ABOUT CLINICAL URINE ANALYSIS OF PINNIPEDS IN CAPTIVITY

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Results of study of some urine properties and composition in gray and harp seals in captivity are presented. It noted differences in physical properties, qualitative reactions and chemical composition of urine in healthy and sick animals. It is proposed to use tests in this study applied to assess the health status of pinnipeds at the level of the norm – pathology.

Keywords: pinnipeds, gray seal, bearded seal, harp seal, care in captivity, blood

Methods of biochemical analysis are used, as a rule, at clinical examination either to make the diagnosis or to examine the severity and kinetics of deseases process. In the first case biochemical analysis must either confirm or reject presumptive diagnosis, in the second case, it should help evaluate effectiveness of methods of treatment and prognostication [1].

Among all diseases occurring in captured sea mammals, proportion of internal uninfectious ones is large. The peculiarity of the last ones that besides obviously sick animals receiving treatment, there are a lot of animals without clinical signs of a disease, but with low level metabolism or with protein, carbohydrate, lipid, vitamin and mineral metabolopathy. These animals also require treatment although they have no marked clinical signs. The main reasons of internal uninfectious diseases are violation of feeding rules, maintenance a usage of animals [2, 3].

In view of this it is necessary to implement regular control of animal health, timely veterinary preventive measures that prevent diseases and provide for normal vital activity of animals. Analysis of urine not only points to this or that condition of kidneys but also permits to think about involvement of some other organs and systems (affection of liver, metabolopathy etc). This analysis is relatively simple to obtain comparing to taking blood analysis for example, and besides it doesn't scare animals. Its easiness and rather full informative range attract attention.

Materials and methods of research

Material for study was obtained from four seals delivered to Murmansk Marine Biological Institute for rehabilitation: gray seal (*Halichoerus grypus*) – "Rik"; bearded seal (*Erignathus barbatus*) – "Gip"; harp seals (*Pagophilus groenlandica*) – "Murka", "Mishka". Animals were housed in plastic tubs 2×2×1 m in flowing seawater. Bath was dried before urine sampling.

Results of research and their discussion

There are only two papers on study of urine properties in northern fur seal [6] and two species of cetaceans [8], as well as general guidelines for urine analysis in marine mammals [1].

But it should be noted that practically there are no data about such investigations in sea mammals.

On basis the above-stated we made it our aim to develop and choose the simplest, most accessible and informative procedures of analyzing urine of captured seals. Besides, as we haven't found data on urine composition of seals in literature available, results obtained for healthy and sick animals, can act as starting point in this course of research.

Taking urine analysis in sea mamals presents certain difficulties because water is their habitat. However, experience shows that maintenance of animals in pools with controlled water flow allows to obtain urine analysis. But only inconvenience about it is that sometimes it takes quits a long time. It is advisable to collect urine in the morning before feeding animals and when animals are dried. To collect urine first water is removed from the pool and it is not poured into until the procedure of obtaining the analysis ends. Usually, if animals aren't scared and are accustomed to people, they allow to approach them during urination without getting excited. Investigator's only task is to carefully collect urine in glass prepared beforehand. Urine is collected in ry clean glass and examined within 30 minutes to 1,5 hours after its collection. Long storage results in changing physical properties, multiplication of bacteria and destruction of deposit If necessary urine can be stored quite long and the best way is its storage in cold. However, in case it is impossible, some chemical agent is supplemented. Toluene is the most convenient substance that is supplemented in the amount to cover the surface of the collected urine with a thin floating layer [4].

Analysis of urine included examination of physical properties (colour, transparency, pH) and chemical analysis. Quantitative tests that allow to reveal compounds, normally not found, are convenient for express-characteristic of urine. Table 1 presents data on the qualitative urine analysis of healthy and sick animals in captivity.

Table 1

Physical properties and quantitative tests of the urine of pinnipeds in captivity

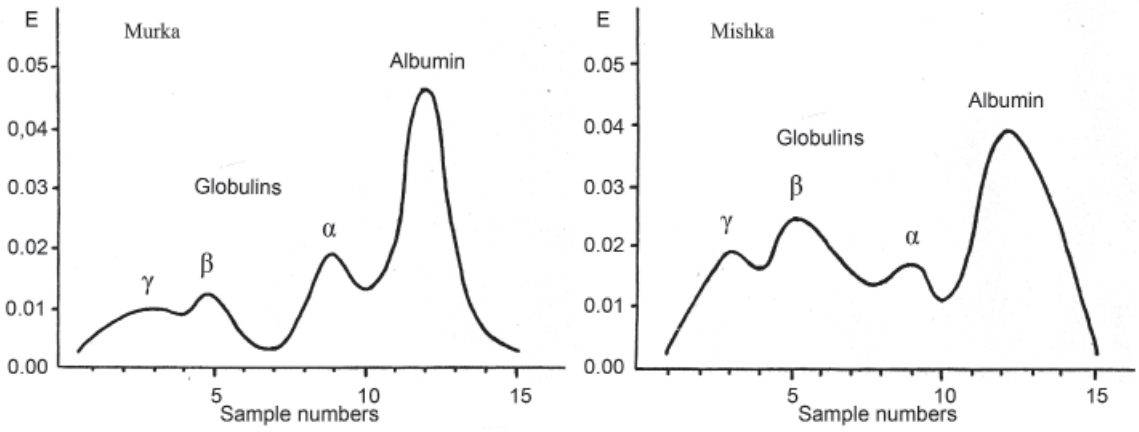
Characteristics	Healthy		Sick	
	Rik	Gip	Murka	Mishka
Colour	straw-yellow	straw-yellow	deep-yellow	rich-yellow
Transparence	clear	clear	clear	cloudy
pH	5,0	5,0	6,0	5,0
Ketones	0	0	0	0
Glucose	0	0	0	0
Hemoglobin	0	0	0	++++
Erythrocytes	G	0	++++	0
Protein assay	negative	negative	positive (0,11 %)	positive (0,18 %)
Kimbarovsky Colour Sedimentary Reaction (KCSR)	negative	negative	positive	positive

Note that pH of seals urine in this study was 5,0 in healthy animals. In the literature, there are reports that in healthy sea lions pH is of about 6,0 [6], as well as in other pinnipeds, cetaceans, sea otters, polar bears, and from 6,0 to 7,5 in manatees [2]. For comparison, the human urine pH is 5,5–6,5 [4], and in representatives of terrestrial mammals – 6,0–6,5 [7].

Urine of sick animals is seen to differ in colour, transparency, pH, presence of hemoglobin, erythrocytes, protein as well as in character of Kimbarovsky Colour Sedimentary Reaction (KCSR) that is nonspecific and is positive in any pathologic process. To determine ketones, glucose, hemoglobin and erythrocytes "La Chema" kit of diagnostic strips for semiqualitative urine analysis was used. Protein was determined with sulfosalycilic acid [4]. Normal urine contains traces of protein and usually clinical protein assays in urine are negative. In sick individuals proteinuria develops, serum albumin being the main protein component as seen from figure. More often kidney disease is the cause of proteinuria. Albuminuria is also observed in various conditions with characteristic disorders of kidney bloodsupply (for example, at stasis of failing heart), in a feverish conditions anemia, liver diseases or different heart anomalies [5].

In Figure, electrophoretic profiles of the urine of sick animals with pathologies of different etiology look unequal.

Murka died of hypotrophy, Mishka's death followed because of systemic aspergillosis with lesions in lungs and kidneys (findings obtained by veterinary surgeon T.B. Yelfimova).



Electrophoretic profiles of proteins in the urine of sick animals

We carried out more detailed examinations of the urine of clinically healthy animals (Rik, Gip) to determine norm for further investigations. Besides, as different species of animals were used in investigations (Rik, a grey seal; Gip – a bearded seal) of certain interest is discovery of specific features of urine composition that reflects peculiarities of specific metabolic processes. Results of investigations are presented in Tables 2 and 3. Different chemical composition of urine of grey seal and of bearded seal mainly consists in presence of nitrogenous components, in particular, total nitrogen, carbamide, aminoacids. These differences are connected both with features of animal feeding habitats [Suzuki et al., 2008], and with peculiarities of nitrogen metabolism. Urine animals studied varied considerably in total content of amino acids (aspartic acid, glutamic acid, glycine, alanine, valine, methionine), and to a lesser extent – on the content of free amino acids (lysine and proline).

Table 2

Chemical composition of the urine of healthy animals

Indexes	Rik	Gip
Dry compounds, %	5,48	4,62
Water, %	94,52	95,38
Total nitrogen, mg %	1541	3283
Carbamide, mg %	3194	6868
Total mineral compounds, %	3,47	5,12
Ca, mg %	14,00	24,00
P, mg %	15,00	39,00
K, g/kg	3,179	2,939
Na, g/kg	11,485	10,489

Table 3

Aminoacid composition of the urine of healthy animals (μmol/l)

Aminoacids	Total		Free	
	Rik	Gip	Rik	Gip
Aspartic acid	851,5	1439,1	196,22	260,18
Threonine	262,5	469,8	99,65	118,86
Serine	452,1	723,8	160,08	167,29
Glutamic acid	741,5	1194,2	128,06	152,42
Proline	trace	trace	108,04	441,22
Glycine	1225,2	4135,5	312,22	276,45
Alanine	716,0	2565,4	170,27	304,84
Valine	157,4	2347,5	87,60	73,75
Methionine	24,4	trace	98,48	152,42
Isoieucine	166,4	297,8	–	–
Leucine	248,1	333,0	trace	trace
Tyrosine	248,9	334,8	31,66	59,68
Phenylalanine	368,4	423,8	12,88	14,30
Histidine	4403,3	5714,8	2713,51	3205,75
Lysine	813,3	1212,8	190.26	466,00
Arginine	367,0	197,0	87,66	83,52
Tryptophan	1071,0	958,3	44,35	67,08

Conclusion

Thus, observations performed resulted in establishing the fact that physical properties and chemical composition of urine can be used for rapid evaluation of the state of sea mammal's health. It is recommended to pay attention to colour, transparence and pH of urine. Among qualitative reactions as shown by investigation, the most distinctive are protein, hemoglobin, erythrocytes assays and KCSR. If protein assay is positive, it is helpful to specify origin of albuminuria by electrophoretic examination of urinary proteins together with analysis of the electrophoretic profile significance. Probably, examination of sick animals having other pathology, will reveal additional tests necessary for evaluation of health, but this question will be the subject of our further research. It is evident that findings obtained for healthy anivals and suggested as standard are helpful for evaluation of distinctive changes in chemical composition of urine in this or that disease.

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