3.0	»
P	20 - 30	»
K	40 - 100	»
SO_4— tot.	30 - 50	»
Allantoin	35 - 45	»
Creatine	10 - 50	»
Creatinine	30 - 80	»
Hippuric acid	34	»
Histamine	0.010 - 0.300	»
Urea	300 - 500	»
Uric acid	4,5	»
N tot.	250 - 800	»
NH_3	30 - 60	»
Keto-body	5 - 6	»
Citric acid	2 - 20	»
Vitamin B_2	0.010 - 0.020	»
Choline	0.200 - 0.500	»
Nicotinamide	0.300 - 0.400	»
Pantothenic acid	0.130	»
N'-Methylnicotinamide	0.090 - 0.800	»
Androgens	0.010 - 0.030	»

TABLE 2 - *Amount of excretion of electrolytes in dog urine* (Biological Handbook 1979)

Electrolytes	Concentration	Excretion ratio	
		mEq/L	mEq/kg/die
NH_3	69 (4-190)	25 (2.9-82)	1.2 (0.2-3.7)
HCO_3	39	18 (0.01-71)	1.3 (0.5-3.2)
Ca	2.12 (0.18-7.70)	1.3 (0.1-7.0)	0.11 (0.08-0.14)
Cl^-	76 (0-289)	40 (0-222)	2.0 (0-10.3)
Mg	8.3 (2.8-26.9)	3.9 (0.7-20.7)	0.21 (0.05-0.53)
PO_4^{---}	(0-120)	7 (0-38)	0.25 (0-1.04)
K	84 (18-234)	31 (3-128)	1.0 (0.1-2.4)
Na	74 (2-189)	32 (1-209)	1.9 (0.04-13)
SO_4^{--}	48 (6-233)	26 (1-48)	1.7 (0.05-3.1)

b) dog alimentation has changed from a carnivorous one to an omnivorous one similar to man's;

c) a dog's average-life span (10-16 years) offers the possibility of working with subjects in the various ages (youth, maturity, old age);

d) urine collecting is relatively easy;

e) dogs, living in close connection with man, offer the possibility to collect certain and complete anamnesis and to follow clinical cases continuously;

f) death in dogs almost always occours in old age.

At first a total of 29 dogs was examined; each subject was clinically examined for an accurate anamnesis. Dog faeces were analyzed in order to point out parassite infectans. A physical-chemical analysis of dog urine and hematological examination were effected in order to find the hematic costants. These results allowed us to subdivide the dogs into three groups:

n. 13 healthy dogs

n. 10 dogs affected by various pathologies

n. 6 cancer affected dogs.

Regarding the third group, diagnosis was completed by surgical anatomic-pathologic observations.

At a second stage urine was collected (Table 3) (200 ml of urine), spontaneously in 13 cases and by vescical cateterism in 16 cases. Out of each sample collected, 100 ml were used for normal chemical-physical analysis and the other 100 ml for SEM observation.

This latter sample of urine, kept in sterilized containers, was brought within 24 hrs. to Biological Laboratory where it was centrifuged at 3500 r/m for 10'. Afterwards the organic substances were removed by washings with ethilic ether and bi-distilled water; the sediment residue, after spontaneous dessiccation at 15-18° C, was placed on the sample-holder of SEM, and metalised. Two metal sprayings were effected for each sample: one with gold for morphological observations and one with graphite to determinate the chemical composition.

The above mentioned analyses were effected by means of SEM (JEOL-JSM-U3) with X-ray dispersive energy Microanalyzer (PGT-716 Ortec - Ortec 6200) and were executed with an energy of about 15 Kv in an interval of 20 K V.

Our attention was focused on microcrystals, on the crystallized, non organic part. Morphological and chemical analyses have been performed by means of SEM and X-microanalyzer.

TABLE 3 - *Data of analytic identification and clinical diagnosis of the considered dogs.*

Specimen	Subject	Age	Sex	Urine	Faeces	Red blood cells	White blood cell	Hema-tocrit %	Hemo-globin gr% ml	Urea nitrogen mg%	Creatinin mg%	Glucose mg%	Amilase u/l	GOT u/l	GPT u/l	Diagnosis
Healthy dogs	Y	6 years	M	—	—	6.300.000	7.500	—	12,5	37	—	74	297	14	12	—
	L	3 »	M	—	—	7.000.000	6.400	—	11,8	29	—	58	375	15	13	—
	B	6 »	M	—	—	5.400.000	9.800	—	19	35	—	69	325	17	12	—
	Bu	6 »	M	—	—	7.800.000	6.300	—	17,5	33	—	58	282	18	17	—
	Ne	2 »	M	—	—	8.000.000	7.800	—	15	30	—	68	308	18	13	—
	Ar	2 »	M	—	—	6.300.000	9.200	40	14,2	21	—	93	407	16	20	—
	Nb	2 »	F	—	—	7.400.000	8.400	41	12,4	28	—	64	210	12	17	
	Br	8 »	F	—	—	8.000.000	6.300	39	11,6	24	—	70	300	18	19	—
	Nr	9 »	F	—	—	7.000.000	8.000	38	12,5	40	—	83	450	15	19	—
	Tf	7 »	M	—	—	7.300.000	6.800	—	11,7	35	—	72	320	15	12	—
	Bt	6 »	F	—	—	5.700.000	8.300	—	12,5	24	—	82	228	11	12	—
	Bo	4 months	M	—	—	7.800.000	8.300	—	12,7	22	—	63	289	11	12	—
	Dr	2 years	M	—	—	5.900.000	8.700	—	11,3	25	—	67	322	11	13	—
Pathological cases	K	12 years	M	albumin blood	—	5.000.000	11.000	—	15	58	1,7	67	380	22	25	Vaginal cist
	Dk	3 »	M	—	anchilostoma Trichiuris	4.800.000	9.700	—	17	27	—	75	390	16	17	Intestine parasites
	Ri	3 »	M	—	—	7.500.000	9.900	—	19	34	—	56	247	22	20	Traumatic injures
	W	13 months	M	—	toxocars	5.400.000	11.500	41	11,8	32	—	82	296	23	26	Intestine parasites
	Set	5 years	F	albumin glucose	-	4.200.000	15.800	36	11,2	59	2,3	128	478	55	63	Abdominal pain
	Sg	6 months	M	—	—	6.400.000	8.300	42	10,7	31	—	63	284	18	17	Abdominal pain
	Pe	13 years	M	albumin glucose	-	4.700.000	12.400	32	10,5	43	1,8	125	400	59	60	Red blood cells and urine alteration
	Oc	1 year	M	—	—	6.800.000	12.800	—	10,9	38	—	79	386	12	18	Pnemonia distemper
	Ros	3 years	M	—	—	5.700.000	8.500	35	10,2	43	1,6	100	295	17	12	Tonsillitis
	Rg	9 »	M	—	—	6.700.000	8.600	37	10,8	41	1,5	90	315	23	30	Pneumonia distemper
Tumours	Bd	9 years	F	albumin glucose	—	6.400.000	7.200	40	10,9	36	—	58	266	26	29	Breast cancer
	Mf	13 years	F	albumin	-	4.800.000	12.100	37	10,9	47	1,8	107	384	23	25	Breast cancer
	On	9 »	F	—	—	6.200.000	10.400	38	11,6	25	1,3	98	320	12,5	19	Breast cancer
	Ch	12 »	F	albumin	—	5.200.000	13.400	36	11,4	32	—	78	256	15	22	Perinous
	Pt	10 »	M	albumin	—	6.500.000	9.500	—	12,3	90	2,2	60	297	38,7	79,4	Cerebral tumour with liver and lung metastases
	Pol	14 »	F	—	—	5.800.000	9.700	40	10,2	39	1,4	81	318	22	22	Spleen tumor

Results

The analysis by SEM and X-ray microanalyzer on micro-crystals of the dog urine sediments have pointed out that in healthy subjects there are regular geometric crystals (photos 1-4).

These geometric forms are really definite and in particular they can be cubic or rhombohedral or prismatic forms and so on.

The crystals can be isolated or in crystalline aggregates.

The crystalline aggregate is in geminate forms never observed in the crystals of human urine sediments.

In the photo n. 5 we can observe the characteristic gemination of some geminate crystals of chemical definite composition and crystalline definite lattice face [III].

In the neoplasia carrier subjects we note an evolution and a degeneration of the crystalline formation as we observed in human beings. The analysis done on the crystalline formations of the dogs affected with neoplasia show an evolution related to the pathologic process. In fact the morphologic crystalline alteration begins with an inflation of the crystal, a lamellar arrangement of the compound and the corners become round.

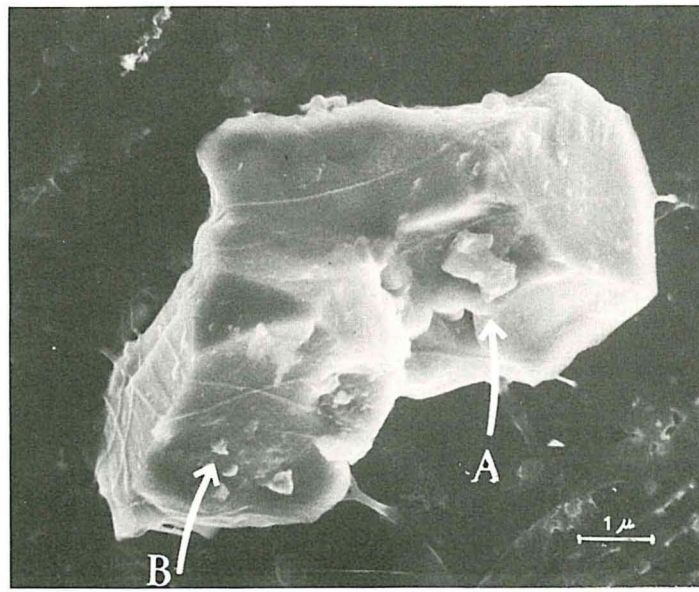

Photo 1 - Full-grown crystal with evident sharp corner and regular, parallel increase. A and B are the zone in which microanalyses are affected (see Tb. 3 for Tf.).

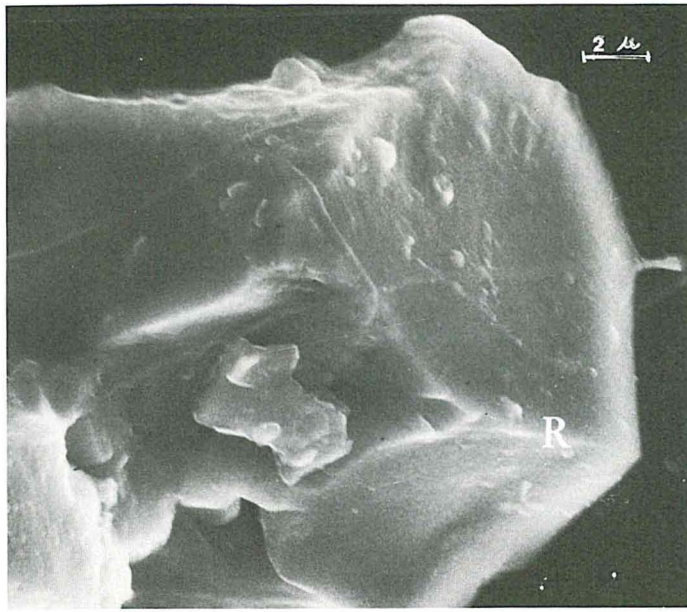

Photo 2 - Detail of Photo 1: A beginning phase of gemination in R is evident throughout the crystallization (see Tb. 3 for Tf.).

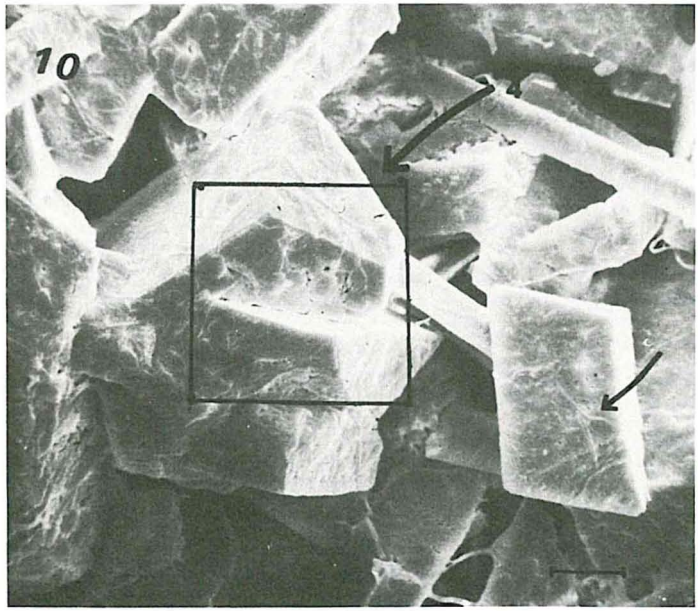

Photo 3 - Regular crystals with parallel increase and crystalline rotation (see Tab. 3 for W.).

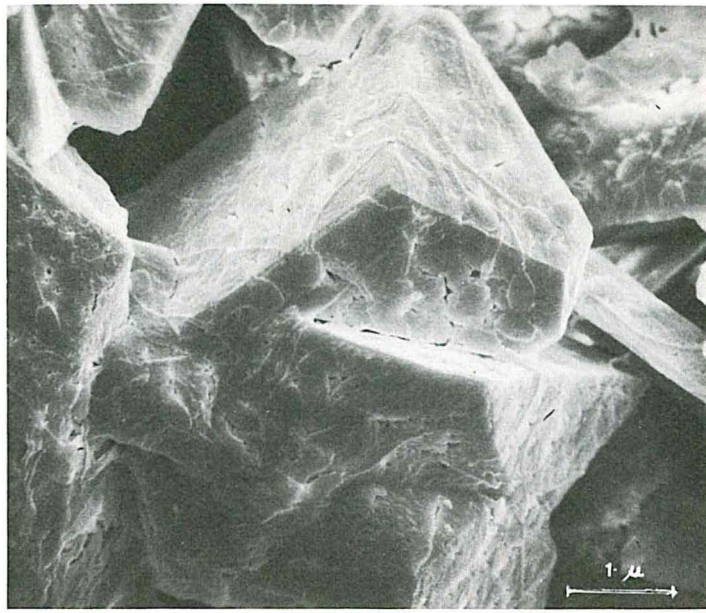

Photo 4 - Detail of photo 3: evident rotation and beginning of gemination.

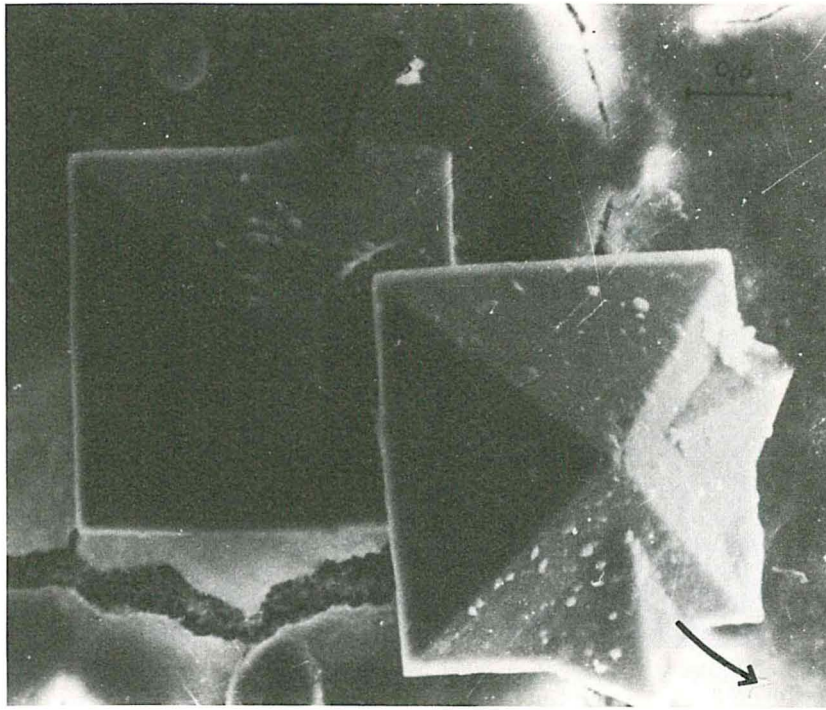

Photo 5 - Evident gemination by penetration. Regular crystals. A slight swelling in B perhaps for a crystallization interaction (See Tab. 3 for Sg.).

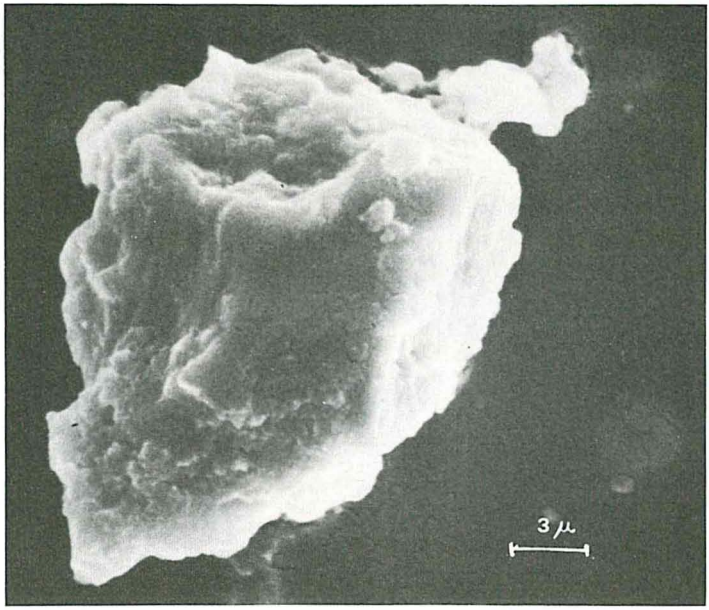

Photo 6 - Abnormal crystallization is beginning, the mass seems to swell and the parallel crystallizations stretch to widen. Gold metallization. Magn. x 4000 (See Tb. 3 for Neb.).

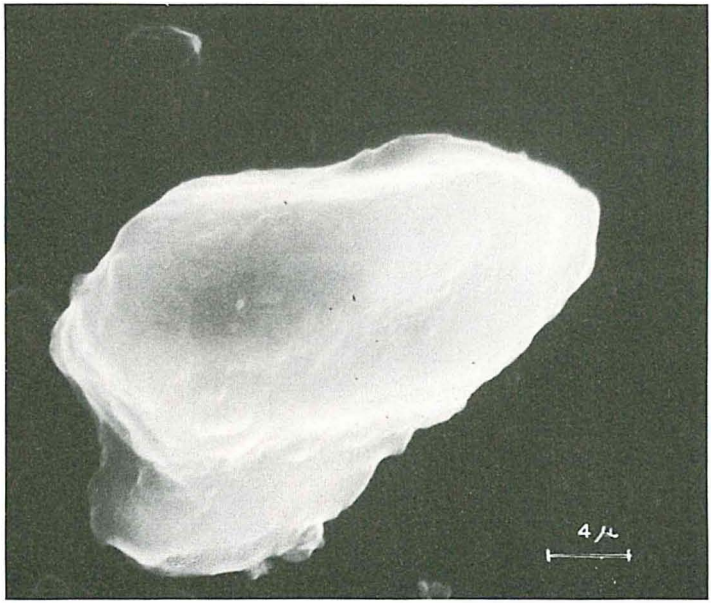

Photo 7 - The phenomenon is like the preceding one. The arrow shows the rounded corners more evidently than in photo 6 (See Tb. 3 for Bd.).

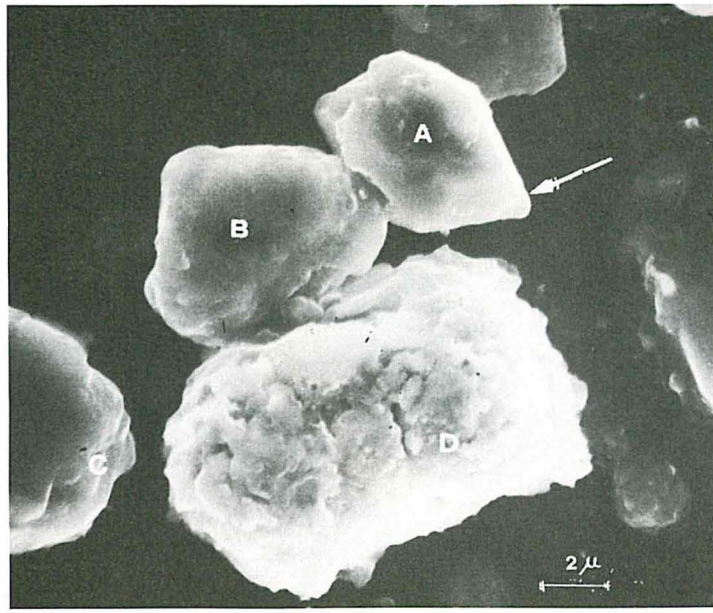

Photo 8 - Two kinds of crystals are evident, in A and B crystallization is somewhat regular. In A it is normal. In C and D it is abnormal). In C the parallel, rounded and enlarged formations are evident. In D the mass is more irregular (See Tb. 3 for Sett.).

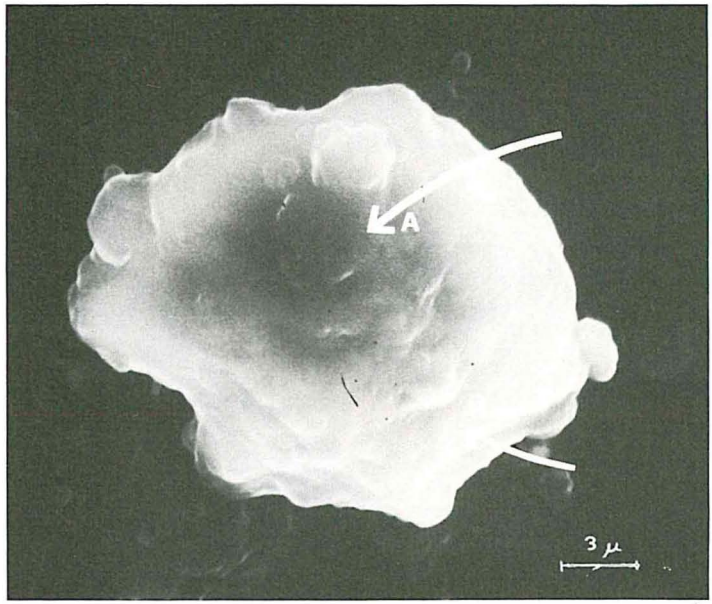

Photo 9 - Anomalous matter: there is no crystallization. The phase is very advanced (See Tab. 3 for Pt.).
Two different zones are shown. The A zone is like photo 8; in the B zone a certain amount of packet crystallization is still visible.

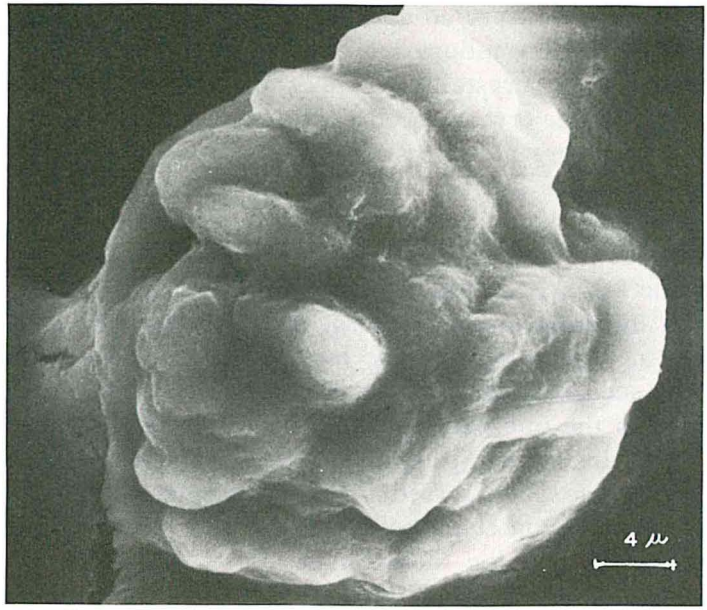

Photo 10 - Final, degenerative phase. Similar phenomenon may be found in a dying man. The matter is undone and chemical composition of crystals is undefinable. The shape is anomalous, with evident heap of cellular matter. These forms are to be found in the whole samples (See Tb. 3 for Pt.).

This is the first step of the degenerative process (photos 8-9). In the most advanced steps of the neoplastic process the crystalline forms accumulate giving anomalous aggregates. In fact we can observe irregular forms which can't be identified (photos 6-7-10).

Chemically the microanalysis have demonstrated that a morphologic alteration corresponds to a transformation of the compound, i.e. a partial substitution $Na^+ \rightarrow K^+$.

In the dogs there are different ions than in man, Ca, Mg, Fe. It is on these ions that the modifications and the exchanges that we are studying occur. As in man in the final step the ion sulphur comes out.

Discussion

The study of the morphology of dog urine sediments has given data which can be compared with the observations done on human beings. In the healthy dogs we found that the chemical and physical-

chemical rules are respected and the crystallographic rules too; the solid state of the crystallury has precise characters which can be observed again. The crystalline forms show that in the healthy dogs the formation of the crystals occurs differently than in man (geminate and crystalline aggregate). Consequently, the ionic concentrations and the reticular bonds are probably different from those of man. In the presence of neoplasia the crystalline formation evolves and degenerates as in human beings but in the dogs we have a first step of alteration followed by the final degenerative step; instead in man we have 3 different steps: the first step of alteration, the second one of chemical modification and the final one of degeneration. Until now the second step has never been observed. The degenerative phenomena in the microcrystals of a dog affected with a cancer is shorter than in a man probably because there are not the intermediate processes observed in human beings.

This phenomenon could be due to the shorter life of a dog and to the differences in the metabolism.

We have also remarked that between data reported in Tb. 3 and the alterations observed by SEM and Micro-X there is a complete parallelism.

The variations we found in the individuals affected with cancer are not estimable as a repetitive and statistic datum.

The crystalline alterations in the urine sediments of the dogs affected with cancer have been extensively demonstrated with these new methodologies and so the information obtained are a new datum of research.

REFERENCES

ALTMAN PL. L. (1979) - Blood and Other Body Fluid. Edited by Dorothy S. Dittmer Washington D.C., 371-376.

BECCARI L. (1948) - Fisiologia degli animali domestici. Vol. 1, 385-411.

GRAZZINI M., CENCIONI R., FORMICONI G. (1984) - Evaluation des substitutions ioniques des composants à l'état solide au moyen du MEB et du microanalyseur à rayon X. J. de Phisique; tome à rayon X. J. de Phisique; tome 45, 741-744.

GRAZZINI M., FORMICONI G., PIOVANELLI A., CENCIONI R. (1980) - Effects de cristallisation dans les sédiments urinaires: étude au moyen du MEB, du microanalyseur à rayon X et de l'analyseur thérmique. *Atti Soc. Tosc. Scienze Nat.*, serie B, V. 87, 234-263.

MARTINI E. (1975) - Fisiologia degli animali domestici. Ed. Tinerelli, Bologna, vol. 2, 738-794.

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