ПРОБЛЕМНАЯ СТАТЬЯ

METAL-LIGAND HOMEOSTASIS AS A POSSIBLE EPIGENETIC FACTOR OF DIFFERENTIATION OF BLASTOMERES AND A CAUSE OF DRUG DISEASES

I.A. Krasnova[1], R.U. Kabirova[1], A.R. Marisyuk[1], G.K. Barashkov[2]*

[1] Central scientific-methodical veterinary laboratory (CSMVL), Oranzhereina str., 23, Moscow, 111622, Russia
[2] I.M. Sechenov First Moscow State Medical Iniversity, Trubetskaya str., 8 bld 2, Moscow, 119992, Russia

ABSTRACT. The analysis of 31 trace elements (TE) content in 20 different organs and tissues of male guinea pig (*Cavia porcellus*) using the optical and mass-spectrometric atomic emission method revealed a marked difference in the ratio of TE. These differences apparently account for causes of drug diseases due to characteristics of functional groups in medicinal preparations. They affect metal-ligand homeostasis (MLH) of individual organs and tissues in different ways. They can act as epigenetic factors (EF) in the differentiation of blastomeres as well.

KEYWORDS: metal-ligand homeostasis (MLH), trace elements (TE), epigenetic factors (EF), drug diseases.

INTRODUCTION

One of the most mysterious phenomena of nature can be considered a process of histo- and ontogenesis in the embryonic development of animals. The differentiation of blastomeres of different germinal layers into cells of tissues and organs is a little-studied phenomenon. The formation of chemically and functionally distinct tissues and organs from the same genome cannot be explained from the standpoint of the genocentric biological paradigm. The chromosome theory of heredity sidesteps this question too. This phenomenon is explained by the influence of hypothetical "epigenetic factors" (EF). They participate in the time and spatial control of gene activity in the developmental process (Holliday, 1990).

Thus, the term "epigenetic" may be used to describe any internal factors that affect the development of an organism except DNA sequence itself. Among the known EF, there have been named the proteins of some genes (Takahashi et al., 2007) and enzymes associated with methylation of nitrogenous bases of DNA and deacetylation of histone nucleosomes (Vanyushin et al., 1968; 1970; Zakiyan et al., 2012). The gene expression is suppressed in both these processes. Other EF are still unknown.

The disclosure of their nature is very important not only in terms of a theory, but also for understanding the nature of side effects of pharmaceuticals and drug diseases caused by them. The development of bioinorganic chemistry (BIC) and research at the ion-molecular (the lowest) level of organization of life processes allowed to assume that metal ions, their composition and ratio are of maximum value among the possible EF.

MATERALS AND METHODS

The laboratory animals, namely, guinea pigs *Cavia porcellus* (3 adult males, 1.5 years) were taken to study trace element contents. After euthanasia with diethyl ether, tissues and organs developed from different germ layers were collected:

From mesoderm: 1) whole blood; 1c) serum; 2) bone (femur); 3) striated muscles (buttocks); 4) kidneys; 5) heart (syncytium); 6) blood vessels (aorta); 7) testes; 8) skin of the neck; 9) spleen; 10) adrenal.

From ectoderm: 12) cerebrum; 13) large (straight) intestine; 14) teeth.

From endoderm: 15) mid-intestine (narrow intestine); 16) lungs; 17) liver; 18) thyroid body; 19) urinary bladder; 20) eye.

All the samples (tissues and organs – about 0.2 g each, blood and serum – 0.5 ml) were placed in polytetrafluoroethylene vessels, therein was added the mixture of H_2O_2, HNO_3 and H_2O (1:3:1) and they were burned in a microwave Berghof MWS-2 according to the program P_4 (T_1 – 160ºC, t_1 – 5 min, p_1 – 80%; T_2 – 210ºC, t_2 – 15 min, p_2 – 90%; T_3 < 100ºC, t_3 – 15 min, p_3 – 0). The sample volume was adjusted to 20 ml with water.

Then the samples were assayed for elements. They were roughly divided into two pools: **pool 1** included 10 macroelements (Na, Mg, Al, P, S, K, Ca, Fe, Cu, Zn), and **pool 2** included 21 trace elements (Li, Be, B, Si, V, Cr, Mn, Co, Ni, Ga, As, Se, Rb, Sr,

* Corresponding author:
Georgy K. Barashkov
E-mail: barachbig@gmail.com

Ag, Cd, I, Cs, Ba, Pb, U). The optical instrument ICP-OES Optima 7300 and mass spectrometer ICP-MS-DRC ELAN(e) (Both devices made by Perkin Elmer) were used for this study.

The results are shown with respect to the reference element. The reference element is chosen to be Ca as the main inorganic messenger (Williams, Frausto da Silva, 1997). The relative values allow excluding non-systematic errors of sampling and sample preparation (Barashkov, 2007). The content of Ca is shown separately.

RESULTS AND DISCUSSION

Upon preparation of supposedly pluripotent cells from mouse fibroblasts, it was found out that the proteins of 4 genes Oct3/4, Sox2, Klf4 and c-Myc are involved in this process (Takahashi et al., 2007). However, the biochemical mechanism is not ascertained. The conclusive evidences of changes in potency of finally obtained "stem" cells were not obtained.

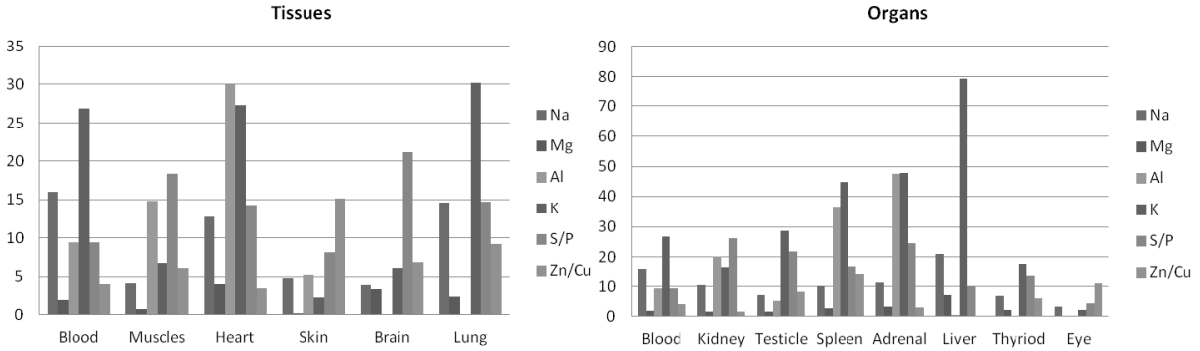
Our results are given in Tables 1–3. The content of macro- and trace elements, respectively, in all objects in the form of relative values (E/Ca). Figures 1 show a histogram of the TE content of the 1st pool in some tissues and organs, as illustrative examples of the whole picture.

The results clearly testify that all the studied organs and tissues contain the markedly differing ratios of all 31 elements. Despite the actual co-involvement of derivatives of all three germinal layers in the differentiation of organs and tissues. The cell differentiation in ontogenesis and histogenesis occurs according to their own "templates" regardless of other structures. Undoubtedly, the same is the case for the remaining elements of Mendeleev's table.

For example, Ca is abundant in brain, kidneys and spleen, its content in teeth is almost 4 times as much as in bones; Zn is eminently abundant in bones and liver; Fe is abundant in spleen, lungs and liver; Cu – in liver, B – in blood, marrow, spleen and adrenal gland; Si – in testes and urinary bladder; Se – in kidneys, adrenal glands and lungs; I is eminently abundant in blood and brain. The details of the TE distribution in humans may slightly differ. Moreover, there are the preliminary data on gender dependence of metal-ligand homeostasis (MLH).

The obtained results may be considered in several aspects: 1) metal-ligand homeostasis and etiology of drug disease; 2) MLH and epigenetic of differentiation of stem cells.

1. The discovery of prions and affected diseases have shown the possibility of transmission of hereditary information «from protein to protein» and independence of metabolism in different brain divisions. Depending on the place where the conversion of a normal prion protein into a virulent one have occurred, 5 different diseases manifest themselves. If in the brain stem – Bovine Spongiform Encephalitis (BSE), in the thalamus – Fatal Familial Insomnia (FFI), in the cerebral cortex – Creutzfeldt-Jakob disease (CJD), in the cerebellum – Kuru disease and Gerstmann–Sträussler–Scheinker (GSS) (Prusiner, 1998). The cover and membrane properties of different parts of the same organ ensure their physiological and chemical peculiarities. One can assume that MLH of different tissues and organs retains its specificity in life activity.



a) *b*)

Fig. 1. *Content of some trace elements in different tissues (a) and organs (b)*

Table 1. ***The content of Ca in all objects (ppm)***

Element	Samples									
	1	**1c**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Ca	63,4	36,8	219591,6	264,5	101,7	55,9	97,1	49,2	195,3	65,3
	10	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Ca	47,2	2887	259,8	778343,5	207,8	129,5	63,1	238,4	156	807,6

Note: here and elsewhere in the table the samples are: **From mesoderm: 1 –** whole blood; **1c** – serum; **2 –** bone (femur); **3 –** striated muscles (buttocks); **4 –** kidneys; **5 –** heart (syncytium); **6 –** blood vessels (aorta); **7** – testes; **8** – skin of the neck; **9 –** spleen; **10** – adrenal. **From ectoderm: 12 –** cerebrum; **13 –** large (straight) intestine; **14 –** teeth. **From endoderm: 15 –** mid-intestine (narrow intestine); **16 –** lungs; **17 –** liver; 18 – thyroid body; **19 –** urinary bladder; **20 –** eye.

Table 2. ***Relative content of elements against Ca (E/Ca), pool 1***

Elements	Samples									
	1	**1c**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Na	16	14,32	0,028	4,17	10,38	12,83	12,9	7,1	4,77	10,1
Mg	1,9	0,26	0,016	0,76	1,64	4,06	2,35	1,7	0,23	2,69
Al	9,4	0,41	0,23	14,81	20,01	30,01	0,25	5,22	5,21	36,5
P	47,4	4,57	0,003	18,22	21,54	49,85	0,26	10,01	13,6	34,1
S	69,7	3,15	0,22	37,24	37,51	80,97	17,64	28,62	11,01	75,5
K	26,9	0,9	0,0012	6,78	16,43	27,31	31,34	28,62	2,24	44,74
Fe	2,33	0,04	0,00001	0,036	0,595	0,95	0,4	0,21	0,041	8,74
Cu	0,14	0,009	0,00044	0,052	0,23	0,31	0,012	0,063	0,09	0,27
Zn	0,2	0,55	21,92	0,046	0,31	0,2	0,092	0,3	0,4	0,2
Na/K	0,97	8,66	26,33	0,12	0,51	0,28	0,41	0,35	1,28	0,2
S/P	9,48	7,31	471,7	18,33	26,1	14,13	68,1	21,66	8,15	16,9
Mg/Al	1,8	144,9	24967,4	13,43	44,88	38,94	5,61	134,5	143,9	25,8
Zn/Cu	4,1	0,6	175,3	6,094	1,71	3,41	7,48	8,23	15,2	14,33
Fe/Zn	168,2	12,1	0,033	3,66	7,1	11,22	4,35	6,96	2,17	72,85
	10	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Na	11,37	3,96	4,31	0,024	11,53	14,58	21,1	6,82	10,55	3,14
Mg	3,16	3,32	2,3	0,073	1,87	2,3	7,15	2,15	2,98	0,18
Al	47,54	0,002	0,05	0,00004	0,028	0,007	0,39	0,02	0,036	0,006
P	37,81	29,5	5,61	0,26	6,73	15,6	36,38	10,91	9,26	0,63
S	96,26	5,92	12,3	0,26	18,5	27,65	77,2	14,7	24,9	2,67
K	47,87	6,11	16,6	0,012	18,69	30,2	79,3	17,76	34,3	2,21
Fe	1,81	0,11	0,35	0,0001	0,32	2,06	2,92	0,26	0,19	0,015
Cu	0,19	0,007	0,01	2,50E-06	0,044	0,011	1,394	0,011	0,01	0,001
Zn	0,19	0,08	0,09	0,0006	0,15	0,11	0,38	0,066	0,18	0,006
Na/K	0,19	0,51	0,26	1,92	0,93	0,49	0,26	0,4	0,3	1,34
S/P	24,63	21,2	12,2	312,2	10,83	14,7	10,3	13,8	6,9	4,3
Mg/Al	101,2	2364,9	59	60274	108,1	1165	19,3	159	78,2	444
Zn/Cu	2,96	6,85	11,2	244,1	4,51	9,3	0,26	6,01	20,6	10,9
Fe/Zn	17,73	2,08	5,28	0,24	2,43	21,4	11,5	5,27	1,5	2,02

Therefore, the same impacts of the environment, elemental composition of foods and drinks, including mineral content, unequally affect different tissues and organs. On the one hand, consumed metals interact with other metals in an organism in compliance with the law of substitution and its consequences (Barashkov, 2011).

Usually the immune system of a healthy organism easily copes with fluctuations in the composition of natural products. The situation changes when chemically synthesized or genetically modified foods and drinks are used. The same is in the case of various diseases and treatment with pharmaceutical drugs in classical medicine (allopathy). In all this preparations there are functional groups with different electron-donating and stereochemical properties which provide a therapeutic effect. The physiologically active ligands enter the organism, behaving in accordance with the composition and the ratio of TE in different organs. As a result of a long-term use, MLH in healthy organs and tissues is disrupted and drug diseases arise.

Only one example: statins blocking the mevalonate pathway of cholesterol synthesis by inhibiting HMG-CoA-reductase are widely used against high cholesterol levels in blood. Because of the high cost of natural statins, there have been launched a

synthesis of their analogs. The active amide and imide groups have appeared in these molecules. Their ligand properties lead to the removal of metal ions from normal metabolism enzymes. As a result, the development of rhabdomyolysis and idiopathic polyneuropathy occurs (Betteridge, Khan, 2001).

Table 3. Relative content of trace elements against Ca (E/Ca), pool 2

Elements	Samples									
	1	**1c**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Li	0,0002	0,00006	0,00000072	0,00009	0,00034	0,00025	0	0,00013	0,000078	0,00016
Be	0	1,00E-06	0,000000017	6,70E-07	5,95E-07	8,80E-07	8,14E-06	3,46E-06	0	0
B	0,0023	0,00054	0,0000052	0,0007	0,0003	0,0008	0	0,00115	0,001400339	0,003
Si	1,3	0,053	0,0006	0,264	0,217	0,37	0,001	3,1	0,575	0,83
V	0,001	0,0002	0,00000018	0,000045	0,00045	0,00031	0,00041	0,00022	0,00022	0,00025
Cr	0,0014	0,00022	0,000000077	0,00015	0,00044	0,00022	0,0022	0,00078	0,00086	0,004
Mn	0,0016	0,000051	0,0000015	0,0005	0,01	0,0086	0,0036	0,0067	0,0012	0,0041
Co	0,00002	7,70E-06	0,0000014	0,000024	0,00032	0,00075	0,000095	0,000076	0,000029	0,00021
Ni	0,00015	0,000037	0,000027	0,000044	0,00024	0,00002	0,0015	0,00021	0,00035	0,0027
Ga	0,00007	9,20E-06	0,0000025	0,000031	9,29759E-05	0,00017	0,00002	0,000016	0,000014	0
As	0,0004	0,0001	0,00000025	0,00002	0,00012	0,000068	0,0001	0,0001	0,000087	0,000077
Se	0,008	0,002	0,0000005	0,0015	0,011	0,006	0,0017	0,0022	0,0009	0,0042
Rb	0,15	0,0073	0,000003	0,07	0,086	0,162	0,0014	0,07	0,027	0,154
Sr	0,0024	0,0016	0,0013	0,00086	0,0016	0,0021	0,001	0,0021	0,0016	0,0018
Ag	0,00003	0	0,00000009	0,000041	4,80E-06	4,10E-06	0	0,000013	0,000012	0,000015
Cd	0,00004	1,10E-06	0,000000044	0,000017	0,016	0,000085	0,00011	0,000081	0,000024	0,00021
I	0,01	0,00058	0	0,00001	0,0016	0,001	0,00031	0,003	0,0008	0,0016
Cs	0,00008	0,000006	4,1E-09	0,00015	0,00023	0,00022	0,00008	0,000097	0,000034	0,00016
Ba	0	0,0003	0,00008	0,00024	0,002	0,00096	0,00094	0,00038	0,00048	0
Pb	0,0017	0,000066	0,000005	0,000045	0,00031	0,00093	0,000092	0,00021	0,00016	0,0073
U	0	0	5,3E-09	0	4,60E-06	0	2,70E-06	3,13E-06	9,30E-07	0
	10	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Li	0,00018	0,000091	0,000036	5,94E-07	0,000045	0,00018	0,00013	0	0,000067	0,000043
Be	0	0	0,0000017	1,20E-08	1,00E-06	8,90E-07	1,30E-06	8,10E-06	5,20E-06	0
B	0,0033	0,0012	0,00044	4,10E-06	0,00035	0,00015	0,0004	0	0,00058	0,00076
Si	0,92	0,65	0,027	0,0009	0,13	0,11	0,18	0,001	1,55	0,29
V	0,00065	0,0006	0,00021	1,46E-07	0,000036	0,00048	0,00029	0,0004	0,00028	0,00026
Cr	0,00086	0,00084	0,00024	5,48E-08	0,0001	0,00047	0,00033	0,0022	0,00059	0,00075
Mn	0,025	0,00096	0,00004	1,39E-06	0,00028	0,0096	0,0063	0,0036	0,006	0,00081
Co	0,00049	0,000014	0,0000066	1,24E-06	0,000016	0,00028	0,0006	0,000095	0,000066	0,000022
Ni	0,00037	0,00011	0,000053	0,000024	0,000024	0,00017	0,00003	0,0015	0,00014	0,00025
Ga	0,000018	0,000034	6,30E-06	2,32E-06	0,000016	0,000079	0,000087	0,000019	0,000023	0,000021
As	0,00014	0,00025	0,00012	2,11E-07	0,0000165	0,00015	0,00008	0,0001	0,0001	0,0001
Se	0,013	0,0051	0,0018	5,72E-07	0,00096	0,0089	0,0047	0,0017	0,0032	0,00072
Rb	0,17	0,086	0,0047	2,73E-06	0,042	0,067	0,11	0,0014	0,078	0,016
Sr	0,0013	0,0018	0,0014	0,0012	0,00051	0,0012	0,0014	0,001	0,0016	0,0011
Ag	3,10E-06	0,000047	0	1,10E-07	0,000021	2,40E-06	2,05E-06	0	6,30E-06	0,000007
Cd	0,0007	0,000024	0,0000017	4,23E-08	0,000014	0,016	0,00008	0,00011	0,000081	0,000021
I	0,0018	0,005	0,0004	0	0,000015	0,0014	0,00074	0,00031	0,0016	0,0005
Cs	0,00033	0,000048	4,70E-06	3,50E-09	0,000092	0,00018	0,00016	0,000079	0,00012	0,000021
Ba	0,00024	0	0,00031	0,000076	0,00012	0,0019	0,00067	0,00093	0,00057	0,0005
Pb	0,00044	0,00094	0,00004	3,80E-06	0,000028	0,00023	0,00051	0,000092	0,00012	0,00012
U	0	0	0	5,00E-09	0	4,10E-06	0	2,70E-06	1,60E-06	8,00E-07

The statins violate biosynthetic processes of selenoproteins of iodinase and glutathione peroxidase as well (Moosman, Behl, 2004), and it leads to a deficiency of Se, Mn, Mg. One can give hundreds or even thousands of such examples. The mortality due to imbalance of MLH in drug diseases increases confidently. Therefore, manufacturers of drugs are not interested in publishing this statistics. Naturally, the homeopathic treatment has less side effects.

2. The second important aspect of MLH study is the epigenetic of animal development at the lowest organizational level of living matter, including the control of gene expression and suppression. For example, I. Bertini in his report on the exchange of copper at the 13th International Conference on BIC referred to the fact of the "awakening" of the gene of protein or Wilson's disease, or Menkes disease. While another gene remains in a "sleep" state (Bertini, Cavallaro, 2008). The reason for this phenomenon has not been studied. Most likely, it results from the different ratio of TE. The importance of studying this problem is that Wilson's disease can be cured but not Menkes.

Here is an another example related to the differentiation of blastomeres. The fantastical ideas of new medical technologies have appeared. For example, in 2012 the Nobel Committee for physiology or medicine recognized the return of differentiated cells into a pluripotent state as possible. Until now, the embryology laws excluded the possibility of artificial cultivation of differentiated cells from other ones. Stem (totipotent) cells disappear after the third division of a zygote and they are never formed again. Fibroblasts can form only one tissue possible for their nature which is a connective tissue. They cannot be transformed into cardiomyocytes for formation of heart syncytium. This also applies to transversal striated and unstriated muscles, nerve tissue and even epithelium. The "Hayflick limit" (Hayflick, Moorhead, 1961) about limited cell divisions of different tissues has not been disproved.

The genome of all the cells at fission, gastrulation, delamination, differentiation of germinal layers, organogenesis and histogenesis is the same. What manages the processes of differentiation and how it occurs is completely unknown. The genetic molecular level of research in the geocentrically paradigm of natural science is insufficient. Our data suggest that MLH, composition and proportion of TE in all tissues and organs are different. This can be a key to understanding the "work" of genes, and how and why the control of a genome occurs as well. Therefore it is necessary to develop research at the ion-molecular level, in particular research related to embryology. The development of "elixir of immortality" is too early.

AKNOWLEDGEMENT

The authors are grateful for help at different work stages to the following employees of CSMVL: G.V. Ivanova, B.A. Krapivkin, L.P. Satykova and L.R. Kabirova.

REFERENCES

Barashkov G.K. [Bases of medicinal bioneoorganics]. Moscow: CheRo, 2007 (in Russ.).

Barashkov G.K. [Medicinal bioinorganics. Bases, analytics, clinics]. Moscow: Binom, 2011 (in Russ.).

Bertini I., Cavallaro G. Metals in the «omics» world: copper homeostasis and cytochromes c oxidase assembly in a new light // JBIC. 2008, 13(1):3–14.

Betteridge D.J., Khan M. Statins and Coronary Artery Disease. Sci. Press Ltd: London, 2001.

Hayflick L., Moorhead P.S. The serial cultivation of human diploid cell strains // Exp Cell Res. 1961, 253:585–621.

Holliday R. Mechanisms for the control of gene activity during development // Biol Rev Cambr Philos Soc. 1990, 65:431–471.

Takahashi K., Tanabe K., Ohnuki M., Narita M., Ichisaka T., Tomoda K., Yamanaka S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors // Cell. 2007, 131(5): 861–872.

Moosman B., Behl C. Selenoprotein synthesis and side-effects of statine // Lancet. 2004, 363:892–894.

Prusiner S.B. Prions // Proceedings of the Nat Acad Sci USA. 1998, 95(23):13363–13383.

Vanyushin B.F., Belozersky A.N., Kokurina N.A., Kadirova D.X. 5-methylcytosine and 6-ethylaminopurine in bacterial DNA // Nature. 1968, 218:1066–1067.

Vanyushin B.F., Tkacheva S.G., Belozersky A.N. Rare bases in animal DNA // Nature. 1970, 225:948–949.

Williams R.J.P, Frausto da Silva J.J.R. The Natural Selection of the Chemical Elements. The Environment and Life's Chemistry. Clarendon Press: Oxford, 1997.

Zakiyan S.M., Vlasov V.V., Dementieva E.V. (eds.) [Epigenetics]. Novosibirsk: Publishing company SO RAN, 2012 (in Russ.).

МЕТАЛЛ-ЛИГАНДНЫЙ ГОМЕОСТАЗ КАК ВОЗМОЖНЫЙ ЭПИГЕНЕТИЧЕСКИЙ ФАКТОР ДИФФЕРЕНЦИАЦИИ БЛАСТОМЕРОВ И ПРИЧИНА ЛЕКАРСТВЕННЫХ БОЛЕЗНЕЙ

И.А. Краснова[1], Р.У. Кабирова[1], А.Р. Марисюк[1], Г.К. Барашков[2]

[1] Центральная научно-методическая ветеринарная лаборатория, Москва
[2] Первый московский государственный медицинский университет имени И.М. Сеченова;
E-mail: barachbig@gmail.com

РЕЗЮМЕ. Исследование содержания 31 микроэлемента (МЭ) в 20 разных органах и тканях, развившихся из всех трёх зародышевых листков, у самцов морской свинки *Cavia porcellus* с помощью оптического и масс-спектрометрического атомно-эмиссионного метода анализа (ICP-OES и ICP-MS) выявило их заметные отличия по соотношению МЭ. Это может играть роль эпигенетического фактора при дифференциации бластомеров из-за разного влияния на МЛГ при гисто- и онтогенезе. Этим же можно объяснить причины лекарственных болезней из-за различий свойств функциональных групп в лечебных препаратах.

КЛЮЧЕВЫЕ СЛОВА: металл-лигандный гомеостаз (МЛГ), микроэлементы (МЭ), эпигенетические факторы (ЭФ), лекарственные болезни.