О. Мась, асп., О. Андрійчук, канд. біол. наук, В. Поліщук, проф.
КНУ імені Тараса Шевченка, Київ

СИСТЕМНИЙ ПІДХІД ПРИ ДОСЛІДЖЕННІ ВІРУСІВ БАКТЕРІЙ АНТАРКТИЧНИХ ҐРУНТОВИХ ЦЕНОЗІВ

Субантарктичний клімат та специфічні геолого-біологічні характеристики створюють унікальні умови для розвитку ґрунтових бактеріальних та вірусних популяцій. В статті розглядаються фактори оточуючого середовища які мають вплив на збереження інфекційності вірусів та які необхідно враховувати при роботі в лабораторії.

Ключові слова: Субантарктичний клімат, бактеріофаги, ґрунтові ценози.

Е. Мась, асп., Е. Андрейчук, канд. биол. наук, В. Полищук, проф.
КНУ имени Тараса Шевченко, Киев

СИСТЕМНЫЙ ПОДХОД ПРИ ИССЛЕДОВАНИИ ВИРУСОВ БАКТЕРИЙ АНТИРКТИЧЕСКИХ ПОЧВЕННЫХ ЦЕНОЗОВ

Субантарктический климат и специфические геолого-биологические характеристики создают уникальные условия для развития почвенных бактериальных и вирусных популяций. В статье рассматриваются факторы окружающей среды, которые влияют на сохранение инфекцинности вирусов и которые необходимо учитывать при работе в лаборатории.

Ключевые слова: Субантарктический климат, бактериофаги, почвенные ценозы.

UDK 612.367:577.175.732

N. Skochko, PhD student, M. Cherpak, student, E. Torgalo, PhD, D. Jankowski, Doctor of Science
Taras Shevchenko National University of Kyiv, Kyiv

AGE RELATED FEATURES OF WATER AND ELECTROLYTES TRANSPORT ACROSS THE EPITHELIUM OF RATS COLON AND THEIR CORRECTION WITH MULTIPROBIOTIC "SIMBITER ® ACIDOPHILUS" CONCENTRATED

The transport of water and electrolytes across the epithelium of the colon in rats of different ages was studied. It was found that at the 21 and 24 month old rats absorption of water and Na^+ and Cl^- ions significantly increased, which is one of the causes of constipation occurancens in aged and elderly rats. Periodic administration of multi-probiotic "Symbiter" (0.14 mg / kg) prevents age-related changes in the transport of water and electrolytes across the epithelium of the colon.

Keywords: colon, the total flow of water and Na^+ and Cl^- ions, multiprobiotic.

More than 60% of people over 60 years old have the problem with constipations. Such problem not only reduce the social activities of people, but also cause pressure increasing in the cavity of intestine, diverticulums, overgrowth and accumulation of endotoxin [3]. It was shown that the main reason of constipation in healthy individuals who are older than 80 years is slowed chyme transport through intestine, which occurs due to decreased of motor activity of colon [10]. Age related changes in colon are associated with degeneration of nerve fibers that innervate its smooth muscles [14]. With age neurotransmitter acetylcholine (main stimulator of smooth muscle motor activity) release decreases, that is the reason of constipations occurrence. Robert et al [11] established another mechanism of acetylcholine release reduction in colon during aging. They showed that aging is accompanied by decrease of calcium appearance in the colon neurons of rats, resulting in acetylcholine release reduction. Ayzman R. I. showed that aging causes changes in intestinal mucosa, which leads to hydrolysis and absorption decrease. There are several reason: surface of membrane digestion decrease because of atrophy, number of operating carriers in transport systems also reduce, Cl^-secretion decrease [1].

Thus, the most of literature sources associated the development of age related constipation with changes in colon motility and much less attention is paid to the study of age-related aspects of water and electrolytes transport across the epithelium of the colon.

Previously, we showed that water, Na + and Cl- absorption increases with age and that is the reason of age-related constipation [6]. These changes begin at the end of mature age. Despite the large number of laxatives drugs, the search of safe preparation and drugs without addiction is still actual. One of the most perspective preparations are probiotics. Especially taking into account that with age colon contamination with opportunistic flora increases [7].

The aim of our work was to study dynamics of water and electrolytes transport across the epithelium of the colon in rats of different ages with standart diet and with periodic consumption of multiprobiotic "Symbiter ® acidophilic" concentrated (Symbiter).

Methods: In chronic experiments the transport of water and electrolytes across the epithelium of the colon were studied on white nonlinear female rats in ontogenesis. Rats were born with different females in one day period and were randomly divided into 2 groups: control, rats were fed a standard maintenance diet, and experimental, with periodically multiprobiotic supplement to diet. Rats of control group at the age of 3, 6, 9, 12, 15 and 18 month old were administered 0.5 ml dechlorinated tap water of room temperature. Rats of experimental group in the first 10 days after birth got 1 drop of Symbiter daily. Hereafter these rats at the age of 3, 6, 9, 12, 15 and 18 month were injected 0.14 ml/kg multiprobiotic Symbiter diluted in 0.5 ml of water. One dose of Symbiter (10 ml) consists of biomass of 14 living probiotic stains in symbiosis: Lactobacillus and Lactococcus – non less than $6.0x10^{10}$ CFU/cm^3, propionic-acid bacteria – $3.0x10^{10}$ CFU/cm^3, bifidobacteria – $1.0x10^{10}$ CFU/cm^3, acetic-acid bacteria – $1.0x10^6$ CFU/cm^3. From each group at the age of 3 month (juvenile age), 6, 9 month (young age), 18 month (mature age), 21 month (eldery) and 24 month (senile) animals were taken for experimental tests. Rats were anaesthetized by urethane at the dose 1.15 g/kg intraperitoneally**.** The transport of water and electrolytes across the epithelium of the colon were measured in acute experiments by method of perfusion of isolated colon. After equilibration period (60 min.), samples were taken each 20 minutes. Total perfusion duration was 180 min. Changes in concentration of unabsorbed phenol red marker determined with photocolorimetric methods (λ=520 nm, λ =560 nm та λ=600 nm) represented the level of water absorption. Concentration of CL^- in perfused solution was measured with ion meter. Measure-

ments of the concentrations of Na^+ and K^+ were carried out using a flame photometric analyzer of liquids [13].

All obtained data were subjected to statistical analysis using software package "Statistics, 8.0". Shapiro-Wilks criterion was used for the analysis of data distribution type. As the data were abnormal distributed independent samples were compared by Mann-Whitney U test. We calculated median (Me) and lower and upper quartiles (Me [L.q... U.q]) [4].

Results. It was found that in 3 months old rats the level of water absorption was 31.01 [5.05... 68.93] ml/g*20 min, Na^+ – 24.81 [2.86... 46.85], K^+ – 0.74 [-3.01... -0.05] and Cl^- – 13.92 [4.46... 26.99] µmol/g*20 min. This results matched with other researchers data obtained in vivo and showed that the absorption of water is accompanied by absorption of Na^+ and Cl^-, K^+ secretion [1, 8, 10] (Fig. 1).

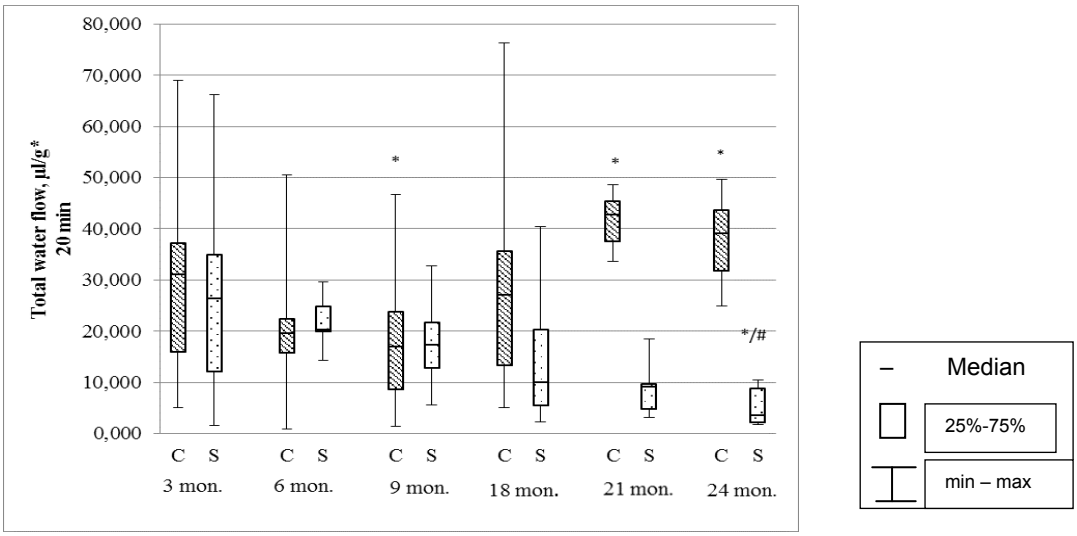

Figure 1. Total flow of water across the epithelium of the colon of different age groups rats (Me [L.q... U.q], n = 8)
K – rats of control group;
C – rats of experimental group that were periodically injected with multyprobiotic Symbiter;
* – p <0,05 compare to 3 months old control group; # – p <0,05 compare to same age control group

The level of water absorption in 6 and 18 months old rats of control group had no significant difference from rats of juvenile age and was equal to19.82 [3.5... 50.57] and 27.16 [12.72... 35.95] ml/g*20 min respectively. In the same time in 21 and 24 month old rats absorption of water and Na^+ and Cl^- ions was increased. Absorbtion of water in eldery rats increased by 33.7% ($p<0.05$), in senile – 26.0% ($p<0.05$) compare to juvenile. Periodic introduction of multiprobiotic decreased water absorption in colon of 18, 21 and 24 months old rats correspondently by 63.0% ($p<0.05$), 79.1% ($p<0.05$) and 90.8% ($p<0.05$) compare to control group of same age.

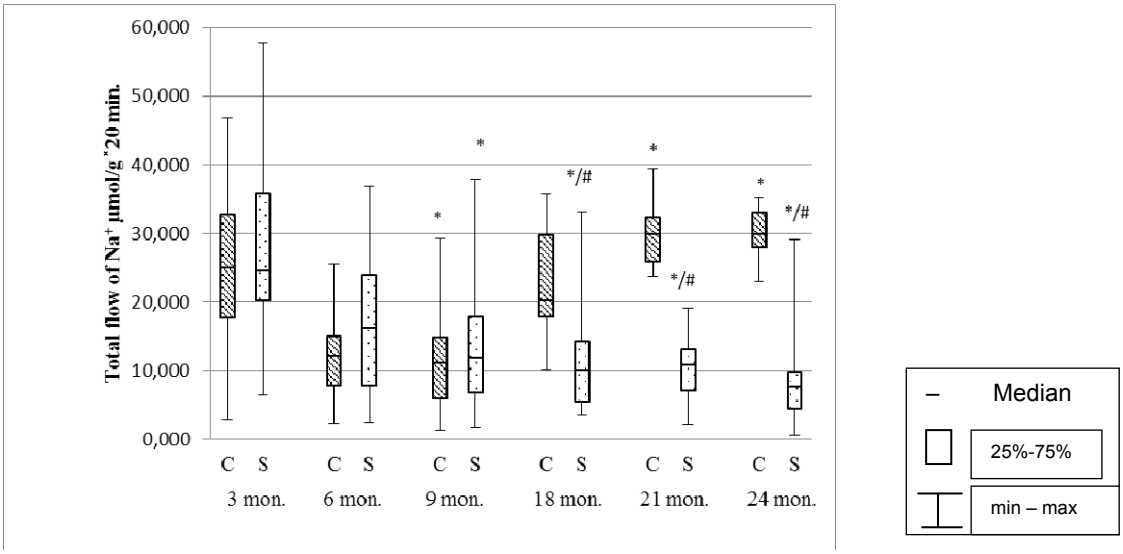

Figure 2. Total flow of Na^+ across the epithelium of the colon of different age groups rats (Me [L.q... U.q], n = 8)
K – rats of control group;
C – rats of experimental group that were periodically injected with multyprobiotic "Symbiter";
* – p <0,05 compare to 3 months old control group; # – p <0,05 compare to same age control group

The total flow of Na^+ in 6 months old rats was lower than in 3 months old, but there was no significant differences (Fig. 2). In 9 month old rats Na^+ absorption decreased by 57.7% ($p<0.05$) compare to rats of juvenile age. Thus, the total flow of electrolytes in 9 month old rats was the smallest. In experimental group same as in control, in 9

months old rats the total flow of ions decreased by 51.99% ($p<0.05$) in comparison with 3 months old control group.

In the eldery (21 months) and senile rats (24 months) total flow of Na^+ was increased by 19.7% ($p<0.05$) and 20.5% ($p<0.05$) compare to 3 month old rats of control group. Thus, the rate of Na^+ total flow, as well as water, was the biggest in the 24 month old rats and was 29.90 [23.04... 32.25] μmol/g*20 min. The total flow in mature, elderly and senile rats of experimental group of ions decreased by 50% ($p<0.05$) and 64.07% ($p<0.05$) and 74.05% ($p<0.05$) compare to control group of same age and was also lower than in 3 month old rats of control group.

The total flow of Cl^- ions in 6 and 18 months old rats did not differ from 3 months old. The lowest level of Cl^- ions absorption was in 9 months and was 9.03 [0.37... 23.43] μmol/g*20 min. In eldery and senile rats significant increase of ions total flow was observed. In 21, 24 month old rats data was equal to 22.73 [17.49... 30.93], 20.38 [10.61…26.92] μmol/g*20 min correspondently, which was higher by 63.3% ($p<0.05$) and 46.4% ($p<0.05$) compare to juvenile rats. As in the case with the level of water and Na^+ total flow periodic Symbiter introduction reduced the total flow of Cl^- ions across the epithelium of colon (Fig. 3).

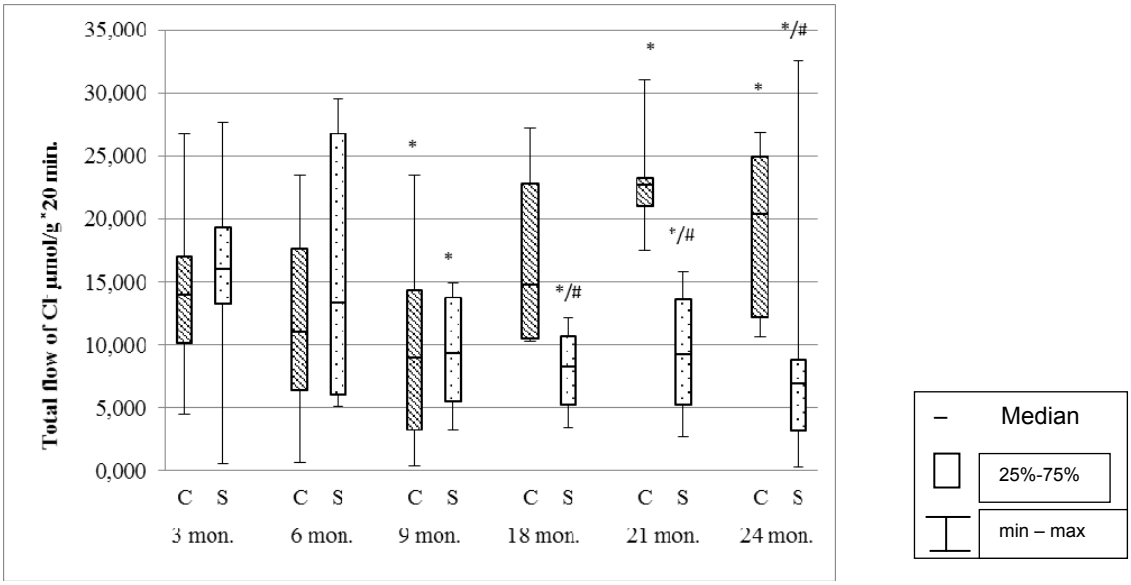

Figure 3. Total flow of Cl^- across the epithelium of the colon of different age groups rats (Me [L.q... U.q], n = 8)
K – rats of control group;
C – rats of experimental group that were periodically injected with multyprobiotic Symbiter;
* – p <0,05 compare to 3 months old control group; # – p <0,05 compare to same age control group

It was found that Na^+ and Cl^- total flow was accompanied with changes in the total flow of water across the epithelium of the colon in different age groups of rats. However, during the ageing the total flow of K^+ ions had no significant changes either in control or in experimental groups. It was clear that at the rest transcellular is the predominant type of water transport across the epithelium of the colon. That occurs passive by diffusion over osmotic gradient of NaCl concentration between intra-and intercellular fluids of intestinal epithelial cells. Our experiments confirm such theory.

Periodic addition of multyprobiotic Symbiter in the diet of rats prevented the reduction of water, Na^+ and Cl^- total flow in elderly and senile rats. We concluded that multiprobiotic consumption had prevented age-related changes in intestinal microflora.

The significance of microflora to the human body is still not full revealed and therefore is often underestimated. But today it is clear that bacteria play an important and a multidimensional role in the various functions of the body. For example they generate significant quantities of various physiologically active compounds. Among them short-chain fatty acids (SCFA) that assure water electrolyte balance and mineral metabolism (the content of carbonates in intestine lumen and pH of the intestinal contents), are the main source of colonocyte nutrition, regulate colon motility [2]. Thus, we consider that SCFA preservation might be the reason of decreased amount of constipations in eldery and senile rats of experimental group.

Conclusions:

1. In elderly and senile rats absorption of water, Na^+ and Cl^- increase.
2. Transport of K^+ ions does not change during ontogenesis.
3. Periodic administration of multiprobiotic "Symbiter ® acidophilic" concentrated prevents the reduction of water, Na^+ and Cl^- total flow in elderly and senile rats.
4. Multiprobiotic "Symbiter® acidophilic" concentrate may be consider an effective preparation for constipations prevention.

References

1. Айзман Р.И. Возрастные особенности транспорта калия в дистальном отделе толстого кишечника крыс / Айзман Р.И., Боровец Е.Н., Герасев А.Д. // Российский физиологический журнал. – 2000. – № 86. – С. 86-94.

2. Белобородова Н.В. Метаболиты анаэробных бактерий (летучие жирные кислоты) и реактивность макроорганизма / Н.В. Белобородова, С.М. Белобородов // Антибиотики и химиотерапия. – 2000. – № 2. – С. 28-36.

3. Белоусова Е.А. Лечение запора у пожилых пациентов / Е.А. Белоусова // Клиническая геронтология. – 2008. – № 1. – С. 58–65.

4. Гланц С. Медико-биологическая статистика / С. Гланц (Пер. с англ.). – М.: Практика, 1998. – 459 с.

5. Приходько В.Ю. Проблема хронічних закрепів у терапевтичній і геріатричній практиці / В.Ю. Приходько, О.В. Давидович, Н.Я. Давидович // Ліки України. – 2008. – № 5. – С. 43-46.

6. Черпак М.Ф. Вікові особливості транспорту води і електролітів через епітелій товстої кишки / М.Ф. Черпак, Т.В. Берегова // Шевченківська весна 2012: 10-а Міжнародна міждисциплінарна науково-практична конференції молодих вчених, 19-23 березня 2012 р., Київ: матеріали конференції. – Київ, 2012. – С. 326-327.

7. Enck P. The effects of ageing on the colonic bacterial microflora in adults / P. Enck, K. Zimmermann, K. Rusch [et al.] // Z Gastroenterol. – 2009. – Vol. 47, № 7. – P. 653-658.

8. Greger R. Regulation of ion transport in colonic crypts / R. Greger, M. Bleich, J. Leipziger [et al] // News Physiol Sci. – 1997. – Vol. 12. – P. 62-66.
9. Huang Z.C. Diagnosis and treatment of slow transit constipation complicated with adult megacolon / ZC. Huang, Q. Liu, SG. Li // Zhonghua Wei Chang Wai Ke Za Zhi. – 2011. –Vol. 14, № 12. – P. 41-44.
10. Madsen JL. Effects of aging on gastrointestinal motor function / JL. Madsen J.Graff // Age, Ageing. – 2004. – Vol. 33. – P. 154-159.
11. Roberts D. Evidence for age-associated reduction in acetylcholine release and smooth muscle response in the rat colon / D. Roberts, D. Gelperin, JW. Wilwey // Am J Physiol. – 1994. – Vol. 267. – P. 515-522.
12. Spring K.R. Routes and mechanism of fluid transport by epithelia / K.R. Spring // Annu Rev Physiol. – 1998. – Vol. 60. – P. 105–119.
13. Sladen G.E. Studies on the effects of unconjugated dyhidroxy bile salts on rat small intestinal function in vivo / G.E. Sladen, J.T. Harries // Biochem Biophys Acta. – 1972. – Vol. 288. – P. 443-456.
14. Voutilainen S. The gastrointestinal tract: safety pharmacology aspects relating to agening, diet and natural/herbal remedies / S. Voutilainen // Journal of Pre-Clinical Research. – 2009. – Vol. 3, № 2. – P. 076-083.
15. Winter D.C. Rapid effects of aldosterone on sodium-hydrogen exchange in isolated colonic crypts / D.C. Winter, M.F. Schneider, G.C. O'Sullivan // J Membr Biol. – 1999. – Vol. 170. – P. 17–26.

Received to editorial board 24.12.13

Н. Скочко, асп., М. Черпак, студ., Є. Торгало, канд. біол. наук, Д. Янковский, д-р біол. наук
КНУ імені Тараса Шевченка, Київ

ВІКОВІ ОСОБЛИВОСТІ ТРАНСПОРТУ ВОДИ ТА ЕЛЕКТРОЛІТІВ ЧЕРЕЗ ЕПІТЕЛІЙ ТОВСТОЇ КИШКИ ЩУРІВ ТА ЇХ КОРЕКЦІЯ МУЛЬТИПРОБІОТИКОМ СИМБІТЕР АЦИДОФІЛЬНИЙ КОНЦЕНТРОВАНИЙ

Досліджено транспорт води і електролітів через епітелій товстої кишки у щурів різного віку. Встановлено, що у віці 21-го та 24-х місяців всмоктування води та іонів Na^+ і Cl^- значуще збільшується, що є однією з причин виникнення закрепів. Періодичне додавання до стандартного корму мультипробіотика "Симбітер® ацидофільний" концентрований (0,14 мл/кг) запобігає віковим змінам у транспорті води і електролітів через епітелій товстої кишки.

Ключові слова: товстий кишечник, сумарний потік води та іонів Na^+ і Cl^-, мультипробіотик.

Н. Скочко, асп., М. Черпак, студ., Е. Торгало, канд. биол. наук, Д. Янковский, д-р биол. наук
КНУ имени Тараса Шевченко, Киев

ВОЗРАСТНЫЕ ИЗМЕНЕНИЯ ТРАНСПОРТА ВОДЫ И ЭЛЕКТРОЛИТОВ ЧЕРЕЗ ЭПИТЕЛИЙ ТОЛСТОГО КИШЕЧНИКА КРЫС И ИХ КОРЕКЦИЯ МУЛЬТИПРОБИОТИКОМ СИМБИТЕР АЦИДОФИЛЬНЫЙ КОНЦЕНТРИРОВАНЫЙ

Исследованы транспорт воды и электролитов через эпителий толстой кишки у крыс разного возраста. Установлено, что в возрасте 21-го и 24-х месяцев всасывания воды и ионов Na^+ и Cl^- значимое увеличивается, что является одной из причин возникновения запоров у крыс пожилого и старческого возраста. Периодическое добавление к стандартному корму мультипробиотика "Симбитер ® ацидофильный" концентрированный (0,14 мл/кг) предотвращает возрастные изменения в транспорте воды и электролитов через эпителий толстой кишки.

Ключевые слова: толстый кишечник, суммарный поток воды и ионов Na^+ и Cl^-, мультипробиотик.

UDK 578.85/.86

T. Mudrak, PGS, G. Korotyeyeva, PhD., T Kompanets, PhD.
Taras Shevchenko National University of Kyiv, Kyiv

DETECTION OF SOME VIRUS PATHOGENS OF CACTUS IN UKRANIAN BOTANICAL GARDENS

Cactus collections of some Ukrainian botanical gardens were analyzed for virus contamination. Different virus-like symptoms including mosaics, chlorosis and local necroses have been detected on cactus plants in these collections. Biological properties of isolated viruses were defined by the methods of bioassay, transmission electron microscopy and indirect ELISA.

Key words: ELISA, isolated viruses, cactus.

Introduction: The *Cactaceae* are mostly spiny succulents with photosynthetic stems comprising 200 genera and more than 2,000 species [1]. Cactuses have been the object of amateur and professional botanical collectors because of unusual structures and exceptionally colorful and beautiful blossoms. Huge variety of cactus species coupled with their distinctive ecological and biological characteristics cause a number of difficulties associated primarily with cultivation of these plants in the greenhouses. Due to the ordinary vegetative propagation and long-term cultivation of cactus plants in the same collections, these plants may serve as reservoirs of different viruses.

In accordance with literary data about 11 viruses are able to affect the members of *Cactaceae* family: *Cactus virus X*(CVX)*, Schlumbergera virus X* (SVX), *Opuntia virus X* (OVX), *Zygocactus virus X (*ZVX), *Saguaro cactus virus* (SCV), *Sammons' Opuntia virus* (SOV), *Cactus virus 2* (CV2), *Cactus mild mottle virus* (CMMoV), *Rattail cactus necrosis-associated virus* (RCNAV), *Impatiens necrotic spot virus* (INSV) and *Tomato spot wilt virus* (TSWV) [3, 4, 5, 6, 7, 8].

That's why the aim of our work was to **analyze collection of cactus in Ukrainian botanical gardens for virus contamination.**

Materials and methods: The material for investigation was collected in different Ukrainian botanical gardens: Donetsk botanical garden of the National Academy of Sciences of Ukraine, Botanical garden of Ivan Franko National university of Lviv, Botanical garden of Odessa I.I. Mechnikov National university, Karazin' Botanic Garden of Kharkiv National University, Nikitsky Botanical Garden – National Scientific Centre. For detection and identification of viruses 67 samples of different cactus cultivars were selected. For the biological characteristics of the pathogen we used the method of bioassay. Infectivity of plant sap was confirmed proved using indicators plants such as *Chenopodium murale, Celosia cristata, Datura stramonium, Gomphrena globosa, Chenopodium murale, Nicotiana tabacum, Nicotiana rustica.* These plants are typical test-plants for majority cactus viruses.

Virus identification was carried out using indirect ELISA [2]. Samples were tested by ELISA with serums to *Tobaco mosaic virus (*TMV) (antiserum obtained at the virology department, the sensitivity and specificity confirmed experimentally), *Potato virus S* (PVS), *Potato virus M (PVM), Potato virus X* (PVX) (Institute of Agricultural Microbiology, Ukrainian Academy of Agrarian Sciences, Chernigiv), *Tomato spotted wilt virus* (TSWV*), Impatiens necrotic spot virus* (INSV) (Loewe, Germany).

The morphology of virions was studied in leaf dip preparations negatively stained with 2% uranyl acetate.